CHAPTER 1

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the main bacterial pathogens responsible for nosocomial and community-acquired infections, including pneumonia, skin and soft-tissue infections, bloodstream infections, osteomyelitis and endocarditis (Perez Vazquez *et al*., 2009). In Malaysia, *S. aureus* was reported as the second most common pathogens isolated from blood specimens, and MRSA contributed to 21% of bacteremia cases due to *S. aureus* infections in year 2008 (Ministry of Health Malaysia, 2008; Ahmad *et al*., 2010).

S. aureus also produces a variety of toxins that cause food poisoning (Staphylococcal food poisoning), and they are classified as one of the leading causes of gastroenteritides due to the consumption of food contaminated by this bacterium (Lee Loir *et al*., 2003; Ray and Bhunia, 2008).

MRSA was reported in Malaysia since early 1970 (Lim and Zulkifli, 1987) and the prevalence of MRSA in Malaysian hospitals has increased from 17% in 1986 (Rohani *et al*., 1999) to 26% in 2008 (Ministry of Health Malaysia, 2008). Elsewhere, an increase of 62% of MRSA was reported in the United States (Klein *et al*., 2007). Since most MRSA is also resistant to many commonly used antibiotics, this has raised a concern over the limited choice of antimicrobial agents for treatment of life-threatening cases. This could lead to prolonged stay in the hospital and increase the cost of health care (Akpaka *et al*., 2006; Udo *et al*., 2006). A death rate of 34% within 30 days was observed among patients with MRSA infections as compared to 27% in methicillin-sensitive *S. aureus* (MSSA) patients (Wyllie *et al*., 2006).

Although there is no report on the presence of vancomycin-resistant *S. aureus* (VRSA) and vancomycin-intermediate *S. aureus* (VISA) in Malaysia, increased of

minimum inhibitory concentrations (MIC) of vancomycin among *S. aureus* strains in Malaysia was observed (Ahmad *et al*., 2010). The increase of MIC in vancomycin is a cause of concern as it could affect the success of treatment using vancomycin (Ahmad *et al*., 2010) and vancomycin treatment failure in vancomycin-susceptible MRSA has also been reported in Malaysia (Norazah *et al*., 2009).

Rifampicin is used with fusidic acid as an alternative therapy of vancomycin against MRSA infections in Malaysia. There is an increasing trend of fusidic acid-resistant MRSA strains in Malaysia from 3 - 5% in the year 1992-1996 (Rohani *et al*., 1999) to 21% in year 2004 (Thong *et al*., 2009). In another Malaysian local report, Sam *et al*. (2008) reported that the MRSA strains in University Malaya Medical Centre (UMMC) were often resistant to erythromycin, gentamicin and ciprofloxacin. The increased resistance to fusidic acid, erythromycin, gentamicin and ciprofloxacin will limit the choice of the antibiotic options for MRSA infections in this country.

Rapid and discriminative subtyping methods are essential for determining the epidemiology of pathogenic strains and are useful in the design of rational pathogen control methods. Several methods are available and these include pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), *mec*-associated direct repeat unit (*dru*) typing, *spa* typing and other PCR-associated typing methods (Goering *et al*., 2008; Park *et al*., 2008).

MLST has been shown to be useful in global epidemiological studies of *S. aureus*. Ghaznavi-Rad *et al.* (2010) reported that over 90% of MRSA infections in a tertiary hospital (HKL) in Malaysia belonged to MLST type ST239 and their latter report (Ghaznavi-Rad *et al*., 2011) also indicated that *mec*-associated *dru* typing can be used to enhance discrimination of ST239.

There is limited information of the molecular evolution of multidrug resistant MRSA in Malaysia. Most of the local studies concentrated on the antimicrobial

resistance rates and the molecular epidemiology of the strains within the same hospital. There aare no studies on the dynamics of MRSA strains over a longer period of time. This is important because understanding of molecular epidemiology of MRSA strains will help in controlling the spread and emergence of MRSA clones. Therefore, in this study I examined a set of strains isolated in year 2003, 2004, 2007 and 2008 to determine the molecular epidemiology of MRSA, their evolutionary relationship and the presence of different resistance and virulence genes among the MRSA strains. Specifically, the objectives of the study are:

1.1 Objectives of this study

1. To determine the antimicrobial susceptibility patterns of MRSA by disk diffusion test and minimum inhibitory concentrations.
2. To determine the presence of selected plasmid-mediated erythromycin, tetracycline, mupirocin and β-lactam resistance genes in MRSA strains by polymerase chain reactions (PCR) and their transferability by transformation.
3. To determine the chromosomal gene mutations, *rpoB* and *fusA* involved in rifampicin and fusidic acid resistance in MRSA strains.
4. To determine the prevalence of virulence gene and *agr* grouping in MRSA strains by PCR.
5. To determine the genomic relatedness of MRSA by SCC*mec* typing, PCR-RFLP of *coa* gene, pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), *mec*-associated *dru* typing and *spa* typing.
6. To investigate the MRSA evolution between the years 2003, 2004, 2007 and 2008 by using combined MLST-*spa*-*dru* typing.

CHAPTER 2

LITERATURE REVIEW

2.1 *Staphylococcus aureus*

2.1.1 Organisms characteristics

Staphylococcus aureus is catalase and coagulase positive, non-motile, non-capsular, non-sporulating, gram positive facultative anaerobe coccus (individual cells approximately 1 µm in diameter) that appears as grape-like clusters when viewed under the microscope (Mims *et al*., 2004; Whitehead *et al*., 2005; Ray and Bhunia, 2008; Plata *et al*., 2009). The name *Staphylococcus* is originates from the Greek staphyle meaning a bunch of grapes whereas kokkos meaning berry (Brown and Grilli, 1998).

S. aureus cells form convex, round and golden-yellow colonies in agar plate and often produce β-hemolysis and appeared as white or golden colonies when grown on the blood agar plate (Mims *et al*., 2004; Plata *et al*., 2009). They also appear as yellow halo colonies in mannitol salt agar (Shimeld and Rodgers, 1998).

The natural habitat of *S. aureus* includes nose, skin, throat and hair (feathers) of healthy human, birds, pig and animals (Ray and Bhunia, 2008; Neela *et al*., 2009b). It is reported that about 20% of individuals are persistent *S. aureus* nasal carriers and around 30% of individuals are intermittent carriers (Wertheim *et al*., 2005).

2.1.2 Clinical significance of *S. aureus*

S. aureus is one of the main nosocomial bacterial pathogens that causes a wide range of illnesses such as boils, pimples, folliculitis, urinary tract infections, bullous impetigo, skin sepsis, septic arthritis, impetigo, carbuncles, staphylococcal scalded skin syndrome, wound infections, traumatic and surgical wounds, ear's infections, mastitis,

pleura (empyema), meningitis, respiratory tract infections, gastrointestinal tract infections, uterus infections, abscesses, pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), chest pain, bacteremia, post-operative wound infection, catheter associated infections, furunculosis, food-borne infection and blood-stream infections (Shimeld and Rodgers, 1998; Scholar and Pratt, 2000; Moore and Lindsay, 2001; Mims *et al*., 2004; Lindsay and Holden, 2006; Perez-Vazquez *et al*., 2009; Plata *et al*., 2009; Yamamoto *et al*., 2010). In Malaysia, *S. aureus* was reported as the second most common pathogens isolated from blood specimens (Ministry of Health Malaysia, 2008; Ahmad *et al*., 2010). Although, there is no report of an outbreak due to nosocomial *S. aureus* infections in Malaysia, outbreaks due to this bacterium have been reported in other countries. Table 2.1 summarizes a few of the outbreaks caused by nosocomial *S. aureus* in other countries.

Table 2.1: Outbreak caused by nosocomial *S. aureus.*

Places/country	Year	Person affected	Infections	References
Health care ward and nursing home in Finland	2003	13 patients	Bacteraemia	Kerttula *et al*., 2005
Hospital of Perigueux, France	2005	7 neonates	Bullous impetigo	Occelli *et al*., 2007
Notre Dame de Bon Secours Hospital, Paris, France	2007	13 neonates	Staphylococcal scaled skin syndrome	El Helali *et al*., 2005
Neonatal intensive care unit in a tertiary hospital, Autralia	2008	5 neonates	Sepsis	Schlebusch *et al*., 2010
Hospital in Nan-Province, Thailand	2008	30 neonates	Bullous impetigo	Pawun *et al*., 2009
Hospital Clı´nico San Carlos, Spain	2008	12 patients	Linezolid-resistant *S. aureus*	Sanchez Garcia *et al*., 2010

Besides, *S. aureus* also produces a variety of toxins that causes food poisoning (Staphylococcal food poisoning), and they are considered to be one of the most frequently food-borne diseases worldwide (Ray and Bhunia, 2008). In addition, *S.*

aureus is classified as one of the leading causes of gastroenteritidis due to the consumption of food contaminated by this bacterium (Le Loir *et al*., 2003).

The only outbreak due to *S. aureus* food-borne disease in Malaysia was reported in Kapar in year 1983 (Rampal, 1983). Outbreaks of staphylococcal food-borne poisoning have also been reported in other countries as summarized in Table 2.2.

Table 2.2: Outbreak of staphylococcal food-borne poisoning in various countries

Country	Year	Person affected	Toxin	References
University Hospital Rotterdam, The Netherland	Nov 1992- Apr 1993	41 persons	-	Kluytmans *et al*., 1995
Brazil	1998	180 persons	Enterotoxin A	Colombari *et al*., 2007
Osaka, Japan	2000	13, 420 cases	Enterotoxin SEA and SEH	Ikeda *et al*., 2005
Brazil	2003	31 persons	Enterotoxin A, B and D	Do Carmo *et a*l., 2003
Taiwan	2003	229 strains from 10 outbreaks	Ten strains positive for *sec* gene	Wang *et al*., 2003
France	2009	23 persons	Enterotoxin SEE	Ostyn *et al*., 2010

'-': no toxin reported

2.2 Virulence determinants in *S. aureus*

2.2.1 The capsule and cell wall structure of *S. aureus*

The staphylococcal cell wall is mainly composed of peptidoglycan, teichoic acid and surface protein (Plata *et al*., 2009). Peptidoglycan is the major component of the staphylococcal cell wall as it makes up of 50% of the cell wall mass whereas teichoic acid contributes around 40% of the cell wall mass (Harris *et al*., 2002). The *S. aureus* cell wall is sensitive to lysostaphin; an enzyme that specifically cleaves the peptidoglycan bridge of the bacterium (Le Loir *et al*., 2003).

The production of capsular polysaccharides by *S. aureus* or better known as virulence factors has allowed the organism to form biofilms leading to the development

of device-related infections. It represents a serious medical problem as a majority of hospitalized patients are given insertion of foreign devices such as catheter tips, pacemakers, artificial heart valves and many mores (El-Din *et al*., 2011). Furthermore, it is known that 20% and 30% of individuals are persistently colonized in the nose and transiently colonized, respectively (Liu, 2009).

2.2.2 Adhesins

Adhesion and colonization of host tissues are the initial step of the pathogenic process, and they are often mediated by proteins on the bacterial surface (adhesions) which bind specifically to complimentary ligands (Podbielska *et al.,* 2011). The surface protein that promotes attachment to the host proteins includes fibronectin, collagen, laminin, bone sialoprotein (BSP) and elastin (Persson *et al*., 2009; Podbielska *et al*., 2011).

Fibronectin is a protein which belongs to the group of microbial surface components recognizing adhesive matric molecules (MSCRAMMs) and it is known to bind at α5β1 Integrin of the host cell surface resulting of a bridge formation between the bacterial FnBP adhesion and host cell Integrin (Plata *et al*., 2009; Podbielska *et al.,* 2011). This fibronectins (FnBP-A and FnBP-B) are crucial for the invasion of host cells and *S. aureus* mutants that lacks fibronectin, which are often impaired by host cells.

Collagen binding protein or better known as CNA is encoded by *cna* gene (Plata *et al*., 2009). The CNA are composed of an N terminal 55-kDa A domain which contains a unique sequence; a B domain that is composed of 1, 2, 3, or 4 repeats of a 25-kDa unit, and a C-terminal domain containing of cell wall attachment site, and finally, a short cytoplasmics segment which is rich in positively charged residues. Presence of *cna* gene is often associated with osteomyelitis and septic arthritis (Podbielska *et al.,* 2011).

2.2.3 The Staphylococcal enterotoxins and its role in pathogenesis

Staphylococcal enterotoxins are short secreted proteins that are usually heat-resistant, and usually resistant to most of the proteolytic enzymes produced by the human body (Ortega *et al*., 2010). These staphylococcal enterotoxins are rich in tyrosine residues, lysine, aspartic acid and glutamic acid (Le Loir *et al.,* 2003). There are more than 20 different types of enterotoxins (SEA to SEE, SEG to SEI, SEIJ, SEIK-SEIQ, SER to SET, SEW, SEIU) (Argudin *et al*., 2010) produced by *S. aureus*, and they are distributed into four or five groups depending on whether SEH should be places within group I or group V. Basically, Group I consist of SEA, SED, SEE, (SEH), SEIJ, SEIN, SEIO, SEIP, SES, Group II consists of SEB, SEC, SEG, SER, SEIU, SEIU2, Group III consists of SEI, SEIK, SEIL, SEIM, SEIQ, SEIV, Group IV consists of SET alone and finally Group V consist of SEH (if this SEH or not placed in Group I) (Argudin *et al.*, 2010).

Staphylococcal enterotoxins genes are known to be located on mobile genetic elements, including plasmids, prophages, *S. aureus* pathogenicity islands (SAPIs), *egc* gene cluster or genomic island *vSa* (Argudin *et al*., 2010). Both staphylococcal enterotoxins and toxic shock syndrome toxins (TSST) are members of the superantigenic toxin family that stimulate nonspecific T-cell proliferation (Ferry *et al.*, 2005; Ortega *et al.*, 2010; Demir *et al*., 2011).

The location of staphylococcal enterotoxins gene and their general properties are summarized in Table 2.3.

Table 2.3: General properties and location of *S. aureus* enterotoxins genes

Toxin	Molecular Mass (kDa)	Emetic Activity	Gene	Accessory Genetic Element
SEA	27.1	Yes	*sea*	ΦSa3ms, ΦSa3mw, Φ252B, ΦNM3, ΦMu50a
SEB	28.4	Yes	*seb*	pZA10, SaPI3
SEC	27.5- 27.6	Yes	*sec*	SaPIn1, SaPIm1, SaPImw2, SaPIbov1
SED	26.9	Yes	*sed*	pIB485-like
SEE	26.4	No	*see*	ΦSab
SEG	27.0	Yes	*seg*	egc1 (vSaβ I); egc2 (vSaβ III); egc3; egc4
SEH	25.1	Yes	*seh*	MGEmw2/mssa476 seh/Δseo
SEI	24.9	Weak	*sei*	egc1 (vSaβ I); egc2 (vSaβ III)); egc3
SElJ	28.5	Nd	*selj*	pIB485-like; pF5
SElK	26.0	Nd	*selk*	ΦSa3ms, ΦSa3mw, SaPI1, SaPI3, SaPIbov1, SaPI5
SElL	26.0	Noa	*sell*	SaPIn1, SaPIm1, SaPImw2, SaPIbov1
SElM	24.8	Nd	*selm*	egc1 (vSaβ I); egc2 (vSaβ III)
SElN	26.1	Nd	*seln*	egc1 (vSaβ I); egc2 (vSaβ III); egc3; egc4
SElO	26.7	Nd	*selo*	egc1 (vSaβ I); egc2 (vSaβ III); egc3; egc4; MGEmw2/mssa476 seh/Δseo
SElP	27.0	nd a	*selp*	ΦN315, ΦMu3A
SElQ	25.0	No	*selq*	ΦSa3ms, ΦSa3mw, SaPI1, SaPI3, SaPI5
SER	27.0	Yes	*ser*	pIB485-like; pF5
SES	26.2	Yes	*ses*	pF5
SET	22.6	Weak	*set*	pF5
SElU	27.1	Nd	*selu*	egc2 (vSaβ III); egc3
SElU2	nd	D	*selu2*	egc4
SElV	nd	Nd	*selv*	egc4

nd, not determined; a Emetic activity demonstrated in rabbits (SElL) or in the small insectivore Suncus murinus (SElP) but not in a primate model; bHypothetical location in a prophage. .
Table 2.3 adapted from Argudin *et al*, (2010).

On the other hand, both toxin shock syndrome toxin-1 (TSST-1) and exfoliative toxin (ETs) A and B, are toxins produced by *S. aureus*, and they are described as being responsible for specific acute staphylococcal toxaemia syndromes, such as toxic shock syndrome (TSS) and staphylococcal scarlet fever (SSF) (Podbielska *et al.*, 2011). It is noted that staphylococcal enterotoxins and TSST-1 toxin shared a common structure

and biological properties suggesting that they might be derived from a common ancestor.

Exfoliative toxin (ETs) A and B are often associated with scalded skin syndromes that resulted in the separation of living layers and superficial dead layers of epidermis cells (Yamaguchi *et al*., 2002). On the other hand, exfoliative toxin D (ETD) is associated with skin and soft-tissue infections such as furuncles, abscesses and finger pulp infections. Most of the exfoliatin-producing strains belong to *agr* group IV (Podbielska *et al.,* 2011).

2.2.4 Panton valentine leukocidin (PVL) and its role in pathogenesis

Panton valentine leukocidin (PVL) is a bicomponent cytotoxin that targets human and rabbit mononuclear and polymorphonuclear cells that lead to leukocyte destruction and tissue necrosis (Lina *et al.,* 1999; Deurenberg and Stobberingh, 2008).

PVL is encoded by *lukF-PV* and *lukS-PV* that produce proteins of 32 and 38 kDA, respectively. These genes are located on four different phages namely x108PVL, wPVL, wSLT and wSA2mw (Ma *et al*., 2006; Deurenberg and Stobberingh, 2008; Feng *et al.,* 2008). PVL with SCC*mec* type IV and V is often associated with CA-MRSA strains (Tristan *et al.,* 2007a; Deurenberg and Stobberingh, 2008).

PVL is often associated with skin and mucous membrane infections such as cellulitis, abscesses, boils and carbuncles, necrotizing pneumonia, urinary tract infections, endocarditis, septic arthritis and bacteremia (Deurenberg and Stobberingh, 2008; Pathirage, 2008).

The prevalence of PVL among invasive (i.e blood, skin and soft-tissue infections) and carriage (nasal swabs from healthy humans) MRSA strains from Malaysia were reported at less than 5% rate and fatality case caused by PVL-producing

hospital-acquired *S. aureus* has also been reported in Hospital Kuala Lumpur (Malaysia) in year 2009 (Neela *et al*., 2009; Al-Talib *et al*., 2011). It is noted that, around 2% of *S. aureus* isolates from general hospital in France in year 1995 are tested positive for PVL (Prevost *et al.*, 1995; Holmes *et al*., 2005). Besides, less than 2% *of S. aureus* isolates from United Kingdom were also tested positive for PVL (Holmes *et al.*, 2005; Pathirage, 2008).

Outbreak caused by *pvl* positive community-acquired MRSA (CA-MRSA) has been reported in Ho Chih Minh city, Vietnam between April 28, 2006 and May 10, 2006 involving nine childrens. All nine childrens developed severe infections ranging from fatal toxic shock syndrome, purulent abscesses, necrotizing soft-tissue infections to fever after they were given out-patients vaccination. Among the nine childrens, eight had been vaccinated by the same health care worker showing that this outbreak was transmitted by an asymptomatic colonised health care worker (Thuong *et al*., 2007).

2.2.5 Accessory gene regulator (*agr*)

An accessory gene regulator (*agr*) is known to be a global regulator of staphylococcal virulon which coordinates the expression of secreted and cell-associated virulence factors (Traber *et al*., 2008).

agr locus of *S. aureus* is composed of two divergent transcriptional units, RNAII and RNAIII, that are driven by P2 and P3 promoters, respectively (Peerayeh *et al*., 2009). Both components will signal the transduction system, leading to down-regulation of surface proteins as well as the up-regulation of secreted proteins during the bacteria in vitro growth (Robinson *et al*., 2005).

The P2 operon encodes for four proteins (AgrA, AgrB, AgrC and AgrD) with AgrA and AgrC shared similar sequence homology with elements of the other bacterial

transduction systems (McNamara *et al*., 2000; Peerayeh *et al*., 2009). AgrC is known to function as a sensor whereas AgrA acts as the response regulator (McNamara *et al*., 2000). Activity by AgrA will result in an increase of P2 and P3 transcription (Peerayeh *et al*., 2009). AgrB is a trans-membrane protein involved in processing and /or secretion of the 8-amino-acid peptide pheromone that are found in AgrD (McNamara *et al*., 2000; Peerayeh *et al*., 2009).

There are four major *agr* types (designated *agr* type I to *agr* type IV) in *S. aureus* based on the sequence diversity in the variable region of AgrA, AgrD and AgrC (Lina *et al*., 2003; Peerayeh *et al.*, 2009)

2.3 Molecular subtyping of *S. aureus*

Various subtyping techniques have been developed in order to track the spread of MRSA. This is important in the design of rational pathogen control procedures and helps the infectious disease physicians in the development of treatment regimens to treat patients affected by MRSA (Singh *et al*., 2006). The commonly used MRSA subtyping methods are pulsed-field gel electrophoresis (PFGE), SCC*mec* typing, *agr, coa* and *spa* typing and multilocus sequence typing (MLST) (Deurenberg *et al*., 2007; Yamamoto *et al*., 2010). Among them, PFGE and MLST are considered as 'gold standards' in typing MRSA strains, although these typing methods are often time-consuming, expensive and laborious (Ishino *et al*., 2007).

Other alternative simpler typing methods such as PCR based subtyping methods, PCR-restriction fragment length polymorphism (RFLP) typing of the coagulase gene (*coa*) and *mec*-associated direct repeat units (*dru* typing) have been used in subtyping of MRSA strains as they are simple, rapid and inexpensive (Olorunfemi *et al*., 2005; Goering *et al*., 2008; Himabindu *et al.*, 2009).

2.3.1 PCR-based method for subtyping of *S. aureus*

Polymerase chain reaction (PCR) is an enzymatic method used to exponentially amplify a specific pre-selected fragment of DNA (Towner and Cockayne, 1993). PCR uses a thermo-stable DNA polymerase to produce multiple copies of a specific nucleic acid region exponentially (Towner and Cockayne, 1993). The procedures require template DNA from the organisms being typed, thermostable DNA polymerase, two synthetic oligonucleotide primers and four standard deoxyribonuclease triphosphate that are incorporated into newly synthesized DNA (Towner and Cockayne, 1993). PCR-based amplification methods such as random amplification of polymorphic DNA (RAPD) and amplified fragment lenth polymorphism (AFLP) have been used widely in the subtyping of various microorganisms, including *S. aureus* (Gardella *et al.*, 2005; Singh *et al*., 2006).

2.3.2 PCR-RFLP of *coa* gene

Coagulase protein is known as an important virulence factor in *S. aureus* and its production act as one of the key's principal criteria used in the clinical laboratory for the identification of *S. aureus* infections (Hookey *et al*., 1998; Tiwari *et al*., 2008). The 3' coding region of coagulase gene (*coa*) comprised of a series of 81 bp tandem short sequence repeats that varied between each strain (Lawrence *et al*., 1996).

The PCR-RFLP of *coa* gene is based on the restriction digest of the heterogenicity of the coagulase region (Himabindu *et al*., 2009). The use of *coa* typing was introduced by Goh *et al.* (1992).

Although PCR-RFLP of *coa* gene is less discriminative when compared to PFGE in subtyping MRSA, this typing method is simple, rapid and inexpensive (Himabindu *et al.*, 2009). This method can be used as a preliminary screening method

for epidemiological studies of infections caused by MRSA because it still provides good discriminatory power (Mitani *et al*., 2005). In addition, this typing method could be useful for epidemiological investigation as PCR-RFLP of *coa* gene can analyze a large number of strains within a short period of time (Himabindu *et al*., 2009).

2.3.3 Pulsed-field gel electrophoresis (PFGE)

PFGE which uses a specialized electrophoresis device to separate chromosomal fragments produced by enzymatic digestions of intact bacterial genomic DNA, is one of the most common genotypic methods used in clinical laboratories for subtyping of MRSA strains (Murchan *et al*., 2003). This subtyping method involves the digestion of small pieces of agarose plugs with *Sma*I restriction enzyme (McDougal *et al*., 2003; Murchan *et al*., 2003).

In order to achieve separation of a broad range of DNA, parameters such as temperature, voltage, agarose concentration and pulse time should be determined. This is because increase of 'pulse' time gradually over a course of the experiment will ensure that each different size range of DNA molecules will be subjected to their respective optimal separation conditions.

The PFGE banding patterns are analyzed by using BioNumerics with the Dice coefficient and un-weight pair group matching analysis (UPGMA) setting according to the criteria of Tenover *et al.* (1995).

PFGE has been found to show a higher discriminatory power than PCR-RFLP of *coa* gene and other PCR-based fingerprinting methods as PFGE enables the entire chromosome to be analyzed whereas the PCR-based fingerprinting methods explore only selected (random) portions of it (Hookey *et al*., 1998; Murchan *et al*., 2003).

2.3.4 Multilocus sequence typing (MLST)

MLST is considered as the rapid method for subtyping MRSA, and has been shown to be useful in global epidemiological studies of *S. aureus* (Deurenberg *et al*., 2007). MLST which is based on the sequence analysis of circa 500 bp fragment of seven housekeeping genes *(arcc, gmk, tpi, aroe, yqil, pta* and *glp*) is a useful and excellent method to study the molecular evolution of *S. aureus* (Deurenberg *et al*., 2007). All the difference sequences of each housekeeping gene are assigned as distinct alleles and sequence type (ST) or allelic profiles is defined based on the combination of seven genes (Deurenberg *et al*., 2006).

The data generated by MLST is highly comparable between laboratories via web-based electronic data (www.mlst.net.). Besides, the software package eBURST in www.mlst.net based on related MLST sequence types can analyze the evolutionary events within *S. aureus* populations (Deurenberg *et al*., 2007). In MLST subtyping, MRSA isolates will be are grouped within a single clonal complex (CC) when five out of seven housekeeping genes in that particular MRSA isolates having identical sequences and isolates with the seven same allelic profiles may be descended from a common ancestor (Deurenberg *et al*., 2006; Campanile *et al*., 2010).

The disadvantage of MLST is the technique is laborious and expensive as one has to sequence seven housekeeping genes in order to get its identity.

2.3.5 *spa* typing

spa typing which is based on the sequence analysis of the polymorphic region X of the *S. aureus* protein A (*spa*) gene is reported to be a highly effective tool in subtyping *S. aureus* (Ruppitsch *et al*., 2010).

The region X of *spa* gene consists of a number of 24 bp repeats sequences, and the diversity of the strains is attributed by duplications and deletions of the sequence in the region X of the gene (Shopsin *et al.,* 1999; Kahl *et al*., 2005; Deurenberg and Stobberingh, 2008).

Both BioNumerics 6.0 (Applied Maths, Kotrijik, Belgium) and Ridom Bioinformatics (Ridom GmBH, Germany) software provides tools for the analysis of *spa* sequences. The nomenclature and identification of different *spa* datas are synchronized via the internet with the central *spa* server (http://www.spaserver.ridom.de/) which is curated by the European SeqNet.org (Deurenburg *et al*., 2006). The *spa* server database also provides global frequencies information regarding mapping of *spa* with the MLST *S. aureus* database (http://www.spaserver.ridom.de/).

The advantage of this typing method is the results generated are easy to interpret, less time-consuming, highly reproducible, less laborious and highly comparable between laboratories via ridom.spa.server (Hallin *et al*., 2006; Deurenberg *et al.*, 2007). Furthermore, this typing method is less expensive when compared to MLST and can be used to the study the molecular evolution of *S. aureus* (Deurenberg *et al*., 2007; Deurenberg and Stobberingh, 2008).

2.3.6 SCC*mec* typing

The characteristics of SCC*mec* can be determined by identifications and determination of the presence of different *ccr* genes and the class of the *mec* complex by PCR. The *ccr* gene complex is composed of *ccr* genes whereas *mec* gene complex is composed of *mecA* gene and its regulatory genes, *mecI* and *mecRI* (Chongtrakool *et al*., 2006; Plata *et al*., 2009).

Several multiplex PCR were available for the characterization and identifications of SCC*mec* types (Oliveira and de Lencastre, 2002; Okuma *et al*., 2002; Zhang *et al*., 2005; Hisata *et al*., 2005; Milheirico *et al*., 2007; Kondo *et al*., 2007).

Oliveira and de Lencastre, (2002) developed a multiplex PCR that uses eight pairs of primers for detection of eight loci (Locus A to Locus H) on SCC*mec* types and one internal primer for detection of *mecA* gene. This multiplex PCR can identify four different SCC*mec* types, including SCC*mec* I to SCC*mec* IV (Oliveira and de Lencastre, 2002).

On the other hand, Okuma *et al.* (2002) developed primers that were specific for SCC*mec* IVa and SCC*mec* type IVb whereas Hisata *et al.* (2005) developed multiplex PCR for the specific identifications of SCC*mec* type IIa, IIb, IVc and IVd.

Zhang *et al.* (2005) and Milheirico *et al*. (2007) also developed two different multiplex PCR for the specific characterization of SCC*mec* types I to SCC*mec* type V. Nine pairs of primers were used in the multiplex developed by Zhang *et al*. (2005) for the identifications of SCC*mec* type I, II, III, IVa, IVb, IVd and V and *mecA* gene while ten pairs of primers were used for the identifications of SCC*mec* type I to SCC*mec* type V using multiplex PCR developed by Milheirico *et al*. (2007).

In the multiplex PCR developed by Kondo *et al.* (2007), six multiplex PCR (M-PCRs) were used for the identifications of *ccr* gene complex (*ccr*), the *mec* gene compex (*mec*) and specific structures in the junkyard (J) regions. Multiplex PCR 1 (M-PCR 1) (using primer set 1) identified five different types of *ccr* genes; Multiplex PCR 2 (M-PCR 2) identified three classes of *mec* gene (Class A to Class C); Multiplex PCR 3 (M-PCR 3) and Multiplex PCR 4 (M-PCR 4) identified the open reading frames in J1 regions of type I to type V SCC*mec* elements; Multiplex PCR 5 (M-PCR 5) identified the transposon ΨTn*554* and Tn*554* which integrated within the J2 regions of type II and

III SCC*mec* elements and finally, Multiplex 6 (M-PCR 6) identified two plasmids (pT181 and pUB110) which integrated within the J3 regions.

2.3.7 *mec* associated direct repeat unit (*dru* typing)

mec-associated direct repeat unit (*dru* typing) which is based on the sequence analysis of *mec* gene in differentiating MRSA strains, which are indistinguishable by PFGE analysis (Georing *et al*., 2008a). The *dru* locus consists of a number of 40 bp repeats sequences located in the hypervariable region between IS431*mec* and *mecA* of the SCC*mec* (Georing *et al*., 2008).

The advantages of *dru* typing over MLST are its simplicity and low costs as this method only involve sequencing of a single locus. Identification and analysis of *dru* types could be carried out by using BioNumerics programme (Applied Maths, Kotrijik, Belgium). In addition, the identification of *dru* types could also be carried out using a 'stand-alone *dru* typing tool' that could be downloaded from url 'http://www.mystrains.com/druid'.

The result from this typing method is highly comparable between laboratories via dru.server (www.dru-typing.org.).

2.3.8 Mutiple-locus variable-number tandem repeat assay

Multiple-locus variable-number tandem repeat assay (MLVA) of *S. aureus* is based on the analysis of five (*sdr, clfA, clfB, ssp* and *spa*) tandem repeat loci composed of seven individual genes (Sabat *et al.,* 2003). The variability in the number of short tandem repeat sequenced is used to create DNA profiles for epidemiological studies.

This method has high level of inter-laboratory reproducibility (Lindstedt, 2005; Collery *et al.,* 2008) and discriminatory and clustering capacity equivalent to PFGE (Malachowa *et al.,* 2005; Collery *et al*., 2008).

The disadvantage of this technique is the results of sizing of PCR products by DNA sequencer standardization may be differed as different laboratories use different type or model of sequencers, different buffers and different capillaries (http://www.mlva.net/saureus/default.asp).

2.4 Methicillin-resistant *Staphylococcus aureus*

2.4.1 Mobile genetic elements Staphylococcal cassette chromosome *mec* (SCC*mec*)

Methicillin resistant *S. aureus* (MRSA) is known to have evolved from methicillin-susceptible *S. aureus* (MSSA) after they acquired the staphylococcal cassette chromosome *mec* (SCC*mec*) element. This SCC*mec* generally consists of two essential components, the *ccr* gene complex (*ccr*) and the *mec* gene complex (*mec*) (Chongtrakool *et al*., 2006).

The *ccr* gene complex is composed of *ccr* genes whereas *mec* gene complex is composed of *mecA* gene and its regulatory genes, *mecI* and *mecRI* (Chongtrakool *et al*., 2006; Plata *et al*., 2009). The *mecA* gene is known to code for 78-kDA penicillin-binding protein (PBP)2a than often resulted in methicillin and other β-lactam antibiotic resistance (Deurenberg *et al*., 2007).

There are three classes of *mec* gene complex (class A, B and C) and four allotypes (type 1, 2, 3 and 5) of *ccr* complex (Podbielska *et al*., 2011). Currently, 11 different SCC*mec* types have been reported worldwide based on the combinations of different complex classes of *mec* gene and allotypes.

Table 2.4: Characteristic of 11 types of SCC*mec* elements

SCC*mec* type	*mec* complex	ccr genes	Size
I	class B-E	*ccrA1B1*	34 kb
II	class A	*ccrA2B2*	52–58 kb
III	class A	*ccrA3B3*	67 kb
IV	class B–E	*ccrA2B2 or ccrA4B4*	20–25 kb
V	class B–E	*ccrC*	28 kb
VI	class B	*ccrB4*	20–25 kb
VII	class C	*ccrC2, ccrC8*	28–30 kb
VIII	class A	*ccrA4, ccrB4*	32 kb
IX	class C2	ccrA1B1	43 kb
X	class C1	ccrA1B6	50 kb
XI	class E	ccrA1B3	30 kb

Table 2.4 adapted from Plata *et al*. (2009); Li *et al*. (2011) and Shore *et al*. (2011)

The predominant SCC*mec* type in Malaysia was SCC*mec* type III (Ahmad *et al*., 2009) and this SCC*mec* type III is also common in Singapore, Indonesia and Thailand (Chongtrakool *et al*., 2006).

2.4.2 Epidemiology and prevalence of MRSA in Malaysia and worldwide

MRSA is known to have evolved from MSSA via an acquisition of chromosomal gene (*mecA*) that encodes for penicillin-binding proteins (PBP2a) (Chambers, 1997; Corrente *et al*., 2007).

The first MRSA isolate which belonged to SCC*mec* type I was reported in an Irish hospital, United Kingdom in year 1961, around two years after the introduction of methicillin antibiotic (Oliveira *et al*., 2002; Deurenberg *et al*., 2006; Deurenberg *et al*., 2007) and they have disseminated to other European countries. In 1970s, MRSA was reported in Australia, USA and Japan (Deurenberg *et al*., 2007). It is known that worldwide spread of MRSA clones is driven by the dissemination of various clones from a specific genetic background (Deurenberg *et al*., 2007).

It is estimated that 20 to 40% of normal individuals are *S. aureus* carriers whereas the prevalence of MRSA carriage on hospital admission ranged between 1% and 12% (Weigelt, 2007).

The MRSA infection rate in a Malaysian tertiary hospital (Hospital Universiti Sains Malaysia) was reported at 10 among 1000 hospital admission (Al-Talib *et al*., 2010).

The incidence of MRSA hospital infections in Japan was between 0.7 and 0.8 per 100 admissions in year 1999 to 2003 (Kobayashi, 2006). Bell *et al.* (2002) indicated that the rate of MRSA infections among hospitalized patients in Asia-Pacific region was around 45.9% whereas the prevalence rate of MRSA in hospitals in some Asia countries, including Taiwan, Korea, Japan and China were reported to be around 70 – 80% (Voss and Doebbeling, 1995; Lee *et al*., 2000; Aires de Sousa *et al*., 2003; Boyce *et al*., 2005; Song *et al*., 2011). In addition, MRSA is also known as one of the most common causes of nosocomial infections which accounting of 40 - 70% of *S. aureus* infections in intensive care units (Zetola *et al.*, 2005).

In Malaysia, the prevalence of MRSA in Malaysian hospitals has increased from 17% in year 1986 (Rohani *et al*., 1999) to 26% in year 2008 (Ministry of Health Malaysia, 2008). Elsewhere, an increase of 62% of MRSA was reported in the United States (Klein *et al*., 2005).

2.4.3 Hospital-acquired MRSA (HA-MRSA) in Malaysia and worldwide

HA-MRSA refers to infection spread from patient to patient by health workers' hands and medical instruments (Udo *et al*., 2008). In Malaysia, Ahmad *et al.* (2009) reported the presence of 11 different MRSA clones (ST22, ST30, ST45, ST80, ST101, ST188, ST1284, ST1285, ST1286, ST1287 and ST1288) from four referral hospitals

(Hospital Kuala Lumpur, Hospital Selayang, Hospital Queen Elizabeth and Hospital Kota Bharu). Ghaznavi-Rad *et al.* (2010) reported that majority (92.5%) of the MRSA strains isolated from the largest government tertiary hospital in Kuala Lumpur, Malaysia belonged to MLST type ST239, *spa* type t037 and SCC*mec* type III (Ghaznavi-Rad *et al*., 2010). This MRSA clone ST239 which is also known as Brazilian/Hungarian clone was evolved from ST30 through the transfer of a 557 kb fragment, and it is cluster under CC8 lineages. Apart from Malaysia, this ST239 clone is also the major HA-MRSA clone in Australasia and China (Ko *et al*., 2005; Xu *et al*., 2009; Howden *et al.*, 2011). Other MRSA clones reported in Hospital Kuala Lumpur, Malaysia included ST1 (CC1), ST7 (CC7), ST22 (CC22), ST188 (CC1) and ST1283 (CC8) (Ghaznavi-Rad *et al*., 2010; Ghaznavi-Rad *et al*., 2011).

In a prospective surveillance study conducted by the Asian Network for Surveillance of Resistant Pathogens (ANSORP) from Sept 2004 to August 2006, HA-MRSA accounted for 64.7% of the total MRSA infection in 17 ANSORP hospitals located in eight ASIAN countries namely Taiwan (n=3), Korea (n=7), Hong Kong (n=1), Thailand (n=2), Philippines (n=1), India (n=1), and Sri Lanka (n=1) (Song *et al*., 2011).

The predominance HA-MRSA clones in Korea and Japan was MLST type ST5 (CC5), *spa* type t002 or t601 and SCC*mec* type II (Ko *et al*., 2005; Song *et al.*, 2011). On the other hand, the most frequent MRSA clone in Taiwan, Thailand, Hong Kong, Vietnam and India was MLST type ST239 (CC8), *spa* type t037 and SCC*mec* type III whereas the predominant MRSA clone in Sri Lanka was MLST type ST368, *spa* type t425 and SCC*mec* type III (Song *et al*., 2011). In China and Indonesia, the most common MRSA clone was MLST ST239 with SCC*mec* type III (Ko *et al*., 2005; Xu *et al.*, 2009).

Enright *et al*. (2002) reported that major MRSA clones within 359 MRSA strains from 20 countries were associated with ST22, clonal complex CC30 or CC45. Furthermore, they also indicated that different SCC*mec* types had been acquired by *S. aureus* strains with different genetic backgrounds.

Deurenberg *et al.* (2007) observed that ST8-MSSA (CC8) was the ancestor of MLST ST250 as they are only differed by a point mutation in the *yqiL* locus while this MRSA clone, ST250 is also related to MRSA clone ST247 as they differs by a point mutation in the *gmk* locus.

Other pandemic clones reported worldwide includes UK-EMRSA-15 (ST22), UK-EMRSA-16 (USA200, ST36), UK-EMRSA-3 (ST5), UK EMRSA-2/-6 (ST8), Southern Germany (ST228), Paediatric (ST5), New York/Japan (ST5), Irish-I (ST8), Iberian (ST247), Berlin (ST45) and Archaic (ST25) (Deurenberg and Stobberingh, 2008).

Tristan *et al.* (2007b) reported that all their representative MRSA clones grouped under CC5 to CC45 shared the same locus, enterotoxin gene cluster (*egc*) that encodes for five superantigenic enterotoxin genes (*seg*, *sei*, *sem*, *sen* and *seo*) and occasionally harboured *tst* gene.

2.4.4 Community-acquired MRSA (CA-MRSA)

CA-MRSA refers to MRSA strains isolated from outpatient setting, of which the patients must have no medical history of MRSA infection or colonization, admission to nursing home, dialysis and surgery (Deurenberg *et al*., 2006). Furthermore, the patients should not have permanent medical devices or indwelling catheters that inserted through the skin (Deurenberg *et al*., 2006). CA-MRSA is known to be more virulent when compared to HA-MRSA as they often associated with virulence factors (Chambers,

2001; Deurenberg and Stobberingh, 2008). CA-MRSA is known to be responsible for a wide range of infections ranging from soft-tissue infections through left-threatening disease, including necrotizing pneumonia, bacteremia and necrotizing fascilitis (O'Brien *et al*., 2009).

Unlike HA-MRSA, CA-MRSA strains are generally susceptible to antibiotic other than β-lactam, and they are known to have different phenotypic and genotypic characterization when compared to HA-MRSA (Deurenberg and Stobberingh, 2008). In Malaysia, CA-MRSA strains were known to be susceptible to gentamicin, mupirocin and vancomycin (National Antibiotic Guidelines Malaysia, 2008).

The origin of CA-MRSA remained unclear. Okuma *et al.* (2002) showed that CA-MRSA has evolved from MSSA after acquisition of SCC*mec* type IV from the community whereas de Sousa and de Lencastre (2003) indicated that CA-MRSA may have originated from the hospital.

Majority of CA-MRSA strains harbour SCC*mec* type IV, V or VII (Deurenberg and Stobberingh, 2008), although some reports showed that CA-MRSA strains harbour SCC*mec* type I, II or III (Chung *et al*., 2004; Wannet *et al*., 2005; Deurenberg *et al*., 2007).

PVL is often associated with CA-MRSA and is known as a stable genetic marker for CA-MRSA (Vandenesch *et al*., 2003; Tristan *et al*., 2007a) although some report dispute this notion (Rossney *et al*., 2007).

2.4.4.1 CA-MRSA in Malaysia and Worldwide

The first CA-MRSA strain was isolated from patient from remote communities in Western Australia in year 1993 (Udo *et al*., 1993) and the first CA-MRSA strain in Malaysia was reported in year 2008 (Nor Shamsudin *et al*., 2008). Ahmad *et al.* (2009)

showed that CA-MRSA from Malaysian hospitals was from MLST type ST30 and ST80 while Sam *et al*. (2008) indicated that multisensitive CA-MRSA strains from another tertiary hospital in Malaysia were from MLST type ST6, ST22, ST30, ST1178 and ST1179.

In a prospective surveillance study conducted by the Asian Network for Surveillance of Resistant Pathogens (ANSORP) from September 2004 to August 2006, CA-MRSA accounted for 25.4% of the total MRSA infections in 17 ANSORP hospitals located in eight ASIAN countries namely Taiwan (n=3), Korea (n=7), Hong Kong (n=1), Thailand (n=2), Philippines (n=1), India (n=1), and Sri Lanka (n=1) (Song *et al*., 2011). In Hong Kong, the most frequent SCC*mec* type of CA-MRSA strain was SCC*mec* type III. The predominant SCC*mec* type among CA-MRSA isolated in Philippines and Korea was SCC*mec* type IV, and they were from *spa* types t019 and t324 (Song *et al.*, 2011). On the other hand, the most frequent SCC*mec* type among CA-MRSA isolated in Taiwan was SCC*mec* type III followed by SCC*mec* type IV with both associated with *spa* type's t002 and t425. Both t002 and t425 *spa* types were also common in Sri Lanka (Song *et al.*, 2011).

The predominant MRSA clones among CA-MRSA in Philippines and Korea was MSLT type ST30, SCC*mec* type IV, *spa* type 019 and MLST type ST72, SCC*mec* type IV and *spa* type t324, respectively (Song *et al*., 2011).

On the other hand, Deurenburg and Stobberingh, (2008) reported that PVL-positive CA-MRSA clones reported worldwide were mostly from MLST type ST1, ST8, ST30, ST59 and ST80. Among them, ST1 is reported in Asia, Europe and USA; ST8 in Europe and USA; ST30 in Australia, Europe and South America; ST59 in Asia and USA; and finally, ST80 reported in Asia, Europe and the Middle-East (Larsen *et al.*, 2008; Deurenberg and Stobberingh, 2008). ST80 has also been reported as the predominant CA-MRSA clone affecting Europe (Larsen *et al*., 2008).

Although there is no report of an outbreak caused in CA-MRSA in Malaysia, outbreaks caused by CA-MRSA has been reported in other countries. For example, 42 persons with history of drug abuse, homeless or incarcerations from the communities were affected in an outbreak caused by CA-MRSA reported in Alberta, USA between January and September 2004 (Gilbert *et al*., 2006). In another community-acquired food-borne illness MRSA outbreak, three adults were affected after they ate contaminated shredded pork barbeque of which they had bought from a convenience shop (Jones *et al.*, 2002). Other outbreaks cause by CA-MRSA was summarized in Table 2.5.

Table 2.5: Outbreak associated with by CA-MRSA

Places/country	Year	Person affected	Infections	References
San Diego, USA	2002	34 persons	Skin infections	Campbell *et al*., 2004
Cancer center, Texas, USA	2003	13 employees	Boils	Kassis *et al*., 2011
North York General Hospital, Toronto, Ontaria, Canada	2004	15 neonates and 8 postpartum mothers	MRSA infections	Saunders *et al*., 2007
High school Athletes in Illinios, USA	2004	10 cases	MRSA skin infections	Borchardt, 2005
Ho Chih Mind city, Vietnam	2006	9 children with 1 fatal case	Severe CA-MRSA infections including fatal toxic shock syndrome, necrotizing soft tissue infections, purulent abscesses to fever with rash	Thuong *et al*., 2007

On the other hand, an outbreak caused by invasive CA-MRSA infections within a family has also been reported in Tennessee, United States. Three members of the family of nine have history of cough, fever and nasal congestion with the mother died of fulminant pneumonia, and the subtyping by PFGE showed that they were infected by

USA300-0114 clone (Jones *et al*., 2006). In addition, two of the seven childrens of this family had boils one month before their mother past away and the other one asymptomatic child also had MRSA. The subsequent subtyping indicated that all three isolates had similar antibiotic susceptibility profiles with their mother isolate (Jones *et al.*, 2006).

Besides, the spread on human CA-MRSA to animal transmission has also been reported between September 1993 and October 1994. In this particular outbreak, 11 horses which have therapeutic procedures in Machigan State University Veterinary Teaching Hospital has developed wound infection at the site of therapeutic procedures and PFGE subtyping of four veterinary health-care workers and 11 horses samples confirmed that they belong to PFGE pattern type B (Seguin *et al*., 1999).

2.5 Phenotypic and genotypic detection of MRSA strains

Various phenotypic methods can be used for detection of MRSA. This includes oxacillin disk diffusion test, oxacillin agar screen test, cefoxitin test, minimal inhibitory concentration (MIC) and CHROMagar MRSA (Corrente *et al*., 2007; Karami *et al*., 2011).

The use of oxacillin disk (1 µg) for screening MRSA was recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (now re-named as Clinical Laboratory Standards Institute) in year 1992 (Krishnan *et al*., 2002). In this test, an organism is considered as MRSA if their zone diameter is ≤ 10 mm (CLSI, 2010). Krishnan *et al*. (2002) reported that oxacillin disk diffusion test is known to show more than 96.5% sensitivity. However, this oxacillin disk diffusion test could not be applied on *S. lugdunensis* and other coagulase-negative staphylococci (CLSI, 2010).

On the other hand, oxacillin agar dilution method for the screening of MRSA has also been proposed by CLSI. An organism is considered as MRSA if they are able to growth on Mueller-Hinton agar supplemented with 2% NaCl and 6 µg/ml oxacillin (CLSI, 2010).

The use of cefoxitin test for detection of MRSA has been recommended by the European Antimicrobial Resistance Surveillance System (EARSS) and CLSI (Corrente *et al*., 2007; CLSI 2010). In this test, cefoxitin disc 30 µg is used and an organism is considered as MRSA if their zone diameter is ≤ 19 mm (Corrente *et al*., 2007). CLSI indicated that cefoxin disk diffusion could be more favourable than oxacillin disk diffusion test as it gave a better prediction of the presence of *mecA* gene when compared to oxacillin disk diffusion test and other oxacillin-based methods (CLSI, 2010). Limitation of the disk diffusion test is the result obtained might be varied between laboratories as it depends on the heterogeneous expression of resistance from the MRSA strains (Kaya *et al*., 2005).

Minimal inhibitory concentration (MIC) of cefoxitin by using E-test for the screening of MRSA has also been proposed by CLSI and strains with MIC > 4 µg/ml should be reported as MRSA (CLSI, 2010). Skov *et al.* (2006) reported that both cefoxitin disk diffusion test and E-test cefoxitin was highly effective in the determination of MRSA and accuracy of the test can be increased by using incubation temperature of 35 - 36°C with incubation time of 18 to 22 hours.

In addition, MRSA strains could also be determined by using CHROMagar-MRSA as this method provides a high sensitivity and specificity result. An evaluation of 294 *S. aureus* strains by using CHROMagar-MRSA showed that this method was 100% sensitive and 97.9% specific (Karami *et al*., 2011).

Besides, commercially available Slidex MRSA Detection (bioMe'rieux, France), Phoenix Oxacillin MIC, Phoenix Cefoxitic MIC, MicroScan oxacillin MIC and MRSA-

Screen (Denka Seiken Co., Ltd, Japan) can also be used for the detection of MRSA strains (Velasco *et al*., 2005).

Molecular detection methods such as PCR-based methods, Real-time PCR and loop-mediated isothermal amplification (LAMP) can be used for the detection of MRSA strains (Huletsky *et al*., 2004; Velasco *et al*., 2005; Xu *et al*., 2011).

PCR-based method for the detection of *mecA* gene is considered as the most reliable and reference method for rapid determination of MRSA strains (Kaya *et al*., 2009; Velasco *et al*., 2005). Although PCR-based method provides fast reliable results, however, this technique is not practical for routine used in the hospital setting as its required special equipment (i.e PCR thermocycler and gel electrophoresis set) (Brown *et al*., 2005).

MRSA could also be determined by using multiplex Real-time PCR assay (Huletsky *et al*., 2004). The advantage of this Real-time PCR is it can be performed directly from non-sterile clinical specimens such as blood and provide the result in less than an hour's time (Huletsky *et al*., 2004). However, this technique is not practical for routine used as it is cost expensive and required a special machine for the amplification of Real-time PCR assay.

Another new technique for determination of MRSA is by using LAMP. This method depends on the auto-cycling strand displacement DNA synthesis using four or six primers (two inner, two outer with or without two loop primers) with *Bst* DNA polymerase large fragment generated under isothermal conditions (Xu *et al*., 2011).

2.6 Problems with antibiotic resistance among MRSA strains

Drug resistance is a difficult problem for hospitals as hospitals are places for critically ill patients who are more vulnerable to infection. In addition, the heavy use of drugs in these patients hastens the mutations among bacteria that harbour drug resistance genes (Levy and Marshall, 2004).

Since most MRSA strains are resistant to many commonly used antibiotics including macrolides, lincosamides, fluoroquinolones, tetracyclines, aminoglycosides and chloramphenicol (Almer *et al*., 2002), this has raised a concern over the limited choice of antimicrobial agents for treatment of life-threatening cases. This could lead to prolonged stay in the hospital and increase the cost of care (Akpaka *et al*., 2006; Udo *et al*., 2006). A death rate of 34% within 30 days was observed among patients with MRSA infections as compared to 27% in MSSA patients (Wyllie *et al*., 2006).

The National Antibiotic Guidelines 2008 in Malaysia indicated that erythromycin, gentamicin, trimethoprim-sulfamethoxazole, rifampicin, fusidic acid and clindamycin were commonly used in 16 different hospitals (Hospital Kangar, Hospital Alor Setar, Hospital Pulau Pinang, Hospital Ipoh, Hospital Kuala Lumpur, Hospital Selayang, Hospital Sungai Buloh, Hospital Klang, Hospital Seremban, Hospital Melaka, Hospital Johor Bharu, Hospital Kuantan, Hospital Terengganu, Hospital Kota Bharu, Hospital Kuching and Hospital Queen Elizabeth) in Malaysia to treat patients with MRSA infections. Other antimicrobial agents used to treat MRSA infections include linezolid, mupirocin and vancomycin. The MRSA resistance rates reported in 15 different hospitals in Malaysia were as follows: erythromycin (84.3%), gentamicin (81.7%), trimethoprim-sulfamethoxazole (78.2%), clindamycin (26.2%), fusidic acid (14.9%) and rifampicin (10.3%) (National Antibiotic Guidelines Malaysia, 2008).

In another Malaysian local report, Sam *et al*. (2008) indicated that the MRSA strains in University Malaya Medical centre (UMMC) are often resistant to

erythromycin, gentamicin and ciprofloxacin. Furthermore, David *et al.* (2008) indicated that HA-MRSA strains are typically resistant to clindamycin and other non-β-lactam antibiotics.

Specifically, the mechanisms of resistance of selected antimicrobial agents are summarized as described in Table 2.6.

Table 2.6: Mechanism of *S. aureus* resistance to selected antimicrobials

Antibiotic	Resistance gene	Gene product (s)	Mechanism(s) of resistance
β-lactam (penicillins)	*blaZ*	β-lactamases	Enzymatic hydrolysis of β-lactam nucleus
	mecA	PBP2a	Reduced affinity for PBP
Glycopeptides (Vancomycin, teicoplanin)	*vanA, vanB*	Altered peptidoglycan	Trapping of vancomycin in the cell wall
		D-Ala-D-Lac	Synthesis of dipeptide with reduced affinity for vancomycin
Quinolones (eg. ciprofloxacin)	*parC*	parC (or GrlA) component of topoisomerase IV	Mutations in the QRDR region, reducing the affinity of enzyme-DNA complex for quinolones
	gyrA or gyrB	GyrA or GyrB components of gyrase	
Aminoglycosides (eg. gentamicin, netilmicin)	Aminoglycoside-modifying enzymes (eg. *aac*, *aph*)	Acetyltransferase, phosphotransferase	Acetylating and/or phosphorylating enzymes modify aminoglycosides
Trimethoprim-sulfamethaxazole	Sulfonamide: *sulA*	Dihydropteroate synthase	Overproduction of p-aminobenzoic acid by enzyme
	TMP: *dfrB*	Dihydrofolate reductase (DHFR)	Reduced affinity for DHFR
Tetracycline	*tetK, tetL, tetM, tetO, tetS*	Tetracycline effux protein	Enzyme inactivation, ribosomal protection's protein and efflux protein
Oxazolidinones (e.g. linezolid)	*rrn, cfrA*	23S rRNA	Mutations in domain V of 23S rRNA component of the 50S ribosome. Interferes with ribosomal binding
Quinupristin-dalfopristin (Q-D) (erythromycin)	Q: *ermA, ermB, ermC*	Ribosomal methylases	Reduce binding to the 23S ribosomal subunit
	D: *vat, vatB*	Acetyltransferases	Enzymatic modification of dalfopristin
Lincosamides (clindamycin)	*msrA*	Ribosomal methylases	Reduce binding to the 23S ribosomal subunit
Mupirocin	*ileS, ileS2, mupA*	Isoleucyl-RNA synthetase (IRS)	Inhibits protein synthesis
Fusidic acid	*fusB, fusC, fusD*	-	protects the staphylococcal translation apparatus
Rifampicin	*rpoB*	-	β-subunit mutational change

Table 2.6 was adapted from Lowy (2003); Yun *et al.* (2003); Rotger *et al.* (2005); Mick *et al.* (2010); Castanheira *et al.* (2010b); Chopra and Roberts (2002).

2.6.1 Mupirocin resistance

Mupirocin (pseudomonic acid A) is a topical antimicrobial agent used for treatment of superficial skin infections (Yun *et al*., 2003). This topical drug binds competitively to bacterial isoleucyl-RNA synthetase (IRS) and inhibits protein synthesis (Yun *et al.*, 2003). High level resistance to mupirocin is often associated with the acquisition of *mupA* gene while low level resistance to mupirocin is due to mutation in endogenous bacterial isoleucyl-tRNA synthetase (Rotger *et al*., 2005).

The increase usage of this agent has led to rapid emergence of mupirocin resistant strains in some parts of the world (Leski *et al*., 1999). Mupirocin drug has been used in Malaysian hospitals since year 1998 and the only report on mupirocin resistance (1.25%) in Malaysia was reported by Norazah *et al*. (2002). However, in UMMC, mupirocin drug is still of limited usage as it is only recommended for out-patients and not for in-patients. This drug is also used for treatment of MRSA colonization in medical staff for five days and then re-tested on the seventh day for ensure clearance.

The presence of high-level mupirocin resistance can be determined by using disk diffusion test or broth micro dilution test. An organism is considered as exhibiting high-level mupirocin resistance if they are resistant to 200 µg mupirocin disks or having MIC 256 µg/ml (CLSI, 2010).

2.6.2 Erythromycin resistance

Erythromycin is a macrolide antibiotic that consists of a large lactone ring. This antibiotic is often used for the treatment of respiratory tract infection (Scholar and Pratt, 2000). Erythromycin resistance is also associated with resistance to other macrolides, lincosamides and type B streptogramin (MLSB).

There are three mechanisms involved in erythromycin resistance, which are (i) the use of an energy-dependent efflux, (ii) production of inactivating enzymes and (iii) alteration of 23S rRNA methylases (Wang *et al*., 2008).

Alteration of 23S rRNA methylases is mediated by the presence of *erm* genes [i.e *erm*(A), *erm*(B) and *erm*(C)] whereas the energy-dependent efflux pump is often affected by membrane proteins encoded by *msr*(A) or *msr*(B) genes (Spiliopoulou *et al*., 2004). The production of inactivating enzymes, which hydrolyze the macrocycles lactone ring of erythromycin, is mediated by *ereA* or *ereB* genes (Schmitz *et al*., 2000).

2.6.3 Clindamycin resistance

Clindamycin belongs to lincosamide A, an antibiotic that is known to have a wide antibacterial spectrum against gram-positive cocci, anaerobe's gram-negative organisms and certain protozoa (Scholar and Pratt, 2000). It is often used to treat osteomyelitis, bone infections related to diabetic foot and decubitus ulcers (Scholar and Pratt, 2000). However, this antibiotic can cause side effect such as diarrhoea, hypersensitivity reactions and may occasionally cause irritation at local thrombophlebitis (Scholar and Pratt, 2000).

Clindamycin resistance can be grouped as either inducible clindamycin resistance (known as inducible macrolides, lincosamides and streptogramin$_B$, iMLS$_B$) or constitutive clindamycin resistance (also known as constitutive clindamycin resistance, cMLS$_B$). An isolate that is resistant to both erythromycin, and clindamycin is considered showing constitutive clindamycin resistance while isolate that was resistant to erythromycin, sensitive to erythromycin and shows flattening of the clindamycin zone of inhibition adjacent to erythromycin disc, forming a D-shape is described as inducible clindamycin resistance (Mallick *et al*., 2009).

The clindamycin resistance mechanism is often associated with the ribosomal modification by methylases encoded by *erm* genes. This *erm* gene will cause in the methylation of 23S rRNA that resulting in a decrease of affinity for clindamycin and type B streptogramins (Lim *et al*., 2006).

Detection of inducible clindamycin resistance can be performed by using D zone test (CLSI, 2010). In D-test, a disk containing erythromycin (15 µg) is placed on the Mueller-Hinton agar plate and disk containing clindamycin (2 µg) are applied 15 - 26 mm distance away from the erythromycin disk (CLSI, 2010).

2.6.4 Rifampicin resistance

Rifampicin which is a semi-synthetic derivative of rifamycin B, is an important antibiotic used in combination therapy for treatment of deep-seated *staphylococcal* infections and tuberculosis (Yu *et al.*, 2005).

Rifampicin resistance is often associated with mutations in the β-subunit of RNA polymerase encoded by the *rpoB* gene. This mutation is related to amino acid changes found in two particular regions of *rpoB* gene, namely cluster I and II (Mick *et al.*, 2010).

In Malaysia, rifampicin is used with fusidic acid as an alternative or switch therapy of vancomycin against *Staphylococcal* infections (Norazah *et al.*, 2002). Little is known about the rifampicin resistance mechanisms in *S. aureus* isolated in Malaysia. Rohani *et al.* (2000) reported rifampicin resistant rate of 3.3% (in year 1996) by using disk diffusion test while Norazah *et al.* (2002) reported a 5% (year 1997 to 1999) rifampicin-resistant rate.

2.6.5 Fusidic acid resistance

Fusidic acid can be used as topical antibiotic or systemically for the treatment of Staphylococcal infections (Alreshidi and Mariana, 2011). This drug binds to elongation factor G (EF-G) on the ribosome and inhibits protein synthesis (Castanheira *et al*., 2010a, b). Fusidic acid in known as bacteriostatic but it can become bacteriocidal when this antibiotic was applied at high concentration (Howden and Grayson, 2006).

Resistance to fusidic acid is often associated with mutations in *fusA* gene leading to the alteration of EF-G structures or to the acquisition of plasmid mediated resistant genes such as *fusB, fusC* or *fusD* gene (Howden and Grayson, 2006; O'Neill *et al*., 2007; Norstrom *et al*., 2007; Lannergard *et al*., 2009; Chen *et al*., 2010). The plasmid mediated *fusB, fusC* and *fusD* resistance genes encode at the EF-G binding proteins and protect the staphylococcal translation apparatus against the inhibitory action of fusidic acid (O'Neill and Chopra, 2006; Chen *et al*., 2011). *fusB* gene is usually carried by plasmid with size of 21 kb (pUB101) (Chen *et al*., 2011). The third mechanism involved in fusidic acid-resistance is mutations in the riboprotein L6 operon of the *rplF* gene (Norstrom *et al*., 2007; Castanheira *et al*., 2010b).

Fusidic acid can also be used together with rifampicin as an alternative or switch therapy of vancomycin against MRSA infections in Malaysia (Norazah *et al*., 2002). In addition, O'Neill *et al.* (2001) also indicated that combination use of rifampicin and fusidic acid gave a wider role in the prevention of MRSA and vancomycin-intermediate *S. aureus* (VISA) infections. The prevalence of fusidic acid resistance among MRSA strains from Malaysia increased from 3.8% in year 1996 (Rohani *et al*., 2000) to 5% in year 1997 - 1998 (Norazah *et al*., 2002).

2.6.6 Linezolid resistance

Linezolid is an oxazolidinone antibiotic that has a board spectrum activity against gram-positive bacteria, including MRSA, VRSA and vancomycin-resistant *Enterococcus* (Longmore *et al*., 2008). This antibiotic inhibits bacterial protein synthesis by binding to the 50 S subunit of the bacterial ribosomes and interacts with the 23S rRNA resulting in the inhibition of 70S initiation complex formation (Hortiwakul *et al.*, 2004; Besier *et al*., 2008). Linezolid is known to be more effective than teicoplanin in treating skin and soft-tissue infections caused by MRSA (Hayman *et al*., 2007).

Linezolid resistance is often associated with mutations in domain V of the 23S rRNA gene, including amino acid alteration of G244T, T2500A and G2576T or by the presence of *cfr* (chloramphenicol-florfenicol resistance) gene that encodes a 23S rRNA methyltransferase of host cells (Arias *et al.*, 2008; Besier *et al.*, 2008).

Outbreak due to linezolid resistant *S. aureus* has been reported in an intensive care department of a 1000-bed tertiary care University teaching hospital located in Madrid, Spain in year 2008 (Sanchez Garcia *et al.*, 2010).

2.6.7 Vancomycin resistance

Vancomycin is glycopeptides antibiotic that act actively against most species gram positive bacilli and coccus, such as *S. aureus*, *Staphylococcus epidermidis*, *Clostrium* species, *Streptococcus pneumoniae*, *Clostridium* species, *Listeria monocytogenes*, *Actinomyces* species, *Lactobacillus* species (Scholar and Pratt, 2000). It binds tightly to the acyl-D-alanyl-D-alanine terminus of the bacterial cells that will lead to cell lysis (Scholar and Pratt, 2000).

Vancomycin resistance is either conferred by the presence of several *van* genes, i.e *vanA*, *vanB* and *vanD* (Sanakal and Kaliwal, 2011) or *S. aureus* cell wall thickening

(Hiramatsu, 2001). It is reported that *vanA* gene often confers inducible high-level of resistance whereas *vanB* confers to various levels of resistance, and finally *vanD* confers resistance to intermediate level of vancomycin resistance (Quintiliani *et al*., 1993; Sanakal and Kaliwal, 2011).

The presence of VRSA or vancomycin intermediate *S. aureus* (VISA) can be determined by using agar dilution test or E-test (CLSI, 2010). An isolate is considered as VRSA if the MIC of vancomycin is ≥ 16 µg/ml or as VISA if the MIC ranges from 4 to 8 µg/ml (CLSI, 2010).

Although there is no report on the presence of VRSA or VISA in Malaysia, the number of MRSA with an increased vancomycin minimum inhibitory concentration among MRSA has been observed (Ahmad *et al*., 2010). Elsewhere, the presence of VISA and VRSA is increasing globally (Tenover *et al*., 2007; Howden *et al*., 2008) as summarized in Table 2.7

Table 2.7: Occurrence of VISA or VRSA in various countries

VRSA/VISA	Country	Author
VISA	Japan	Hiramatsu *et al*., 1997
VISA	Korea	Kim *et al*., 2000
VISA	Vietnam, China, Indonesia, Philippines, Saudi Arabia, Singapore, Sri Lanka and Taiwan	Song *et al*., 2004
VISA	Thailand	Lulitanond *et al*., 2009
VRSA	Iran	Saderi *et al*., 2005
VRSA	Northern India	Tiwari and Sen, 2006
VRSA	Kokalta hospital, India	Saha *et al*., 2008
VRSA	Michigan, USA	Zhu *et al*., 2008
VRSA	Nigeria	Taiwo *et al*., 2011

2.6.8 Gentamicin resistance

Gentamicin is a broad-spectrum antibiotic from aminoglycoside antibiotic, often used to treat serious infections due to gram-negative bacilli and is also active against *S. aureus* (Scholar and Pratt, 2000).This antibiotic works by binding to the 30S subunit of the bacterial ribosome and thus interrupt the synthesis of protein.

In *S. aureus*, the main mechanism involved in gentamicin resistance is by the production of aminoglycoside modifying enzymes (AME) (Klingenberg *et al.,* 2004). Three AME, including aminoglycoside-6′-N-acetyltransferase/2″-O-phosphoryltransferase [AAC(6′)/APH(2″)], aminoglycoside-4′-O-nucleotidyltransferase I [ANT(4′)-I] and aminoglycoside-3′-O-phosphoryltransferase III [APH(3′)-III] responsible for gentamicin resistance in *S. aureus* (Miller *et al*., 1997; Schmitz *et al.*, 1999; Livermore *et al*., 2001; Klingenberg *et al.*, 2004). Among the three AME genes, aminoglycoside-6′-N-acetyltransferase/2″-O-phosphoryltransferase [AAC(6′)/APH(2″)] is encoded by transposon Tn*4001* (Rouch *et al*., 1987).

2.6.9 Netilmicin resistance

Netilmicin is an aminoglycoside antibiotic that is known to be active against gentamicin-resistant *Escherichia coli, Klebsiella* and *Enterobacter* (Scholar and Pratt, 2000). They are known to be less ototoxic when compared to other aminoglycosides in animal models (Scholar and Pratt, 2000).

Resistance to netilmicin is often associated with the presence of aminoglycoside-modifying enzymes (AME) which are encoded within the mobile genetic elements (Vakulenko and Mobashery, 2003; Hauschild *et al*., 2008). Presence of this AME will result in drug inactivation (Hauschild *et al.*, 2008).

2.6.10 Tetracycline resistance

Tetracycline is a broad-spectrum antibiotic often used to treat sinuses, acne, respiratory and urinary tract infections, intestines and used for the treatments of gonorrhoea for patients who are allergic to macrolides and β-lactam (Chopra and Roberts, 2001; El-Mahdy *et al*., 2010).

Tetracycline resistance is mediated by enzyme inactivation, ribosomal protection's proteins and efflux proteins (Chopra and Roberts, 2001; Villedieu *et al*., 2003). In the efflux proteins, the *tet* efflux genes are able to encode for membrane-associated proteins and thus removing the tetracycline from the cells (Dorsch, 2007) whereas ribosomal protection's proteins are cytoplasmics proteins that protect the bacterial ribosome against tetracycline (Dorsch, 2007) and finally enzyme inactivation refers to the inactivation of tetracycline by enzymatic modifications (Dorsch, 2007).

2.6.11 Teicoplanin resistance

Teicoplanin is a lipoglycopeptide antibiotic that has mechanisms of action very similar to vancomycin (Scholar and Pratt, 2000; Svetitsky *et al*., 2009). This antibiotic is safely administered intramuscularly and has long life-times ranging from 33 to 190 hours or even longer, thus can be used on once-daily maintenance dosing (Scholar and Pratt, 2000).

Teicoplanin is used as an alternative to vancomycin, and it appears to be affective against vancomycin-resistant enterococcal infections (Scholar and Pratt, 2000). Teicoplanin resistance is often associated with bacterial cell wall thickening or over expression of penicillin binding proteins 2 (PBP2) in bacterial cells (Hiramatsu, 2001). On the other hand, teicoplanin resistance can also be conferred by vancomycin resistance genes (*van*) such as *vanA* and *vanB* (Sanakal and Kaliwal, 2011). Outbreak

involving MRSA strains with reduced susceptibility towards teicoplanin has been reported between January and March, 2000 in France (Heym *et al*., 2002).

2.6.12 Ciprofloxacin resistance

Ciprofloxacin, a synthetic chemotherapeutic antibiotic from the flouroquinolone drug class is often used to treat adult cystic fibrosis, and it is known to be active against *Salmonella* and *Campylobacter* infections (Longmore *et al*., 2008). It is also being used for the topical treatment of recurrent purulent otitis (Sachse *et al*., 2008).

Ciprofloxacin resistance is often associated with mutations in the topoisomerase IV or DNA gyrase, or by the induction of multidrug efflux pump (Lowy, 2003). Nucleotide mutation that leads to amino acid changes in topoisomerase IV, DNA gyrase A will reduce the quinolone affinity to its targets and thus confer the resistance (Lowy, 2003).

2.6.13 Trimethoprim-sulfamethoxazole resistance

Trimethoprim-sulfamethoxazole or also known as co-trimoxazole, a combination of trimethoprim and sulfamethoxazole in the ratio of one to five, is often used in the treatment of upper and lower respiratory tract infections, skin and wound infections, septicemias, renal and urinary tract infections caused by bacterial (Masters *et al.*, 2003).

Resistance to trimethoprim is associated with mutations in the chromosomal gene for dihydrofolate reductase or by uptakes of transposon Tn*4003*-borne *dfrA* gene or Tn*559*-borne *dfrK* gene (Rouch *et al.,* 1989; Dale *et al*., 1993; Qi *et al*., 2005; Kadlec and Schwarz, 2010). Resistance to sulfonamides is associated with mutations in the

chromosomal dihydropteroic synthase gene (Rouch *et al.*, 1989; Dale *et al*., 1993; Qi *et al*., 2005).

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Bacterial Strains

Initially, all the MRSA strains from year 2003 to 2008 stock cultures were included in this retrospective study conducted in year 2009. However, no MRSA strains from year 2005 and 2006 were available as they were not viable. Therefore, a total of 188 methicillin-resistant *Staphylococcus aureus* (MRSA) strains was examined in this study. All MRSA strains were obtained from University Malaya Medical Centre (UMMC). The clinical strains were isolated from various types of specimens.

All strains were identified by standard biochemical methods by laboratory staff of the microbial diagnosis laboratory of UMMC. Briefly, all the clinical specimens were streaked on blood and MacConkey agars and inoculated overnight at 35°C. The suspected *S. aureus* colonies in blood agar (showed β-haemolysin with golden-yellow colonies) were further tested with coagulase test and cefoxitin disk diffusion test following CLSI guidelines (2010). The strains were identified as MRSA if zone diameter were ≤ 21 mm and tested positive in coagulase test. Upon receipt, the purity of the bacterial cultures was determined by streaking on mannitol-salt agar (Oxoid Ltd, Basingtoke, Hampshire, UK).

Bacterial cultures of the strains were stored in Tryptone Soy Broth (TSB) supplemented with 50% glycerol at -20^{o}C and -85^{o}C as well as in Tryptone Soy Agar (TSA) stab cultures at room temperature. Details of the strains used in this study are listed in Appendix 1.

S. aureus ATCC25923 was used as positive control for antimicrobial susceptibility test, whereas strains NCTC10442, N315, 85/2082, JCSC4744, JCSC2172,

JCSC4469, JCSC4788, and W15 were used as positive controls for SCC*mec* type I, II, III, IVa, IVb, IVc, IVd and V, respectively. For transformation experiments, *E. coli* strain DH10B was used as positive control whereas *S. aureus* ATCC29213 was used as the recipient (Table 3.1).

Table 3.1: Details of *E. coli* strain used in this study

Bacterial Strain	Genotype/Phenotype	Source
E. coli DH10B	F^- *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC)ϕ80*lac*Z Δ*lac*X74 *rec*A1 *end*A1 *ara*D139 Δ (*ara, leu*)7697 *gal*U *gal*K λ^- *rps*L *nup*G *ton*A	Invitrogen, USA

3.1.2 Chemicals, Reagents and Consumables

All chemicals and solvents used were of Analar grade purchased from Sigma Chemical Co., U.S.A., BDH Chemicals Ltd., England, and Invitrogen™., USA.

Seakem gold agarose powder for DNA plug preparation was purchased from Camrex Bio Science Rockland, Inc, USA. Low EEO agarose powder and Promega LE Analytical for standard gels were from Sigma Aldrich, USA and Promega, Madison Wis, USA.

Yeast extracts; bacteriological agar, Mueller-Hinton agar, Brain-Heart Infusion agar, Manittol-Salt Agar, Typtone Soy agar, sucrose and tryptone for bacterial culture media were from Oxoid Ltd, Basingstoke, Hampshire, England. Glycerol was purchased from Invitrogen, California, USA. Sodium chloride, potassium dihydrogen phosphate, potassium hydrogen phosphate, sodium hydrogen maleate, maleic anhydride was from Merck, USA whereas congo red was from VWR Prolabo, Fonteay-sous-Bois, France.

All the antimicrobial disks used were purchased from Oxoid Ltd., Basingstoke, Hampshire, England. E-test strips were purchased from Ab Biodisk, Sweden.

Rifampicin, oxacillin, trimethoprim-sulfamethoxazole, vancomycin, erythromycin, tetracycline, ciprofloxacin, lysozyme, lysostaphin and bovine albumin's powders were purchased from Sigma, USA. All medias, buffers and solution preparations are listed in Appendix 2.

3.1.3 Restriction enzymes

Restriction endonucleases (RE) used in this study are listed in Table 3.2.

Table 3.2: List of enzymes used in this study

Enzyme	Sources
*Eco*RI	Promega Madison Wis, USA
*Xba*I	Promega Madison Wis, USA
*Sma*I	Promega Madison Wis, USA
*Alu*I	Promega Madison Wis, USA

3.1.4 DNA molecular weight markers

DNA molecular weight markers used in this study are listed in Table 3.3.

Table 3.3: List of DNA molecular weight markers used in this study

DNA marker	Range (bp)	Source
100 bp DNA marker	100 - 1,500	Promega Madison Wis, USA
1kb DNA marker	250 - 10,000	Promega Madison Wis, USA
Lambda DNA/*Hin*dIII Marker	125 - 23,130	Promega Madison Wis, USA

3.1.5 Primers and Oligonucleotides

Commercially synthesized primers and oligonucleotides were purchased from Bioneers, Korea. All primers and oligonucleotides used in this study were salt-free purified. Primers that amplify the 16S ribosomal RNA genes were included as a positive control in all the PCR detection for erythromycin, tetracycline and mupirocin resistant genes. The primer sequences are as follows: Primer 1 (5'- AGT TTG ATC ATG GCT CAG-3') and primer 2 (5'-GGA CTA CCA GGG TAT CTA AT-3') (Shukla *et al*., 2003; Varela *et al*., 2004) which yield an expected amplicon size of 720 bp.

3.1.6 Commercial Kits for extraction of genomic DNA and purification of PCR products

Commercially DNA purification kits used in this study are listed in Table 3.4.

Table 3.4: List of commercial kits used in this study

Name	Source
QIAquick Gel Extraction Kit	Qiagen GmBH, Germany
QIAprep Spin Miniprep Kit	Qiagen GmBH, Germany
Wizard Genomic DNA purification kit	Promega Madison Wis, USA
PCRquick-spin PCR product purification kit	Intron Biotechnology, Korea

3.1.7 Softwares

The various softwares used in this study are listed in Table 3.5.

Table 3.5: List of software used in this study

Software	Sources
BioRad Imager	BioRad, USA
Primer 3	http://frodo.wi.mit.edu/primer3/
In-silico PCR programme	http://insilico.ehu.es/PCR/
Mega 4	The Biodesign Institute, USA
BioNumerics 6.0	Applied Maths, Kortrijk, Belgium
Simpson's index	http://insilico.ehu.es/mini_tools/discriminatory_power/index.php
Statistica 8.0	StatSoft, Inc, USA
MLST	http://saureus.mlst.net
BURST algorithm	http://eburst.mlst.net
BLAST search program	http://www.ncbi.nih.gov/BLAST

3.2 Methods

3.2.1 Antibiotic Susceptibility Tests

The antimicrobial susceptibility of MRSA strains to 14 antimicrobial agents [vancomycin (30 μg), oxacillin (1 μg), mupirocin (5 μg), ciprofloxacin (5 μg), tetracycline (30 μg), erythromycin (15 μg), fusidic acid (75 μg), netilmicin (30 μg), teicoplanin (30 μg), gentamicin (10 μg), linezolid (30 μg), rifampicin (5 μg), trimethoprim-sulfamethoxazole (75 μg) and clindamycin (2 μg) (Oxoid Ltd.,

Basingstoke, Hampshire UK)] were determined by the disk diffusion method according to Clinical Laboratory Standard Institutes guidelines (CLSI, 2010).

The minimum inhibitory concentration (MIC) for vancomycin and fusidic acid was confirmed using Etest (Ab Biodisk, Sweden) whereas MIC for erythromycin, tetracycline, oxacillin, rifampicin and ciprofloxacin was done by using agar microdilution test according to CLSI guidelines (CLSI, 2010). Briefly, various concentrations of stock solutions (5120 µg/ml, 640 µg/ml, 80 µg/ml and 10 µg/ml) were prepared according to CLSI guidelines (CLSI, 2010). Intermediate (10x) antimicrobial agent solutions of serial twofold dilutions were prepared by referring to Table 3.6 and the antimicrobial solutions were added to molten Mueller-Hinton agar (45°C to 50°C). The agar and antimicrobial solution was mix thoroughly before being poured into petri dishes on a level surface in an agar depth of 4 mm. The agar was allow to solidify at room temperature and can be either used immediately or store at 4°C for up to five days.

Table 3.6: Scheme for preparing dilutions of antimicrobial agents to be used in agar dilution susceptibility tests.

Antimicrobial solutions						
Step	Concentration (µg/ml)	Source	Vol (ml)	Diluent (ml)	Intermediate concentration (µg/ml)	Final concentration at 1:10 dilution in agar (µg/ml)
1	5120	Stock	2	2	2560	256
2	5120	Stock	1	3	1280	128
3	5120	Stock	1	7	640	64
4	640	Step 3	2	2	320	32
5	640	Step 3	1	3	160	16
6	640	Step 3	1	7	80	8
7	80	Step 6	2	2	40	4
8	80	Step 6	1	3	20	2
9	80	Step 6	1	7	10	1
10	10	Step 9	2	2	5	0.5
11	10	Step 9	1	3	2.5	0.25
12	10	Step 9	1	7	1.25	0.125

Table 3.6 adapted from CLSI, (2010).

The bacterial suspension used for MIC test was adjusted to the 0.5 McFarland standards (turbidity of 0.08 to 0.10). Aliquots of inoculum were applied on the agar surface by using replicators with 1 mm pins, and the agar plates were incubated at 37°C for 16 to 20 hours.

D-zone test method, which is used for the detection of inducible clindamycin resistance (known as inducible macrolides, lincosamides and streptograminB, i-MLSB) and constitutive clindamycin resistance (known as constitutive macrolides, lincosamides and streptograminB (cMLSB) were performed on all erythromycin-resistant strains according to established protocols (Mallick *et al*., 2009). Briefly, erythromycin (15 µg) disk was placed at a distance of 15 mm (center to center) from clindamycin (2 µg) disk on Mueller-Hinton agar (Oxoid Ltd, Basingstoke, Hampshire, UK) and incubated at 37°C for overnight. Any enhancement of inhibition that produces a D-shape of the clindamycin zone is an indication of inducible-clindamycin resistance (Mallick *et al.*, 2009). Strains that were resistant to both clindamycin, and erythromycin are defined as showing constitutive clindamycin resistance (Mallick *et al*., 2009).

S. aureus ATCC25923 was used as quality control strain for susceptibility testing as recommended by CLSI (CLSI, 2010).

3.2.2 DNA template preparation

3.2.2.1 Genomic DNA preparation

DNA template from bacterial strains was prepared for PCR amplification by simple boiling method and by using Wizard, Genomic DNA purification kit (Promega, Madison Wis, USA).

3.2.2.2 Crude lysate DNA template preparation by simple boiling method

One loopful of bacterial colonies was picked and suspended in 100 µl ddH_2O in a microcentrifuge tube containing 2 µl of lysostaphin (1 mg/ml). The microcentrifuge tube containing a loopful of bacterial colonies and lysistaphin was incubated for 5 min at 37°C before being boiled for 10 min at 100°C and immediately snap-cooled on ice for 10 min. The cell lysates were then centrifuged at 10, 000 X *g* or 90 seconds. Five microlitres (approximately 50 ng measured by using Eppendorf Biophotometer) of the supernatant were used in the PCR assay. The lysate was stored in -20°C and could be used for several PCR reactions.

3.2.2.3 Genomic DNA preparation by using a commercial Genomic DNA purification kit

One loopful of bacterial colonies from overnight culture was picked and suspended in 480 µl of 0.5 M EDTA in a microcentrifuge tube containing 10 µl of lysozyme (10 mg/ml) and 10 µl of lysostaphin (1 mg/ml). The mixture was incubated for 30 min at 37°C before being centrifuged for 5 min at 4°C. After centrifugation, all supernatant was removed and 600 µl of nuclei lysis solution (Promega Madison Wis, USA) was added into the microcentrifuge tube, and the solution was re-suspended gently. The tube was incubated at 80°C for 5 minutes in order to lyse the cells and then cool to room temperature.

Three microliters of RNase solutions (10 mg/ml) were added into the microcentrifuge tube and the tube was inverted for 2 to 5 times before being incubated at 37°C for 30 min. After incubation, 200 µl of protein precipitation solution (Promega Madison Wis, USA) was added to RNase-treated cell lysate, and the microcentrifuge tube was vortex vigorously at high speed for 20 sec before being incubated on ice for 5

min. The microcentrifuge tube was centrifuged at 13, 000 x *g* for 3 min and supernatant containing the DNA was transferred to a clean 1.5 ml microcentrifuge tube which contained 600 µl room temperature isopropanol. The microcentrifuge tube was then centrifuged at 13, 000 x *g* for 2 min and all supernatant was carefully removed, and the DNA was washed with 70% ethanol.

The dried pellet was resuspended in 100 µl of deonised water, and the genomic DNA was stored at - 20°C for PCR reaction.

3.2.3 Plasmid extraction by alkaline lysis method

Plasmid extraction was carried out as described by Zuccarelli *et al*. (1990) with minor modifications. Briefly, a single bacterial colony was inoculated into 20 ml of TSB broth and incubated overnight at 37°C with shaking at 180 rpm. The overnight culture was harvested by centrifugation at 5000 x *g* for 10 min at 4°C. The supernatant was discarded, and the pellet was resuspended in 1 ml of TES Solution I (10 mM Tris, 1 mM EDTA and 0.1 mM NaCl, pH 8.0) and 10 µl of lysozyme (10 mg/ml) plus 10 µl of lysostaphin (1 mg/ml) were added to the mixture before being incubated for 30 min at 37°C with shaking at 180 rpm. One mililitre of Solution II (0.2 M NaOH, 1% SDS) was added into the mixture and incubated at ice for 10 min. After that, 1 ml of Solution 3 (3 M Kac) was added into the mixture and incubated at ice for another 10 min. The mixture was centrifuged at 10 000 x *g* for 10 min at 4°C. Following that, the supernatant was transferred into a 15 ml polypropylene tube containing of 2 µl of RNase (100 mg/ml) and incubated at 37°C for 1 hour.

After addition of 3 ml of phenol-chloroform, the polypropylene tube was inverted for 20 times and then centrifuged at 8 000 x *g* for 10 min at 4°C. The upper aqueous phase was transferred to a new polypropylene tube that containing equal

volume of room temperature isopropanol and left for 2 min before being centrifuged at 10 000 x *g* for 10 min. After centrifugation, the supernatant was carefully discarded. The pellet was washed with 200 µl of 70% ethanol and centrifuged at 10 000 x *g* for 5 min at 4°C. The supernatant was discarded carefully, and the DNA pellet was air-dried. The dried pellet was resuspended in 50 µl of pre-warmed (60°C) sterile deonised water and stored at -20°C for PCR amplification and transformation.

3.2.4 PCR detection of β-lactam, mupirocin, gentamicin, vancomycin, linezolid, erythromycin, tetracycline and fusidic acid resistance genes

PCR was used to detect various resistant genes encoding for penicillin (*blaZ*), mupirocin (*mupA, ileS*), gentamicin [*aac(6')-aph(2'')*], vancomycin (*vanA, vanB*), linezolid (*cfr*), erythromycin (*ermA, ermB, ermC, msrA*), tetracycline (*tetK, tetL, tetM, tetO, tetS*) and fusidic acid (*fusB, fusC, fusD*). The primer pairs used to amplify the genes of interest as well as the conditions used for PCR amplification are listed in Table 3.7.

Table 3.7: Primer pairs, their respective sequences and amplification conditions for the various resistant genes

Resistant gene	Primer name	Primer sequence (5'- 3')	PCR condition	References
blaZ	blaZ-F	TACAACTGTAATATCGGAGGG	1 cycle for 5 min at 95ºC; 30 cycles of 1 min at 95ºC, 1 min at 55ºC, 1 min at 72ºC; 1 cycle of 5 min at 72ºC	Vali *et al*., 2008
	blaZ-R	AGGAGAATAAGCAACTATATCATC		
mupA	Mup 1	CCCATGGCTTACCAGTTGA	same as *blaZ*	Ramsey *et al*., 1996
	Mup 2	CCATGGAGCACTATCCGAA		
ileS	MupA	TATATTATGCGATGGAAGGTTGG	same as *blaZ*	Anthony *et al*., 1999
	MupB	AATAAAATCAGCTGGAAAGTGTTG		
aac6-aph2	aac(6)-aph(2)-1	TTGGGAAGATGAAGTTTTTAGA	same as *blaZ*	Martineau *et al.*, 2000
	aac(6)-aph(2)-2	CCTTTACTCCAATAATTTGGCT		
vanA	vanA F	CATGAATAGAATAAAAGTTGCAATA	same as *blaZ*	Tiwari and Sen, 2006
	vanA R	CCCCTTTAACGCTAATACGACGATCAA		
vanB	*vanB* F	GTGACAAACCGGAGGCGAGGA	same as *blaZ*	Tiwari and Sen, 2006
	vanB R	CCGCCATCCTCCTGCAAAAAA		
cfr	cfr-fw	TGAAGTATAAAGCAGGTTGGGAGTCA	same as blaZ	Kehrenberg and Schwarz, 2006
	cfr-fw	ACCATATAATTGACCACAAGCAGC		
ermA	ermA-1	TATCTTATCGTTGAGAAGGGATT	same as *blaZ*	Martineau *et al.*, 2000
	ermA-2	CTACACTTGGCTTAGGATGAAA		
ermB	ermB-1	CTATCTGATTGTTGAAGAAGGATT	same as *blaZ*	Martineau *et al.*, 2000
	ermB-2	GTTTACTCTTGGTTTAGGATGAAA		

Table 3.7 (continue)

Resistant gene	Primer name	Primer sequence (5'- 3')	PCR condition	References
ermC	ermC-1	CTTGTTGATCACGATAATTTCC	same as *blaZ*	Martineau *et al.*, 2000
	ermC-2	ATCTTTTAGCAAACCCGTATTC		
msrA	msrA-1	TCCAATCATTGCACAAAATC	same as *blaZ*	Martineau *et al.*, 2000
	msrA-2	AATTCCCTCTATTTGGTGGT		
tetK	tetK -1	TCGATAGGAACAGCAGTA	same as *blaZ*	Ng *et al*., 2001
	tetK -2	CAGCAGATCCTACTCCTT		
tetL	tetM -1	GTGGACAAAGGTACAACGAG	same as *blaZ*	Ng *et al*., 2001
	tetM- 2	CGGTAAAGTTCGTCACACAC		
tetM	tet(L)-1	TCGTTAGCGTGCTGTCATTC	same as *blaZ*	Ng *et al*., 2001
	tet(L)-2	GTATCCCACCAATGTAGCCG		
tetO	tet(O)-1	AACTTAGGCATTCTGGCTCAC	same as *blaZ*	Ng *et al*., 2001
	tet(O)-2	TCCCACTGTTCCATATCGTCA		
tetS	tet(S)-1	CATAGACAAGCCGTTGACC	same as *blaZ*	Ng *et al*., 2001
	tet(S)-2	ATGTTTTTGGAACGCCAGAG		
fusB	fusB-1F	TCATATAGATGACGATATTG	same as *blaZ*	Castanheira *et al*., 2010b
	fusB-1R	ACAATGAATGCTATCTCGAC		
fusC	fusC-1F	GATATTGATATCTCGGACTT	same as *blaZ*	Castanheira *et al*., 2010b
	fusC-1R	AGTTGACTTGATGAAGGTAT		
fusD	fusD-1F	TGCTTATAATTCGGTCAACG	same as *blaZ*	Castanheira *et al*., 2010b
	fusD-1R	TGGTTACATAATGTGCTATC		

PCR amplification for *mupA, ileS, aac6'-aph2", vanA, vanB, cfrA* and *msrA* genes was performed in a final volume of 25 µl containing 5 µl DNA template (approximately 20 ng), 0.3 µM of each primer, 35 µM of each deoxynucleoside triphosphate, 1.4 mM $MgCl_2$ and 0.5 U *Taq* DNA polymerase (Promega, Madison Wis., USA). PCR detection of *blaZ, ermA, ermB* and *ermC* genes was performed by using multiplex-PCR in a final volume of 25 µl containing 5 µl DNA template (approximately 20 ng), 0.3 µM of each primer, 50 µM of each deoxynucleoside triphosphate, 1.8 mM $MgCl_2$ and 1.0 U *Taq* DNA polymerase (Promega, Madison Wis., USA) whereas detection of tetracycline resistance gene (*tetK, tetL, tetM, tetO, tetS*) was performed by using multiplex-PCR in a final volume of 25 µl containing 5 µl DNA template (approximately 20 ng), 0.3 µM of each primer, 50 µM of each deoxynucleoside triphosphate, 1.8 mM $MgCl_2$ and 1.0 U *Taq* DNA polymerase (Promega, Madison Wis., USA).

Representative products were purified by using the PCR Mega-Quick Spin™ product purification kit (Intron, Biotechnology, Korea). Briefly, 100 µl of binding buffer was added into a 1.5 ml microcentrifuge tube containing the PCR-amplified product and mixed well. Meanwhile, a spin column was placed in a 2 ml collection tube. The suspension was transferred into the spin column and centrifuged for 1 min at 10, 000 x *g* at room temperature. After centrifugation, the flow-through was discarded and 500 µl of washing buffer (containing ethanol) was added into the column and centrifuged for 1 min at 10 000 x *g* at room temperature. The column was washed again with washing buffer and the flow-though was discarded. In order to remove the residual buffer, centrifugation was repeated again for additional 1 min.

The DNA bound to the column was eluted by addition of 40 µl of ddH_2O onto the column and allowing it to stand for 1 min, and subjecting the tube with column to

centrifugation at 10, 000 x *g* for 1 min at room temperature. The column was discarded after centrifugation and the flow-through containing the desired PCR product was collected in the 1.5 ml micro-centrifuged tube. The extracted PCR product was kept in -20°C.

The purified PCR products were outsource to a commercial company (1st Base Pte Ltd, Malaysia) for sequencing. Sequences obtained were aligned and compared with archived database sequences for gene identification using BLAST.

3.2.5 PCR detection of tetracycline and gentamicin resistance transposons

Detection of tetracycline (Tn*916*, Tn*5801*) and gentamicin (Tn*4001*) transposon-associated genes were performed by PCR amplification on genomic and plasmid DNA as template. The primers used for the detection of the transposon-associated genes, their sequences and amplification parameters are shown in Table 3.8.

Table 3.8: Primer pairs, their respective sequences and amplification conditions for transposons associated integrases

Transposon-encoding gene	Primer name	Primer sequence (5'- 3')	PCR conditions	Reference
Tn*916*	327(Tn916-1)	GCCATGACCTATCTTATA	1 cycle for 5 min at 95ºC; 30 cycles of 1 min at 95ºC, 1 min at 55ºC, 1 min at 72ºC; 1 cycle of 5 min at 72ºC	de Vries *et al*., 2009
	328 (Tn916-2)	CTAGATTGCGTCCAA		
Tn*5801*	1811(Intcw459-1)	CCGATATTGAGCCTATTGATGTG	Same as Tn*916*	de Vries *et al*., 2009
	1812(Intcw459-2)	GTCCATACGTTCCTAAAGTCGTC		
Tn*4001*	Primer 1	TGAAAAGCGAAGAGATTCAAAGC	Same as Tn*916*	Kozitskaya *et al*,. 2004
	Primer 2	CTAAACCGTGCATTTGTCTTA		

PCR amplification was performed in a final volume of 25 µl containing 5 µl DNA template (approximately 20 ng), 0.4 µM of each primer, 35 µM of each deoxynucleoside triphosphate, 1.4 mM $MgCl_2$ and 0.5 U *Taq* DNA polymerase (Promega, Madison, Wis., USA).

The amplification product was purified and outsource to a commercial company (1st Base Pte Ltd, Malaysia) for sequencing. Sequences obtained were aligned and compared with archived database sequences for gene identification using BLAST.

3.2.6 Transfer of Antibiotic Resistance determinant by transformation.

3.2.6.1 Preparation of electro-competent *S. aureus* cells

Electro-competent *S. aureus* cells were prepared according to method described by The SunLab Homepage (http://sunlab.ustc.edu.cn/protocol/gene_deletion_in_SA.htm) with minor modifications. Briefly, one single colony of *S. aureus* ATCC29213 was inoculated into 10 ml TSB and incubated at 37°C with vigorous shaking overnight. After that, 10 ml of the overnight grown culture was transferred to 100 ml TSB medium in a 500 ml flask and incubated at 37°C with vigorous shaking until an OD_{610} reading of about 0.4.

The flask was transferred to an ice bath for 5 min following which the cultures were transferred into 50 ml polypropylene tubes, centrifuged at 2 500 x *g* at 4°C for 10 min. The resulting supernatant was discarded, and the cell pellet was re-suspended in 10 ml of 0.5 M sucrose and kept on ice for an additional 5 min. The cell was harvested at 2 500 x *g* at 4°C for 5 min. The cell pellet was re-suspended in 10 ml of 0.5 M sucrose and kept on ice for an additional 5 min before being harvested at 2500 x *g* at 4°C for 5 min.

The cell pellet was again re-suspended in 1 ml of ice-cold 0. 5 M sucrose and the suspension was kept on ice for 15 min. The cell suspension was aliquoted into several pre-chilled microcentrifuged tubes (40 µl) on ice. The aliquots were ethanol-freeze (dip into ice-cool ethanol) and stored at 80°C or used immediately.

3.2.6.2 Preparation of electro-competent *E. coli* cells

Electro-competent *E. coli* cells were prepared according to method described by Sambrook and Russel (2001). Briefly, one single colony of *E. coli* DH10β was inoculated into 10 ml of TSB medium and incubated at 37°C with vigorous shaking overnight. After that, 5 ml of the overnight grown culture was transferred to 100 ml of fresh TSB medium in a 500 ml flask. The flask was incubated at 37°C with vigorous shaking until an OD_{610} reading of about 0.4. The flask was transferred to an ice-bath for 30 min following which the cultures were transferred into 50 ml polypropylene tubes, which were centrifuged at 2500 x *g* for 15 min at 4°C and the resulting supernatant was discarded. The cell pellet was then re-suspended in 100 ml of ice-cold sterile deonised water, and the cell was harvested at 2500 x *g* at 4°C for 20 min. Again, the cell pellet was re-suspended with 50 ml of ice-cold 10% glycerol to wash and harvested at 2500 x *g* for 20 min at 4°C. The cell pellet was then re-suspended in 2 ml of ice-cold 10% glycerol.

The cells were again harvested by centrifugation at 2 500 x *g* for 20 min at 4°C. The supernatant was carefully removed from the tubes and 100 µl of ice-cold GYT [10% (v/v) glycerol; 0.125% (w/v) yeast extracts; 0.25% (w/v) tryptone] medium were added in. The cell suspensions were diluted to a concentration of 2 X 10^{10} to 3 X 10^{10} cells/ml with ice-cold GYT medium. The cell suspension was aliquoted into several pre-chilled microcentrifuge tubes (40 µl) of ice. The aliquots were ethanol-freeze and stored at 80°C or used immediately.

3.2.6.3 Transformation of electro-competent *S. aureus* cells

Frozen stocks of 40 µl of electro-competent *S. aureus* ATCC29213 cells were thawed at room temperature. After the electro-competent *S. aureus*, ATCC29213 cells

(*blaZ*, MSSA) reached room temperature, 10 µl of plasmid DNA (approximately 100 pg/µl) was added into the cell solution and left at room temperature for 30 min.

The electroporation apparatus (Biorad, USA) was set to deliver an electrical pulse of 50 µF capacitance, 2.5 kV and 200 Ω resistance. The cuvette was placed in the electroporater device, and the pulse button was press once. The electroporation cuvette was removed and 1 ml of SMMP broth (at room temperature) was added to the cuvette, and the cell was transferred to a 15 ml polypropylene tube and incubated at 37°C for an hour with gentle rotation.

After an hour, serial dilutions (10^{-1} to 10^{-4}) were carried out and 10 µl of the diluted electroporated cells were transferred onto TSB agar supplemented with either 50 µg/ml erythromycin or 50 µg/ml tetracycline. The plates were inverted and incubated at 37°C overnight, and a same volume of untransformed competent cells was plated on TSB agar plates with and without selective antibiotics to serve as negative controls.

$$\text{CFU/µg} = \frac{\text{CFU on plate}}{\text{pg of the plasmid DNA}} \times \frac{1\text{x}10^{6}\ \text{pg}}{\text{µg}} \times \frac{\text{volume of transformants}}{\text{volume plated}} \times \text{dilution factor}$$

3.2.7 PCR detection of chromosomal associated fusidic acid and rifampicin resistance gene

PCR was used for the amplification of chromosomal associated fusidic acid (*fusA* and *fusE*) and rifampicin (*rpoB*) genes. The primers pairs used to amplify the genes of interest as well as the conditions used for PCR amplification is listed in Table 3.9.

Table 3.9: Primer pairs, their respective sequences and amplification conditions for the *fusA*, *fusE* and *rpoB* genes

Resistance gene	Primer name	Primer sequence (5'- 3')	PCR conditions	References
rpoB	Reverse	TCAACTTTACGATATGGTTC	1 cycle for 5 min at 95ºC; 30 cycles of 1 min at 95ºC, 1 min at 53ºC, 1 min at 72ºC; 1 cycle of 5 min at 72ºC	Mick *et al*., 2010
	Forward	GTCGTTTACGTTCTGTAGGTG		
fusA	rpsU	ATGGCTGGTACCAACAAAGCATTTGCTCACTA	same as *rpoB*	O'Neill *et al*., 2007
	tufL	GCTGTGAGCTCTGTTTTACCATGGTCAACGTG		
fusA	fusA-F2	CTCGTAAYATCGGTATCATG	same as *rpoB*	Castanheira *et al*., 2010b
	fusA-R2	GCATAGTGATCGAAGTAC		
fusA	FusA-F	TTTACCCTGAGTGTGTTCT	same as *rpoB*	Chen *et al*., 2010
	FusA-R	TACATTTAAGCTCACCTTGT		
fusE	fusE(rplF)-1F	CCTAGTGACGTAACAGTAAC	same as *rpoB*	Castanheira *et al*., 2010b
	fusE(rplF)-1F	CGGCGWACRTATTCACCTTG		

PCR amplification for *rpoB and fusE* genes was performed in a final volume of 25 µl containing 5 µl DNA template (approximately 20 ng), 0.3 µM of each primer, 35 µM of each deoxynucleoside triphosphate, 1.4 mM $MgCl_2$ and 0.5 U *Taq* DNA polymerase (Promega, Madison Wis., USA).

All amplified products were purified and out-source to a commercial company (1st Base Pte Ltd, Malaysia) for DNA sequencing. Nucleotide sequences of *rpoB* and *fusA* obtained were compared to the *rpoB* wild-type sequence from *S. aureus* (GenBank accession-number: X64172) and *fusE* sequence from *S. aureus* MW2 (GenBank accession-number: NC_003923.1) as previously described (Mick *et al*. 2010) using Mega 4 software (The Biodesign Institute, USA).

3.2.7.1 Primer design of *fusA* gene

Initially, PCR detection of *fusA* gene was performed by using primers obtained from published protocols (O'Neill *et al*., 2007; Castanheira *et al*., 2010a,b; Chen *et al*., 2010) as shown in Table 3.9. However, despite repeated PCR tests, no amplified product was obtained. Hence, new primers were designed for the detection of *fusA* gene.

Briefly, *fusA* sequence from *S. aureus* (GenBank accession-number: NC_003923.1, NC_007622.1 and NC_ 002745. 2 were retrieved from GenBank. Two pairs of primers [FusA1-F (5'-CGGTATCATGGCTCACATTG-3'), FusA1-R (5'-AGCTGAATCGTCTGCTTTCG-3'), FusA3-F (5'-CGAAAGCAGACGATTCAGCT-3') and FusA1,2,3-R (5'-GTACCGCGACCTTGA GTGTT-3'] were designed based on the sequence using Primer 3 programme. The specificity of these primers was initially tested by using *in-silico* PCR programme.

PCR amplification for *fusA* gene was performed in a final volume of 25 µl containing 5 µl DNA template (approximately 20 ng), 0.3 µM of each primer, 35 µM of each deoxynucleoside triphosphate, 1.4 mM $MgCl_2$ and 0.5 U *Taq* DNA polymerase (Promega, Madison Wis., USA).

All amplified products were purified by using the PCR Mega-Quick Spin^{TM} product purification kit (Intron, Biotechnology, Korea) and out-source to a commercial company (1st Base Pte Ltd, Malaysia) for DNA sequencing. Nucleotide sequences of *fusA* obtained were compared to the *fusA* sequence from *S. aureus* MW2 (GenBank accession-number: NC_003923.1) as previously described (Mick *et al*. 2010) using Mega 4 software (The Biodesign Institute, USA).

3.2.8 Congo red agar (CRA) method for detection of biofilm formation phenotype

Detection of slime production was done according to the protocol by Freeman *et al*. (1989). The media constituted of brain heart infusion agar (Oxoid Ltd., Basingtoke, Hamphsire UK) 52 g/l, Congo red stains 0.8 g/l and sucrose 50 g/l. Inoculated agar was incubated aerobically at 37°C for 24 hours. The biofilm positive strains produced black colour colonies whereas biofilm negative strains remained pink/red in colour.

3.2.9 PCR detection of virulence genes

PCR was used to detect 21 different virulence genes, including enterotoxins (*sea* to *see, seg* to *sej, tst*), exfoliative-toxins (*eta, etb, etd*), cytotoxin (*pvl*), adhesions (*cna, hlg, ica, sdrE, efb, fnbA, fnbB*). The primer pairs used to amplify the genes of interest as well as the conditions used for PCR amplification are listed in Table 3.10.

Table 3.10: Primer pairs, their respective sequences and amplification conditions for the various virulence genes

Virulence gene	Primer name	Primer sequence (5'- 3')	PCR conditions	References
sea	SEA-1	GAAAAAAGTCTGAATTGCAGGGAACA	1 cycle for 5 min at 95ºC; 30 cycles of 1 min at 95ºC, 1 min at 55ºC, 1 min at 72ºC; 1 cycle of 5 min at 72ºC	Jarraud *et al*., 2002
	SEA-2	CAAATAAATCGTAATTAACCGAAGGTTC		
seb	SEB-1	ATTCTATTAAGGACACTAAGTTAGGGA	same as *sea*	Jarraud *et al*., 2002
	SEB-2	ATCCCGTTTCATAAGGCGAGT		
sec	MpSEC-1	GTAAAGTTACAGGTGGCAAAACTTG	same as *sea*	Jarraud *et al*., 2002
	MpSEC-2	CATATCATACCAAAAAGTATTGCCGT		
sed	SED-1	GAATTAAGTAGTACCGCGCTAAATAATATG	same as *sea*	Jarraud *et al*., 2002
sed	SED-2	GCTGTATTTTTCCTCCGAGAGT	same as *sea*	Jarraud *et al*., 2002
see	SEE-1	CAAAGAAATGCTTTAAGCAATCTTAGGC	same as *sea*	Jarraud *et al*., 2002
	SEE-2	CACCTTACCGCCAAAGCTG		
seg	SEG-1	AATTATGTGAATGCTCAACCCGATC	same as *sea*	Jarraud *et al*., 2002
	SEG-2	AAACTTATATGGAACAAAAGGTACTAGTTC		
seh	SEH-1	CAATCACATCATATGCGAAAGCAG	same as *sea*	Jarraud *et al*., 2002
	SEH-2	CATCTACCCAAACATTAGCACC		
sei	SEI-1	CTCAAGGTGATATTGGTGTAGG	same as *sea*	Jarraud *et al*., 2002
	SEI-2	AAAAAACTTACAGGCAGTCCATCTC		
sej	MpSEJ-1	TAACCTCAGACATATATACTTCTTTAACG	same as *sea*	Jarraud *et al*., 2002
	MpSEJ-2	AGTATCATAAAGTTGATTGTTTTCATGCAG		
tst	TST-1	TTCACTATTTGTAAAAGTGTCAGACCCACT	same as *sea*	Jarraud *et al*., 2002
	TST-2	TACTAATGAATTTTTTTATCGTAAGCCCTT		

Table 3.10 (continue)

Virulence gene	Primer name	Primer sequence (5'- 3')	PCR conditions	References
eta	MpETA-1	ACTGTAGGAGCTAGTGCATTTGT	same as *sea*	Jarraud *et al*., 2002
	MpETA-3	TGGATACTTTTGTCTATCTTTTTCATCAAC		
etb	MpETB-1	CAGATAAAGAGCTTTATACACACATTAC	same as *sea*	Jarraud *et al*., 2002
	MpETB-2	AGTGAACTTATCTTTCTATTGAAAAACACTC		
etd	ET-14	AACTATCATGTATCAAGG	same as *sea*	Yamaguchi*et al*., 2002
	ET-15	CAGAATTTCCCGACTCAG		
pvl	luk-PV-1	ATCATTAGGTAAAATGTCTGGACATGATCCA	same as *sea*	Lina *et al*., 1999
	luk-PV-2	GCATCAASTGTATTGGATAGCAAAAGC		
cna	cna-F	AGTGGTTACTAATACTG	same as *sea*	Kumar *et al*., 2009
	cna-R	CAGGATAGATTGGTTTA		
hlg	hlg-F	GCCAATCCGTTATTAGAAAATGC	same as *sea*	Kumar *et al*., 2009
	hlg-R	CCATAGACGTAGCAACGGAT		
ica	ica-F	GATTATGTAATGTGCTTGGA	same as *sea*	Kumar *et al*., 2009
	ica-R	ACTACTGCTGCGTTAATAAT		
sdrE	sdrE-F	AGTAAAATGTGTCAAAAGA	same as *sea*	Kumar *et al*., 2009
	sdrE-R	TTGACTACCAGGCTATATC		
efb	efb-1	AACATTAGCGGCAATAGG	same as *sea*	Moore and Lindsay, 2001
	efb-2	ATTCGCTCTTGTAAGACC		
fnbA	fnbA-1	GATACAAACCCAGGTGGTGG	same as *sea*	Arciola *et al.,* 2005
	fnbA-2	TGTGCTTGACCATGCTCTTC		
fnbB	fnbB-1	TGTGCTTGACCATGCTCTTC	same as *sea*	Arciola *et al.,* 2005
	fnbB-2	AGTTGATGTCGCGCTGTATG		

Four multiplex-PCR were performed for the identification of virulence genes, and this includes; 1) multiplex A for detection of *sea, seb, sec, sed* and *see* genes; 2) multiplex B for detection of *seg, seh, sei* and *sej* genes; 3) multiplex C for detection of *efb, hlg* and *cna* genes and 4) multiplex D for detection of *eta, etb, etd* and *tst* genes. All four multiplex-PCR were performed in a final volume of 25 µl containing 5 µl DNA template (approximately 20 ng), 0.3 µM of each primer, 35 µM of each deoxynucleoside triphosphate, 1.8 mM $MgCl_2$ and 1.5 U *Taq* DNA polymerase (Promega, Madison Wis., USA).

Meanwhile, monoplex PCR was used for detection of *ica, sdrE, fnbA, fnbB* and *pvl* genes in a final volume of 25 µl containing 5 µl DNA template (approximately 20 ng), 0.3 µM of each primer, 35 µM of each deoxynucleoside triphosphate, 1.4 mM $MgCl_2$ and 0.5 U *Taq* DNA polymerase (Promega, Madison Wis., USA).

Representative products were purified and outsource to a commercial company (1st Base Pte Ltd Malaysia) for sequencing.. Sequences obtained were aligned and compared with online database sequences for gene identification using BLAST.

3.2.10 *agr* grouping

Multiplex-PCR was used for the subgrouping of *agr* types as described by Lina *et al*. (2003) with minor modifications. Briefly, the multiplex was performed in a final volume of 25 µl containing 5 µl DNA template (approximately 20 ng), 0.4 µM of each primer, 35 µM of each deoxynucleoside triphosphate, 1.4 mM $MgCl_2$ and 0.5 U *Taq* DNA polymerase (Promega, Madison Wis., USA) and the cycling parameters are: initial denaturation for 5 min at 95°C, followed by 30 cycles of denaturation (95°C for 1 min), annealing (53°C for 1 min), elongation (72°C for 1 min) and a final elongation for 5 min at 72°C. The primers used for *agr* grouping are shown in Table 3.11.

Representative products were purified and outsource to a commercial company (1st Base Pte Ltd Malaysia) for sequencing. Sequences obtained were aligned and compared with archived database sequences for gene identification using BLAST.

Table 3.11: Primers sequences and their respective size for *agr* grouping

Primer name	Primer sequence (5'- 3')	Product length
agr1-4sa-1	ATGCACATGGTGCACATGC	
agr1sa-2	GTCACAAGTACTATAAGCTGCGAT	439
agr2sa-2	TATTACTAATTGAAAAGTGCCATAGC	572
agr3sa-3	GTAATGTAATAGCTTGTATAATAATACCCAG	321
agr4sa-2	CGATAATGCCGTAATACCCG	657

3.2.11 SCC*mec* typing and further sub-grouping of SCC*mec* type IV

Multiplex-PCR was used for the sub-grouping of SCC*mec* types as described by Milheirico *et al*. (2007) with minor modifications. Briefly, multiplex was performed in a final volume of 50 µl containing 5 µl DNA template (approximately 20 ng), 0.3 – 1.0 µM of each primer, 50 µM of each deoxynucleoside triphosphate, 2.25 mM $MgCl_2$ and 1.5 U *Taq* DNA polymerase (Promega, Madison Wis., USA) and the cycling parameters are: Initial denaturation for 6 min at 94°C, followed by 30 cycles of denaturation (94°C for 30 sec), annealing (53°C for 30 sec), elongation (72°C for 1 min) and a final elongation for 5 min at 72°C.

All SCC*mec* type IV strains were further subgrouped to SCC*mec* type IVa to IVd by using primers as described by Hisata *et al.* (2005) and Okuma *et al.* (2002) and were listed in Table 3.12.

Briefly, multiplex PCR was performed in a final volume of 25 µl containing 5 µl DNA template (approximately 20 ng), 0.4 µM of each primer, 50 µM of each deoxynucleoside triphosphate, 1.80 mM $MgCl_2$ and 1.0 U *Taq* DNA polymerase (Promega, Madison Wis., USA) and the cycling parameters are: Initial denaturation for

5 min at 94°C, followed by 30 cycles of denaturation (94°C for 1 min), annealing (50°C for 1 min), elongation (72°C for 1 min) and a final elongation for 5 min at 72°C.

Representative products were purified and outsource to a commercial company (1st Base Pte Ltd Malaysia) for sequencing. Sequences obtained were aligned and compared with archived database sequences for gene identification using BLAST.

Table 3.12: Primers sequences and their respective size for SCC*mec* typing

Primer name	Primer sequence (5'- 3')	Product length (bp)	References
CIF2 F2	TTCGAGTTGCTGATGAAGAAGG	495	Milheirico
CIF2 R2	ATTTACCACAAGGACTACCAGC		*et al*., 2007
ccrC F2	GTACTCGTTACAATGTTTGG	449	Milheirico
ccrC R2	ATAATGGCTTCATGCTTACC		*et al*., 2007
RIF5 F10	TTCTTAAGTACACGCTGAATCG	414	Milheirico
RIF5 R13	ATGGAGATGAATTACAAGGG		*et al*., 2007
SCCmec V	TTCTCCATTCTTGTTCATCC	377	Milheirico
J1F SCCmec	AGAGACTACTGACTTAAGTGG		*et al*., 2007
V J1 R			
dcs F2	CATCCTATGATAGCTTGGTC	342	Milheirico
dcs R1	CTAAATCATAGCCATGACCG		*et al*., 2007
ccrB2 F2	AGTTTCTCAGAATTCGAACG	311	Milheirico
ccrB2 R2	CCGATATAGAAWGGGTTAGC		*et al*., 2007
kdp F1	AATCATCTGCCATTGGTGATGC	284	Milheirico
kdp R1	CGAATGAAGTGAAAGAAAGTGG		*et al*., 2007
SCCmec III J1	CATTTGTGAAACACAGTACG	243	Milheirico
F SCCmec III	GTTATTGAGACTCCTAAAGC		*et al*., 2007
J1 R			
mecI P2	ATCAAGACTTGCATTCAGGC	209	Milheirico
mecI P3	GCGGTTTCAATTCACTTGTC		*et al*., 2007
mecA P4	TCCAGATTACAACTTCACCAGG	162	Milheirico
mecA P7	CCACTTCATATCTTGTAACG		*et al*., 2007
4a1	TTTGAATGCCCTCCATGAATAAA	458	Okuma *et*
4a2	AT		*al*., 2002
	AGAAAAGATAGAAGTTCGAAAGA		
4b1	AGTACATTTTATCTTTGCGTA	994	Okuma *et*
4b2	AGTCATCTTCAATATCGAGAAAG		*al*., 2002
	TA		
4c1	TCTATTCAATCGTTCTCGTATTT	678	Hisata *et*
4c2	TCGTTGTCATTTAATTCTGAACT		*al*., 2005
4d1	TTTGAGAGTCCGTCATTATTTCTT	1010	Hisata *et*
4d2	AGAATGTGGTTATAAGATAGCTA		*al*., 2005

3.2.12 DNA fingerprinting of *S. aureus* strains

3.2.12.1 PCR-RFLP of *coa* gene

PCR amplification of *coa* gene was performed as previously described by Hookey *et al*. (1998) using *coa*-F primer (5'-ATAGAGATGCTGGTACAGG-3') and *co*a-R primer (5'-GCTTCCGATTGTTCGATGC-3') with minor modification. Briefly, PCR was carried out in a final volume of 25 µL containing 0.4 µM of each primer pair (Operon Biotechnologies GmbH, Germany), 5 µL of DNA template (approximately 20 ng), 35 µM of each deoxynucleoside triphosphate, 1.5 mM $MgCl_2$ and 0.5 U *Taq* DNA polymerase (Promega, Madison, Wis., USA).

The cycling parameters are: initial denaturation (94°C for 5 min) followed by 30 cycles of denaturation (95°C for 1 min), annealing (56°C for 1 min), elongation (72°C for 3 min) and a final elongation (72°C for 5 min).

The amplicon of *coa* was digested with *Alu*I enzyme (Promega Madison Wis, USA) as described by Hookey *et al.* (1998). An aliquot of 10 µl of PCR product was incubated at 37°C for 2 hours with 5 U of *Alu*I enzyme supplemented with 0.5 U BSA. The digested products were separated in 1.5% agarose gel at 90 V for 3 hours. Gels were photographed under UV light after staining with ethidium bromide (0.5 µg/ml).

3.2.12.2 Pulsed-field Gel Electrophoresis (PFGE)

PFGE was performed according to an established protocol (Murchan *et al*., 2003) with minor modifications. Briefly, a single colony was streaked on Tryptone-Soy agar and incubated at 37°C for overnight. The next day, the cell culture was transferred to 2 ml of cell suspension buffer (CSB), and cell density was adjusted to OD_{610} = 1.8. An aliquot of 100 µl of standardized cell suspension buffer was then transferred to a 1.5 ml micro-centrifuged tube and 15 µl of lysozyme (10 mg/ml stock solution) and 2.5 µl

of lysostaphin (1 mg/ml stock solution) were added to the suspension. The suspension was incubated at 37°C for 15 min and 1 µl of proteinase K (10 mg/ml stock solution) was added into the microcentrifuged tube. A total of 100 µl of 1% Seakem Gold agarose (Cambrex Bio Science Rockland, Inc, USA) were mixed with 100 µl of the cell-standardized suspension to form plugs. The bacterial cells were lysed within the plugs with cell lysis buffer (50mM Tris; 50 mM EDTA [pH 8.0], 1% Sacrosine, 1 mg/ml proteinase K) and incubated at 54°C for 3 hours. The plug was then washed thoroughly with sterile deionised water (twice) and TE buffer (5 times). The plug can be used immediately or stored at 4°C.

For restriction endonuclease digestion, 1.5 mm slices of the agarose plugs were incubated overnight with 10 U of *Sma*I enzyme supplemented with 1 U BSA at room temperature. As for the H9812 *Salmonella* Braenderup marker, 1.5 mm slice of the agarose plug was incubated overnight with *Xba*I enzyme supplemented with 1 U BSA at 37°C. The plug was then loaded onto a 1.0% agarose gel (Sigma Type I, USA).

The electrophoresis was performed on CHEF-Mapper (Bio-Rad, Hercules, CA) with 0.5 X TBE as running buffer using condition as follows: switch times 5 sec to 60 sec for 22 hours and angle was set at 120° and electrophoresis was carried out at a 6.0 V/cm gradient at a temperature of 14°C.

After electrophoresis, the gel was stained in 300 ml of ethidium bromide (1 µg/ml) for 10 min and destained with 300 ml of 0.5X TBE buffer for 2 hours. The gel image was captured on a UV transilluminator using a gel documentation system.

3.2.12.3 *spa* typing

spa typing was performed on all MRSA strains as described by Harmsen *et al.* (2003) using primer 1095F (5'-AGACGATCCTTCGGTGAGC-3') and 1517R (5'-GCTTTTGCAATGTCATTTACTG-3'). Briefly, monoplex-PCR was performed in a

final volume of 25 µl containing 5 µl DNA template (approximately 20 ng), 0.3 µM of each primer, 35 µM of each deoxynucleoside triphosphate, 1.40 mM $MgCl_2$ and 0.5 U *Taq* DNA polymerase (Promega, Madison Wis., USA) and the cycling parameters are: initial denaturation for 10 min at 95°C, followed by 30 cycles of denaturation (95°C for 30 sec), annealing (60°C for 30 sec), elongation (72°C for 45 sec) and a final elongation for 10 min at 72°C.

The amplicons of *spa* was purified by using commercial DNA purification kit (Intron Biotechnology, Korea) and sequenced to validate their identities. Nucleotide sequences of *spa* amplicons and cluster analysis were analyzed using BioNumerics 6.0 software (Applied Maths, Belgium).

3.2.12.4 Heteroduplex PCR for detection of MLST type ST239 and multilocus sequence typing (MLST)

In order to save cost and time, heteroduplex PCR was performed on all the strains using two pairs of primers as described by Feil *et al.* (2008) for rapid detection of pandemic clone, ST239. PCR amplification of heteroduplex PCR was performed in a final volume of 25 µl containing 5 µl DNA template (approximately 20 ng), 0.4 µM of each primer, 35 µM of each deoxynucleoside triphosphate, 1.4 mM $MgCl_2$ and 1.0 U *Taq* DNA polymerase (Promega, Madison Wis., USA).

MLST was conducted on two representatives ST239 (detected by using heteroduplex PCR) and all non ST239 strains. The PCR amplification for MLST was typically performed in a final volume of 25 µl containing 5 µl DNA template (approximately 20 µg), 0.3 µM of each primer, 35 µM of each deoxynucleoside triphosphate, 1.4 mM $MgCl_2$ and 0.5 U *Taq* DNA polymerase (Promega, Madison Wis., USA). The primer pairs used to amplify the seven *S. aureus* housekeeping genes of

interest are listed in Table 3.13 whereas cycling temperature for both heteroduplex PCR and MLST are: initial denaturation for 7 min at 95°C, followed by 30 cycles of denaturation (95°C for 1 min), annealing (55°C for 1 min), elongation (72°C for 1 min and 30 sec) and a final elongation for 6 min at 72°C.

Amplification products were purified by using commercial purification kits (Intron, Biotechnology, Korea) and outsource to a commercial company (1st Base Pte Ltd, Malaysia) for sequencing. The nucleotides of each housekeeping gene were trimmed by using Mega4 software, and the allelic number and sequences' types (STs) were assigned using the *S. aureus* MLST database (http://saureus.mlst.net).

The clustering of related STs (defined as clonal complexes, CCs) was analyzed with the BURST algorithm (http://eburst.mlst.net) and by using BioNumerics 6.0 software (Applied Maths, Belgium).

Table 3.13: Primers sequences and their respective size for MLST

Primer name	Primer sequence (5'- 3')	References
arcC-Up	TTGATTCACCAGCGCGTATTGTC	Enright *et al.*, 2000
arcC-Dn	AGGTATCTGCTTCAATCAGCG	Enright *et al.*, 2000
aroE-Up	ATCGGAAATCCTATTTCACATTC	Enright *et al.*, 2000
aroE-Dn	GGTGTTGTATTAATAACGATATC	Enright *et al.*, 2000
glpF-Up	CTAGGAACTGCAATCTTAATCC	Enright *et al.*, 2000
glp-Dn	TGGTAAAATCGCATGTCCAATTC	Enright *et al.*, 2000
gmk-Up	ATCGTTTTATCGGGACCATC	Enright *et al.*, 2000
gmk-Dn	TCATTAACTACAACGTAATCGTA	Enright *et al.*, 2000
pta-Up	GTTAAAATCGTATTACCTGAAGG	Enright *et al.*, 2000
pta-Dn	GACCCTTTTGTTGAAAAGCTTAA	Enright *et al.*, 2000
tpi-Up	TCGTTCATTCTGAACGTCGTGAA	Enright *et al.*, 2000
tpi-Dn	TTTGCACCTTCTAACAATTGTAC	Enright *et al.*, 2000
yqiL-Up	CAGCATACAGGACACCTATTGGC	Enright *et al.*, 2000
yqil-Dn	CGTTGAGGAATCGATACTGGAAC	Enright *et al.*, 2000
SA031F	TCGCACTCTCGTTGAACA	Feil *et al*., 2008
SA0317R	AAATCCGCTTCGACAAACATT	Feil *et al*., 2008
SA2003F	CACTTTAAATACTGACGAAAAT	Feil *et al*., 2008
SA2003R	TTGAAAATTGATCATTCAGCAA	Feil *et al*., 2008

3.2.12.5 *mec*-associated direct repeat unit (*dru*) typing

mec-associated *dru* typing was performed on all MRSA strains as described by Goering *et al.* (2008) using primer *dru*-F (5'-GTTAGCATATTACCTCTCCTTGC- 3') and *dru*-R (5'-GCCGATTGTGCTTGATGAG-3'). Briefly, a monoplex-PCR was performed in a final volume of 25 µl containing 5 µl DNA template (approximately 20 ng), 0.3 µM of each primer, 35 µM of each deoxynucleoside triphosphate, 1.40 mM $MgCl_2$ and 0.5 U *Taq* DNA polymerase (Promega, Madison Wis., USA) and the cycling parameters are: initial denaturation for 5 min at 95°C, followed by 30 cycles of denaturation (95°C for 1 min), annealing (53°C for 1 min), elongation (72°C for 1 min) and final elongation for 5 min at 72°C.

The amplicons of *dru* was purified by using commercial DNA purification kit (Intron Biotechnology, Korea) and sequenced to validate their identities. Nucleotide sequences of *dru* amplicons and cluster analysis were analyzed using BioNumerics 6.0 software (Applied Maths, Belgium).

3.2.13. Fingerprint pattern analysis for PCR-RFLP of *coa* gene, PFGE *spa* and *mec*-associated *dru* typing

The banding patterns generated by PCR-RFLP of *coa* gene and PFGE were analyzed with BioNumerics 6.0 (Applied Maths, Kortrijk, Belgium). All the DNA fingerprints were assigned arbitrary designation and analyzed by defining similarity (Dice) coefficient $F = 2n_{xy}/(n_x + n_y)$ where n_x = number of fragments for isolate X, n_y = number of fragments for isolate Y, and n_{xy} = number of shared fragments between isolates X and Y (Thong *et al*., 2007). Gel photos were converted into JPEG images prior to export into BioNumerics 6.0 for cluster analysis which was carried out based on

the unweighted pair group method with arithmetic averages (UPGMA) using the position tolerance of 0.15 (Fontana *et al*., 2003).

Nucleotide sequences of *spa,* MLST and *mec*-associated *dru* typing were analyzed by BioNumerics 6.0 (Applied Maths, Kortrijk, Belgium). The cluster analysis settings for the minimum spanning tree (MST) was set to 25% duplicate extension, 25% duplicate creation, 50% gap extension cost, 250% gap creation cost, maximum duplication length of three repeats and bin grouping distance of 0.5%. Based on the interpretation scheme recommended by Shore *et al.* (2010), two strains are considered closely related if two *spa* or *dru* types are at a MST distance value of ≤ 3 (corresponding to > 98.5% similarity). The distance between each node represents the similarity level between two entries, i.e two entries that had a similarity of between 99.5 to 100%, had a distance of 0.

3.2.15 Statistical Analysis

Statistica software (version 8.0) was used for data analysis. Comparison of certain variables was determined by Fisher's exact test. The associations between different virulence factors or resistance genes were determined by Spearman's rank order correlation coefficient test. The *P* value < 0.05 (two-tailed) was taken as the level of significance for Fisher' exact test whereas R value was taken as the type of association between the variables. The breakpoints for the association of virulence factors are defined as follows: perfect association with R = 1, no association with R = 0 and invert correlation with R = -1.

The association of mutations and resistance level for erythromycin and fusidic acid was determined by Kruskal-Wallis test (where all the categories of mutation consist of at least 5 entries).

CHAPTER 4

RESULTS

4.1 Bacterial Strains

One hundred eighty-eight non-repeat MRSA strains from 184 individual patients and four staff nurse were selected in this study (52 from year 2003, 9 from year 2004, 16 from year 2007 and 111 from year 2008). All MRSA strains that could be revived from stock cultures were included for analysis. The organisms were isolated from respiratory samples, such as nasal swabs (n = 43; 23%), sputum (n = 23; 12%) and nasopharyngeal secretion (n = 9; 5%)], tissue (n = 16; 8%), wound swabs (n = 34; 18%), urine (n = 6; 3%), pus (n = 12; 6%), body fluids (n = 24; 13%), catheter tips (n = 3; 2 %), bone (n = 4; 2%), blood (n = 13; 7%), chest tube "drainage" (n = 1; 1%) (Figure 4.1; Appendix 1).

All strains were obtained from the following wards: orthopaedic (n=58), medical (n=46), surgical (n=34), intensive care unit (n=20), dialysis (n=8), paediatric (n=9), others (n=6), cardiac care unit (n=4), obstetrics and gynecology (n=2) and psychiatry (n=1) (Figure 4.2; Appendix 1).

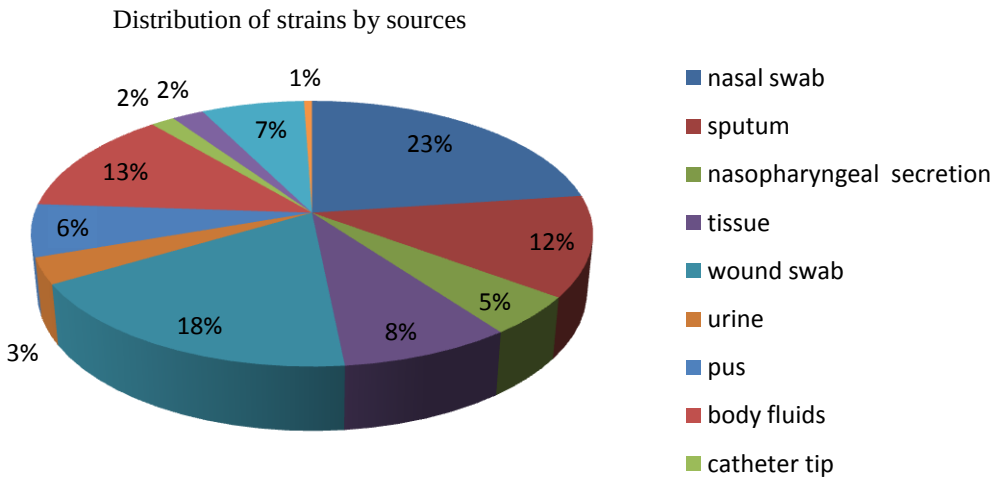


Figure 4.1 Distribution of the 188 MRSA strains by sources

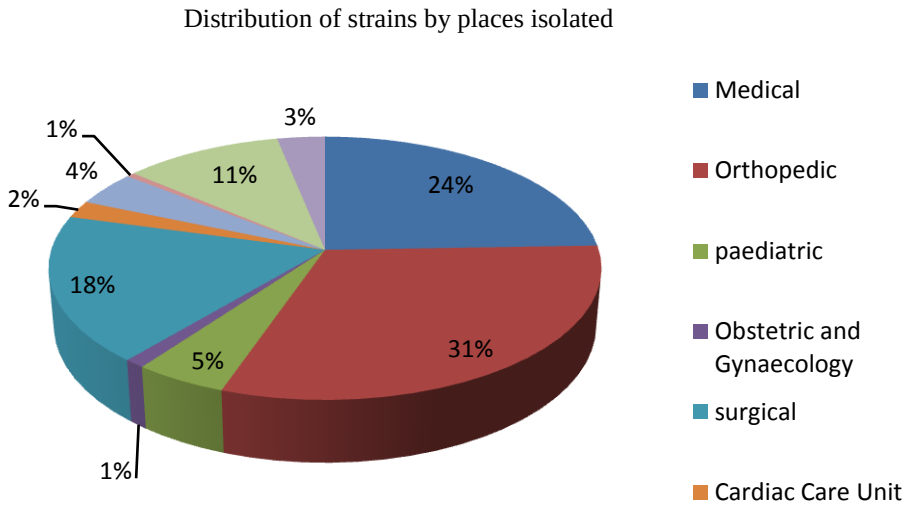


Figure 4.2 Distribution of the 188 MRSA strains by location

4.2 Antimicrobial Susceptibility Profiles

All MRSA strains were sensitive to vancomycin but resistant to oxacillin. Using the disk diffusion method, the resistant rates are as follows: erythromycin (95%); clindamycin (94%); ciprofloxacin (92%); gentamicin (83%); trimethoprim-sulfamethoxazole (59%); tetracycline (50%); netilmicin (42%); fusidic acid (10%); mupirocin (5%); rifampicin (4%); linezolid (2%); and teicoplanin (1%) (Figure 4.3A; Table 4.1; Appendix 3).

A total of 124 strains (66%) were multidrug-resistant (MDR) (resistant to more than three classes of antimicrobial agents). There was a significant increase in the rates of resistance towards trimethoprim-sulfamethoxazole ($P < 0.01$), netilmicin ($P < 0.01$) and tetracycline ($P < 0.01$) for 2008 strains compared with the 2003 strains (Table 4.2). There was no significant difference in the resistance rates in 2003 and 2008 for the following antimicrobials: erythromycin (96% in year 2003 and 2008), ciprofloxacin (96% in 2003 to 89% in 2008), gentamicin (90% in 2003 to 82% in 2008), rifampicin (6% in 2003 to 4% in 2008), fusidic acid (10% in 2003 to 12% in 2008), teicoplanin

(4% in 2003 to 0% in 2008), mupirocin (2% in 2003 and 4% in 2008), clindamycin (94% in 2003 to 96% in 2008) and linezolid (2% in 2003 to 1% in 2008) ($P > 0.05$). The resistance rates for erythromycin remained the same (Table 4.2).

The MIC for oxacillin, erythromycin, ciprofloxacin and tetracycline ranged from 4.0 to 512 µg/ml, 0.25 to 256 µg/ml, 0.5 to 512 µg/ml and 0.5 to 256 µg/ml, respectively (Figure 4.3B, C). The temporal changes in the MIC of four antimicrobials for MRSA are summarized in Table 4.3. There was a significant increase ($P < 0.05$) of high-level erythromycin (128 and 256 µg/ml), medium (16 µg/ml) to high-level tetracycline (256 µg/ml) and medium-level ciprofloxacin (64 µg/ml) resistant strains between year 2003 and 2008.

Based on Spearman's rank correlation coefficient test, the correlation between erythromycin and ciprofloxacin resistance was observed ($R = 0.607$, $P < 0.05$). Similarly, correlations between erythromycin and tetracycline ($R = 0.1922$, $P < 0.05$), ciprofloxacin and tetracycline ($R = 0.0795$, $P < 0.05$) were also observed. The values indicate that tetracycline-resistant strains were most likely to show co-resistance towards ciprofloxacin and erythromycin. Similarly, these values also indicate that erythromycin-resistant strains were most likely to show co-resistance towards ciprofloxacin and tetracycline.

Based on the D-zone test, 96% (170/178) and 3% (6/178) of the erythromycin-resistant strains showed inducible clindamycin resistance and constitutive clindamycin resistance, respectively (Figure 4.3D, E). Two erythromycin resistant strains did not have any flattening of the clindamycin zone adjacent to the erythromycin disk.

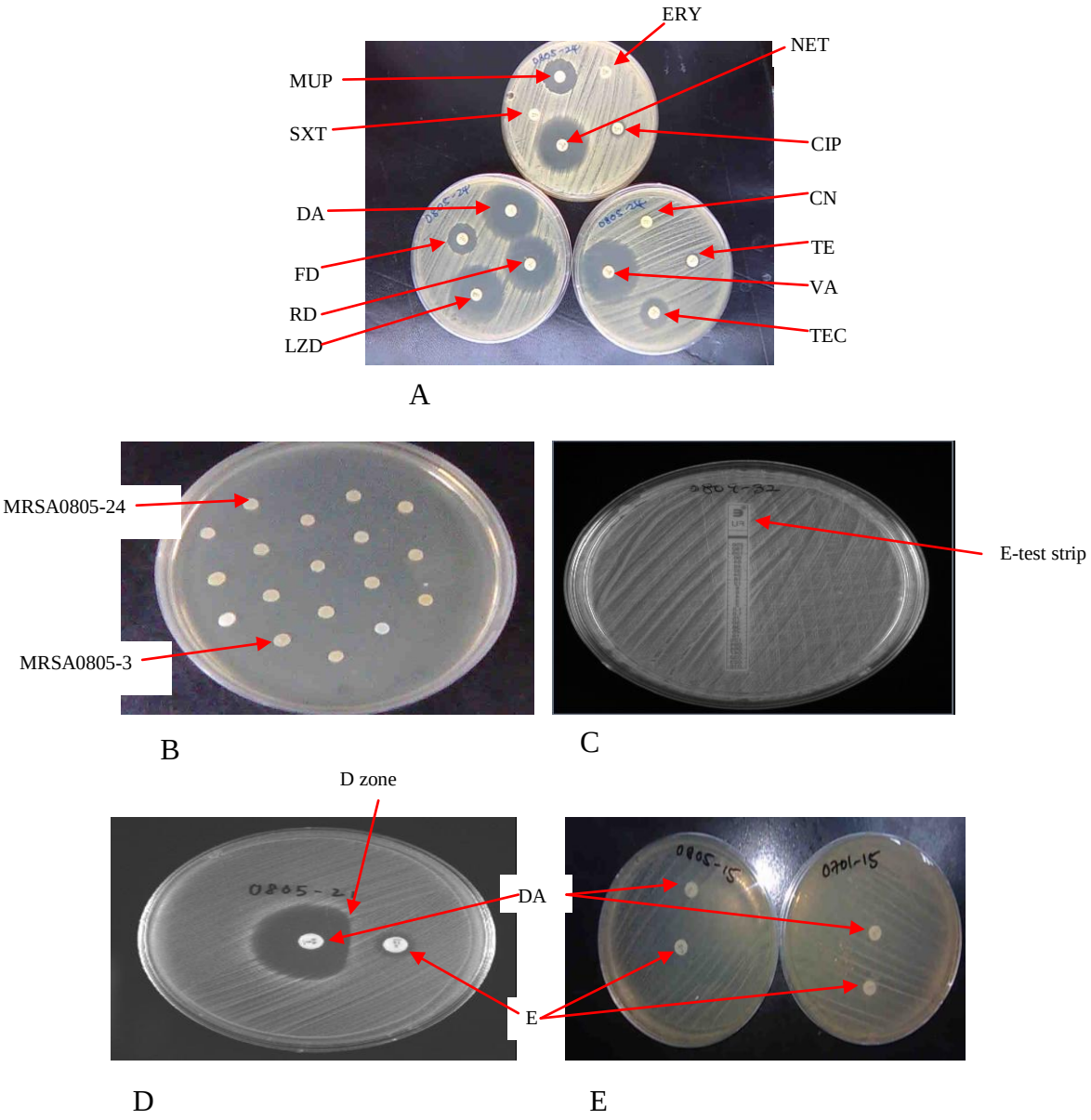


Figure 4.3 (A) MRSA strain (MRSA0805-24) in a representative plate used for the disk diffusion Antibiotic Susceptibility Test, (B) Nineteen MRSA strains in a representative plate used for MIC for tetracycline using agar microdilution method, (C) MRSA strain (MRSA0809-32) in a representative plate used for MIC using E-test strips, (D) MRSA strain (MRSA0805-21) in a representative plate used for D-zone test indicating of inducible-clindamycin resistance and (E) MRSA strains (MRSA0805-15 and MRSA0701-15) in two representative plate used for D-zone test indicating of constitutive-clindamycin resistance.

Abbreviations: MUP, mupirocin; SXT, trimethoprim-sulfamethoxazole; RF, rifampicin; FD, fusidic acid; NET, netilmicin; ERY, erythromycin; DA, clindamycin; CN, gentamicin; VA, vancomycin; CIP, ciprofloxacin; TE, tetracycline; LZD, linezolid; TEC, teicoplanin

Table 4.1: Antimicrobial resistance of MRSA strains tested for 14 antimicrobial agents

Antimicrobial agents	Number of strains (%)		
	Sensitive	Intermediate	Resistant
Ciprofloxacin	10 (5)	6 (3)	172 (92)
Clindamycin	11 (6)	0 (0)	177 (94)
Erythromycin	9 (4)	1 (1)	178 (95)
Fusidic acid	166 (88)	4 (2)	18 (10)
Gentamicin	30 (16)	2 (1)	156 (83)
Linezolid	185 (98)	0 (0)	3 (2)
Netilmicin	68 (36)	42 (22)	78 (42)
Rifampicin	179 (95)	1 (1)	8 (4)
Teicoplanin	179 (95)	7 (4)	2 (1)
Tetracycline	80 (43)	13 (7)	95 (50)
Trimethoprim-sulfamethoxazole	77 (41)	0 (0)	111 (59)
Vancomycin	188 (100)	0 (0)	0 (0)
Mupirocin	178 (95%)	0 (0)	10 (5)

Table 4.2: Resistance rates of Malaysian MRSA strains in year 2003 and 2008

Year	2003 n =52 (%)	2008 n =111 (%)	Total (%)	*P* value
Antimicrobials				
Ciprofloxacin	50 (96)	99 (89)	149 (91)	0.23
Clindamycin	49(94)	106 (96)	155 (95)	0.71
Erythromycin	50 (96)	106 (96)	156 (96)	1.00
Fusidic acid	5 (10)	13 (12)	18(11)	0.79
Gentamicin	47 (90)	91 (82)	138 (85)	0.24
Linezolid	1 (2)	1 (1)	2 (1)	0.54
Netilmicin	14 (27)	55 (50)	69 (42)	$P < 0.01$
Rifampicin	3 (6)	4 (4)	7 (4)	0.68
Teicoplanin	2 (4)	0 (0)	2 (1)	0.10
Tetracycline	10 (19)	71 (64)	81 (50)	$P < 0.01$
Trimethoprim-sulfamethoxazole	19 (37)	80 (72)	99 (61)	$P < 0.01$
Vancomycin	0 (0)	0 (0)	0 (0)	1.00
Mupirocin	1 (2)	4 (4)	5 (3)	1.00

Table 4.3: MIC values of oxacillin, erythromycin, ciprofloxacin, tetracycline, rifampicin and fusidic acid-resistant MRSA strains

Year	MIC	2003-2004 (n=61) n	2007-2008 (n=127) n	Total (n=188)	*P* value
Antibiotic					
Oxacillin	4	6	11	17	0.79
	8	5	12	17	1
	16	3	13	16	0.27
	32	2	2	4	0.59
	64	5	10	15	1
	128	10	24	34	0.83
	256	29	50	79	0.34
	512	1	5	6	0.67
Total resistance		61 (100%)	127 (100%)	188 (100%)	1
Ciproflox-	0.5	0	2	2	1
acin	1	2	7	9	0.75
	2	0	5	5	0.17
	8	8	12	20	0.45
	16	3	10	13	0.55
	32	4	8	12	1
	64	18	18	36	0.02*
	128	5	12	17	1
	256	21	46	67	0.87
	512	-	7	7	0.09
Total resistance		59 (97%)	113 (89%)	172 (91%)	0.09
Erythrom-	0.25	0	1	1	1
ycin	0.5	2	6	8	1
	4	0	1	1	1
	8	4	2	6	0.08
	16	0	5	5	0.17
	32	4	9	13	1
	64	13	19	32	0.30
	128	14	9	23	0.003*
	256	24	75	99	0.01*
Total resistance		59 (97%)	119 (94%)	178 (95%)	0.50
Tetracycli-	0.25	1	0	0	0.33
ne	0.5	4	0	4	0.10
	1	6	11	17	1
	2	11	14	25	0.25
	4	19	14	33	0.002*
	8	3	9	12	0.75
	16	6	30	36	0.01*
	32	4	17	21	0.43
	64	4	14	17	0.43
	128	3	7	10	1
	256	0	11	11	0.01*
	Total	17 (28%)	78 (61%)	95 (51%)	0

Table 4.3 (continue)

Year		2003-2004	2007-2008	Total	*P* value
		n	n		
	MIC	n=61	n=127	n=188	
Rifampicin	0.5	58	122	180	0.72
	2	0	1	1	1
	4	1	0	1	0.32
	8	2	4	6	1
	Total	3 (5%)	5 (4%)	8 (4%)	0.72
Fusidic Acid	0.5	56	114	170	0.79
	6	2	2	4	0.60
	16	0	1	1	1
	64	0	3	3	0.55
	96	1	2	3	1
	256	2	5	7	0.59
	Total	5 (8%)	13 (10%)	18 (10%)	0.79
Mupirocin	0.5	56	122	178	0.3
	8	1	0	1	0.3
	16	1	0	1	0.3
	32	2	4	6	1
	256	1	1	2	0.54
	Total	5 (8%)	5(4%)	10 (5%)	0.3

* Indicative more $P < 0.05$

4.3. Prevalence of β-lactam, mupirocin, gentamicin, vancomycin, linezolid, erythromycin, tetracycline and fusidic acid resistance genes in MRSA strains

PCR amplification for the detection of resistance genes for erythromycin (*ermA, ermB, ermC* and *msrA*), tetracycline (*tetK, tetL, tetM, tetO* and *tetS*), β-lactams (*blaZ*), mupirocin (*mupA* and *ileS2*) and gentamicin [*aac(6')-aph(2")*] were carried out using genomic DNA isolated from erythromycin, tetracycline, oxacillin, mupirocin and gentamicin resistant strains.

All the oxacillin-resistant MRSA strains harboured *blaZ* gene. On the other hand, all mupirocin-resistant strains harboured *ileS2* gene whereas all gentamicin-resistant strains harboured *aac(6')-aph(2")* genes. Furthermore, all erythromycin-resistant strains harboured either *ermA, ermC* or *msrA* genes while all tetracycline-resistant strains harboured either *tetK* or *tetM* genes. Specifically, *ermA, aac(6')-aph(2"), tetM, ermC, tetK, ileS2, msrA* and *mupA* specific amplicons were detected in

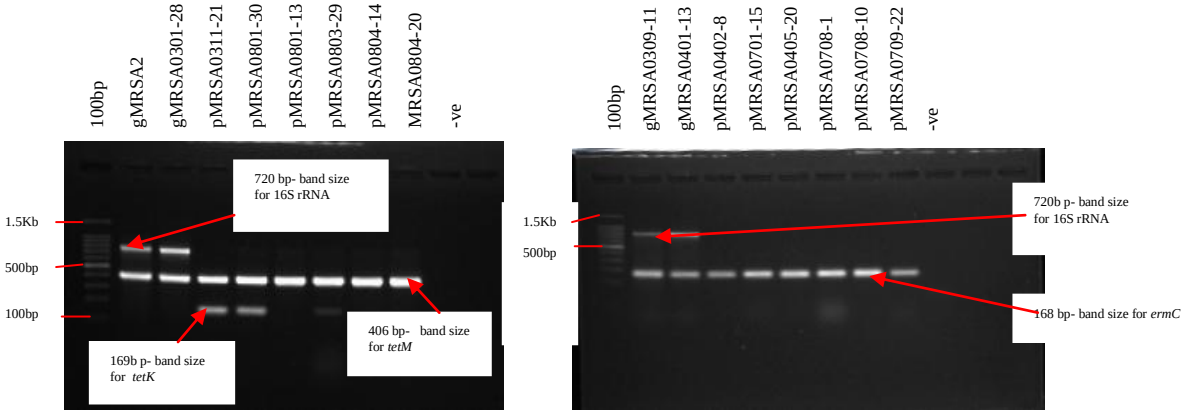
157 (84%), 156 (83%), 92 (49%), 40 (21%), 39 (21%), 10 (5%), 4 (2%) and 2 (1%) strains respectively (Figure 4.4; Appendix 4-12). However, no amplicon was obtained with primers that were specific for the *ermB, fusB, fusC, fusD, cfrA, vanA, vanB, tetL, tetO* and *tetS* genes despite repeated attempts.

Based on Kruskal-Wallis test, no significant difference was found between level of erythromycin resistance for 2008 strains and the presence of different types of erythromycin-resistance genes ($H = 5.29$, $df = 2$, $P = 0.071$).

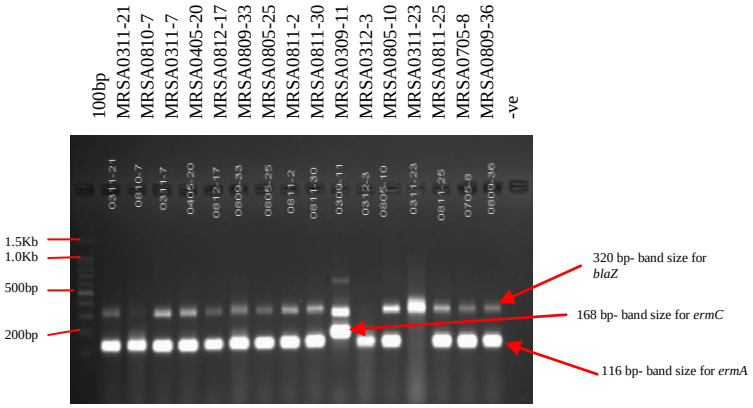
PCR amplifications using plasmid DNA as templates were carried out in parallel with additional 16S rRNA primers to preclude chromosomal DNA contamination. The presence of 720 bp amplicon indicative of either chromosomal DNA contamination or the resistance gene was chromosomal-encoded. Initially, detection of *ermA, ermC* and *blaZ* genes were carried out by using multiplex PCR. As 720 bp amplicon was detected among the MRSA strains which was also tested positive for *ermA, ermC* and *blaZ* genes, therefore the detection of *ermA, ermC* and *blaZ* genes were carried out using monoplex PCR in order to determine which of the resistant genes were plasmid encoded. Results from monoplex PCR showed thal all *blaZ* (n=188) and *ermC* (n=40) genes were plasmid encoded whereas *ermA* (n=157) was chromosomal-encoded (Figure 4.4B; Table 4.4).

The subsequent PCR detection of *tetM, tetK, msrA, ileS2* and *mupA* genes were carried out by using the same batch of plasmids DNA that were used to detected *ermA, ermC* and *blaZ* genes in order to eliminate the possibility of chromosomal contamination. Results showed that *tetM* (n=92), *tetK* (n=39), *ileS2* (n=10) and *mupA* (n=2) genes were plasmid-borne as no 720 bp amplicon was presence in the PCR (Figure 4.4A, D; Tabe 4.4). *msrA* and *aac(6')-aph(2")* genes were associated with chromosome as 16S rRNA gene was detected along with *msrA* and *aac(6')-aph(2")* genes.

Sequencing of the PCR amplicons obtained (selected representatives from *ermA*, *ermC*, *msrA*, *mupA*, *ileS2*, *tetK*, *tetM*, *aac(6')-aph(2")* and *blaZ* amplified products) indicated complete identity to their respective sequences in the NCBI database (Appendix 4-12).



A B



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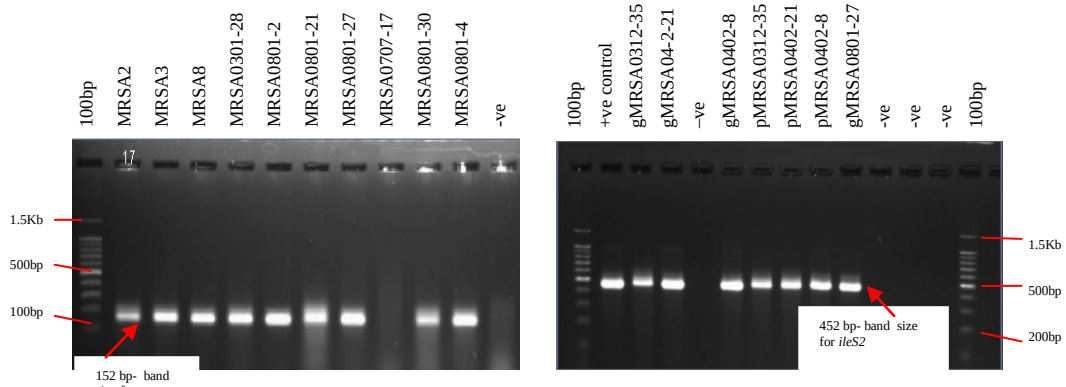

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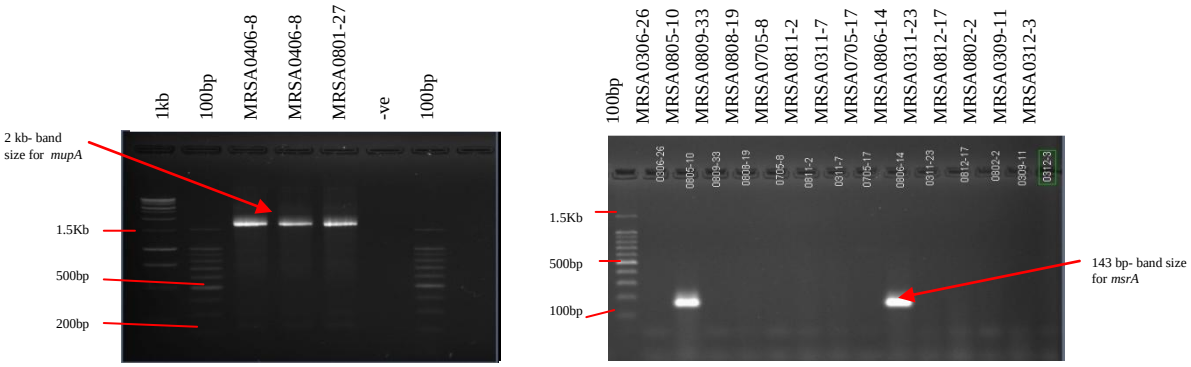


F G

Figure 4.4 Representative agarose gels of PCR-amplified products using different specific primers for detection of various resistant genes for the MRSA strains. (A) *tetM* and *tetK*-specific primers with genomic and plasmid DNA as template; (B) *ermC*-specific primer with genomic and plasmid DNA as template; (C) *ermA*, *ermC* and *blaZ*-specific primers with genomic DNA as template; (D) *aac(6')-aph(2")*-specific primers with genomic DNA as template; (E) *ileS2*-specific primers with genomic and plasmid DNA as template; (F) *mupA*-specific primers with genomic DNA as template; (G) *msrA*-specific primers with genomic DNA as template. Sterile deonised water was used as PCR control.

g: genomic DNA; p: plasmid DNA

Table 4.4: Resistance genes detected in the 188 MRSA strains

Strain No	Resistance Gene	
	Genomic DNA	Plasmid DNA
MRSA2	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA3	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA4	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA8	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0301-1	*ermA, blaZ*	*blaZ*
MRSA0301-28	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0302-4	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0304-16	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0305-10	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0305-18	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0305-23	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0306-10	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0306-14	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0306-15	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0306-18	*blaZ*	*blaZ*
MRSA0306-26	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0306-7	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0307-1	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0307-10	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0307-14	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0307-20	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0307-23	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0307-25	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0307-5	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0307-9	*ermA, blaZ*	*blaZ*
MRSA0308-1	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0308-10	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0308-22	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0308-23	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0308-24	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0308-28	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0309-10	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0309-11	*ermC, blaZ, aac(6')-aph(2")*	*ermC, blaZ*
MRSA0309-9	*ermA, blaZ*	*blaZ*
MRSA0310-19	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0310-23	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0310-26	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0310-9	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0311-1	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0311-21	*ermA, tetk, tetM, blaZ*	*tetk, tetM, blaZ*
MRSA0311-23	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0311-4	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0311-7	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0311-8	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0311-9	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0312-13	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0312-15	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*

Table 4.4 (continue)

Strain No	Resistance Gene	
	Genomic DNA	Plasmid DNA
MRSA0312-17	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0312-2	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0312-3	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0312-30	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0312-35	*tetK, blaZ, ileS2*	*blaZ, ileS2*
MRSA0401-13	*ermA, ermC, tetM, blaZ, aac(6')-aph(2")*	*ermC, tetM, blaZ*
MRSA0402-21	*ermA, tetM, blaZ, ileS2*	*tetM, blaZ, ileS2*
MRSA0402-8	*ermA, ermC, tetM, blaZ, aac(6')-aph(2"), ileS2*	*ermC, tetM, blaZ, ileS2*
MRSA0403-20	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0405-20	*ermA, ermC, blaZ, aac(6')-aph(2")*	*ermC, blaZ*
MRSA0406-8	*ermA, tetM, blaZ, aac(6')-aph(2"), ileS2, mupA*	*tetM, blaZ, ileS2, mupA*
MRSA0408-33	*ermA, blaZ, aac(6')-aph(2"), ileS2*	*blaZ, ileS2*
MRSA0408-34	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0409-17	*ermA, tetM, blaZ*	*tetM, blaZ*
MRSA0701-15	*ermA, ermC, blaZ, aac(6')-aph(2")*	*ermC, blaZ*
MRSA0701-26	*blaZ*	*blaZ*
MRSA0703-8	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0704-15	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0704-18	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0704-20	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0704-3	*ermA, tetM, blaZ, aac(6')-aph(2"), ileS2*	*tetM, blaZ, ileS2*
MRSA0705-13	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0705-17	*ermA, ermC, blaZ, aac(6')-aph(2")*	*ermC, blaZ*
MRSA0705-7	*blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0705-8	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0707-17	*blaZ*	*blaZ*
MRSA0707-26	*ermC, tetK, tetM, blaZ, aac(6')-aph(2")*	*ermC, tetK, tetM, blaZ*
MRSA0708-1	*ermC, blaZ*	*ermC, blaZ*
MRSA0708-10	*ermC, blaZ*	*ermC, blaZ*
MRSA0709-22	*ermC, blaZ*	*ermC, blaZ*
MRSA0801-1	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0801-13	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0801-16	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0801-2	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0801-21	*ermA, ermC, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0801-26	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0801-27	*ermA, ermC, tetM, blaZ, aac(6')-aph(2"), ileS2, mupA*	*ermC, tetM, blaZ, ileS2, mupA*
MRSA0801-30	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0801-4	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0801-9	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0802-14	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0802-19	*blaZ*	*blaZ*
MRSA0802-2	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0802-3	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*

Table 4.4 (continue)

Strain No	Resistance Gene	
	Genomic DNA	Plasmid DNA
MRSA0803-28	*ermC, blaZ*	*ermC, blaZ*
MRSA0803-29	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0803-30	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA003-35	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0804-1	*ermC, blaZ*	*ermC, blaZ*
MRSA0804-14	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0804-20	*ermA, ermC, tetM, blaZ, aac(6')-aph(2")*	*ermC, tetM, blaZ*
MRSA0804-24	*ermA, ermC, blaZ, aac(6')-aph(2")*	*ermC, blaZ*
MRSA0805-1	*ermC, blaZ*	*ermC, blaZ*
MRSA0805-10	*blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0805-11	*ermA, ermC, tetM, blaZ, aac(6')-aph(2")*	*ermC, tetM, blaZ*
MRSA0805-15	*ermA, ermC, tetM, blaZ, aac(6')-aph(2"), ileS2*	*ermC, tetM, blaZ, ileS2*
MRSA0805-17	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0805-19	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0805-20	*ermA, tetM, blaZ, aac(6')-aph(2"), ileS2*	*tetM, blaZ, ileS2*
MRSA0805-21	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0805-22	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0805-23	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0805-24	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0805-3	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0805-4	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0805-5	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0805-6	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0805-9	*ermC, blaZ*	*ermC, blaZ*
MRSA0806-1	*ermC, tetM, blaZ, aac(6')-aph(2")*	*ermC, tetM, blaZ*
MRSA0806-11	*blaZ*	*blaZ*
MRSA0806-13	*blaZ*	*blaZ*
MRSA0806-14	*msrA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0806-18	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0806-21	*ermC, blaZ*	*ermC, blaZ*
MRSA0806-22	*ermA, tetK, blaZ, aac(6')-aph(2")*	*tetK, blaZ*
MRSA0806-26	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0806-33	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0807-1	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0807-13	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0807-14	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0807-19	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0807-7	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0807-8	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0808-17	*ermA, ermC, msrA, tetK, tetM, blaZ, aac(6')-aph(2"), ileS2*	*ermC, tetK, tetM, blaZ, ileS2*
MRSA0808-19	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0808-21	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0808-24	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0808-25	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0808-26	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*

Table 4.4 (continue)

Strain No	Resistance Gene	
	Genomic DNA	Plasmid DNA
MRSA0808-35	*ermA, ermC, tetK, tetM, blaZ, aac(6')-aph(2")*	*ermC, tetK, tetM, blaZ*
MRSA0809-1	*ermC, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0809-10	*ermA, tetM, blaZ*	*tetM, blaZ*
MRSA0809-14	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0809-15	*ermA, tetK, blaZ, aac(6')-aph(2")*	*tetK, blaZ*
MRSA0809-24	*ermA, ermC, tetM, blaZ, aac(6')-aph(2")*	*ermC, tetM, blaZ*
MRSA0809-25	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0809-27	*ermA, blaZ*	*blaZ*
MRSA0809-30	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0809-32	*ermA, ermC, tetM, blaZ, aac(6')-aph(2")*	*ermC, tetM, blaZ*
MRSA0809-33	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0809-36	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0809-38	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0810-10	*ermC, blaZ*	*ermC, blaZ*
MRSA0810-13	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0810-15	*ermA, ermC, blaZ, aac(6')-aph(2")*	*ermC, blaZ*
MRSA0810-16	*ermC, blaZ, aac(6')-aph(2")*	*ermC, blaZ*
MRSA0810-17	*ermA, ermC, blaZ*	*ermC, blaZ*
MRSA0810-18	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0810-2	*ermA, tetM, blaZ*	*tetM, blaZ*
MRSA0810-22	*ermC, blaZ*	*ermC, blaZ*
MRSA0810-23	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0810-6	*ermC, blaZ*	*ermC, blaZ*
MRSA0810-7	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0810-9	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0811-10	*ermA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0811-11	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0811-13	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0811-16	*ermC, tetM, blaZ, aac(6')-aph(2")*	*ermC, tetM, blaZ*
MRSA0811-2	*ermA, blaZ*	
MRSA0811-22	*ermC, blaZ*	*ermC, blaZ*
MRSA0811-24	*ermA, msrA, blaZ, aac(6')-aph(2")*	*blaZ*
MRSA0811-25	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0811-26	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0811-28	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0811-30	*ermA, ermC, tetK, blaZ*	*ermC, tetK, blaZ*
MRSA0811-5	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0811-8	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0812-1	*ermC, blaZ*	*ermC, blaZ*
MRSA0812-11	*blaZ*	*blaZ*
MRSA0812-15	*ermA, tetM, blaZ, aac(6')-aph(2")*	*ermC, blaZ*
MRSA0812-17	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0812-2	*ermC, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0812-22	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0812-23	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*
MRSA0812-27	*ermA, ermC, tetM, blaZ, aac(6')-aph(2")*	*ermC, tetM, blaZ*
MRSA0812-30	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*

Table 4.4 (continue)

Strain No	Resistance Gene	
	Genomic DNA	Plasmid DNA
MRSA0812-31	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0812-33	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0812-35	*ermA, tetK, tetM, blaZ, aac(6')-aph(2")*	*tetK, tetM, blaZ*
MRSA0812-36	*ermA, ermC, msrA, blaZ, aac(6')-aph(2")*	*ermC, blaZ*
MRSA0812-37	*ermA, tetM, blaZ, aac(6')-aph(2")*	*tetM, blaZ*

4.4 Determination of tetracycline and gentamicin transposon-associated genes by PCR

PCR amplification was carried out on genomic DNA isolated from all *tetM*-positive tetracycline and gentamicin-resistant strains in order to determine if they are associated with tetracycline transposon-associated genes (Tn*916* and Tn*5801*) and gentamicin transposon-associated genes (Tn*4001*), respectively.

The results showed that Tn*5801*-like *int* was detected in 78 *tetM*-positive strains, and no Tn*916*-like *int* was detected (Figure 4.5; Appendix 13). Fourteen *tetM*-positive strains (MRSA0310-23, MRSA0402-8, MRSA0409-17, MRSA0801-13, MRSA0806-26, MRSA0809-14, MRSA0809-25, MRSA0811-11, MRSA0811-26, MRSA0811-5, MRSA0812-23, MRSA0812-27, MRSA0812-33 and MRSA0812-37) did not harbour Tn*5801* and Tn*916*-like *int*. Similarly, no Tn*4001*-like transposon was detected among gentamicin and netilimicin-resistant strains.

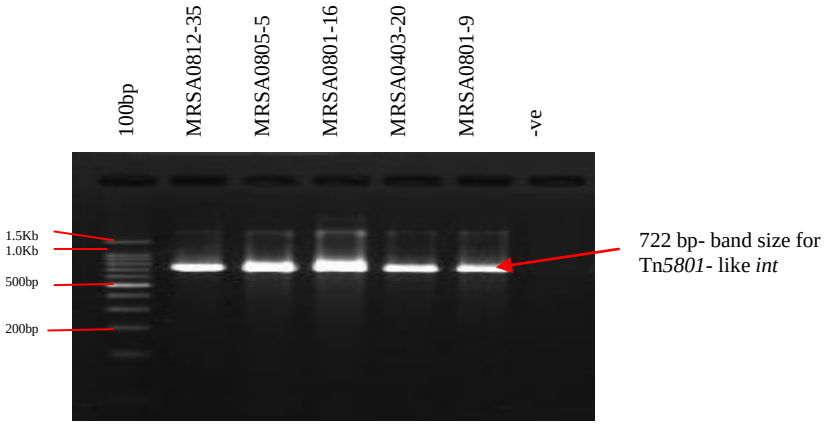


Figure 4.5 Representative agarose gel of PCR-amplified products using primers specific for Tn*5801*-like *int* for t*etM*-positive strains. Sterile deonised water was used as PCR control.

4.5 Transferability of Antibiotic Resistance determinant by transformation

Transformation studies were carried out using plasmid DNA for a subset of 30 randomly selected erythromycin and tetracycline-resistant MRSA strains that harboured the plasmids in order to investigate if erythromycin and tetracycline resistance determinants were transferable.

Although *blaZ* (penicillin resistance) was carried on plasmids, no selection of penicillin-resistance was carried out. This is because the marker used for selection, ATCC29213 is known to harbour *blaZ* gene.

Plasmids (previously used for the PCR detection of plasmid associated resistance genes) extracted from the 30 MRSA strains were electroporated into MRSA ATCC29213 cells, and the transformants were initially selected on TSA plates supplemented with either tetracycline or erythromycin.

Only six out of 30 erythromcyin-resistant MRSA strains could be transferred and no transfer of tetracycline resistance was observed (Figure 4.6). Further analysis

showed that all resultant transformants were resistant to erythromycin (MIC 50 - 256 µg/ml) (Table 4.5).

Subsequent antibiogram profiling of the transformants showed that all six transformants were sensitive to tetracycline and mupirocin.

PCR amplifications showed that only *ermC* gene was successfully transferred into the recipient *S. aureus* ATCC29213 suggesting that this resistant determinant was likely plasmid-encoded. Digested plasmids of sizes ranging from 1.1 to 18.5 kb were detected in six transformants (Figure 4.7, Table 4.5). Identical *Eco*RI restriction profiles were obtained from two plasmids extracted from donor MRSA (MRSA0812-1 and MRSA0804-1) and their respective transformants (Figure 4.7). The other plasmids extracted from transformants were smaller than plasmids from the donors. All the transformants which carried *ermC* contained plasmids with sizes of ~2.5 to 2.7 kb.

Although MRSA0801-27 also harboured *ileS2*, *mupA* and *tetM* genes, none of the resistant genes were successfully transferred. Similarly, no *tetM* or *tetK* genes were successfully transferred even though the MRSA0812-33 and MRSA0806-1 haboured tetracycline resistant genes.

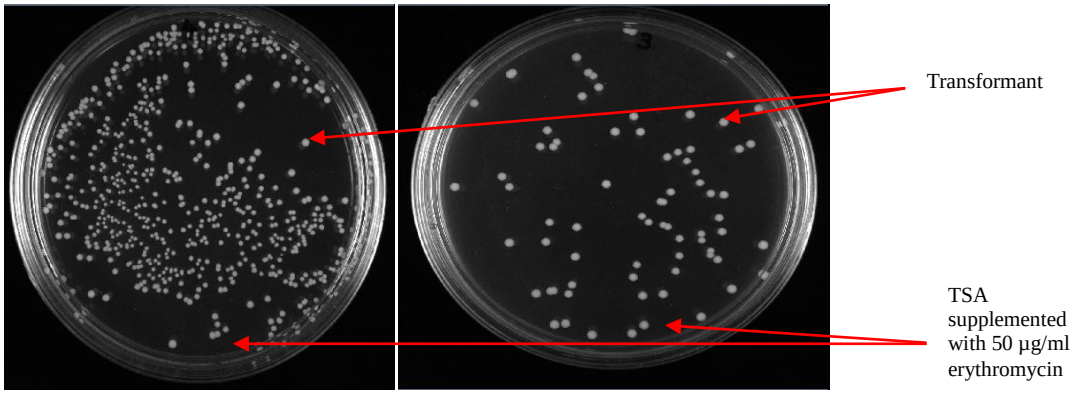


Figure 4.6 Representative plate supplemented with 50 µg/ml erythromycin used for the transformation test (A) transformant MRSA0801-27 and (B) transformant MRSA0803-28.

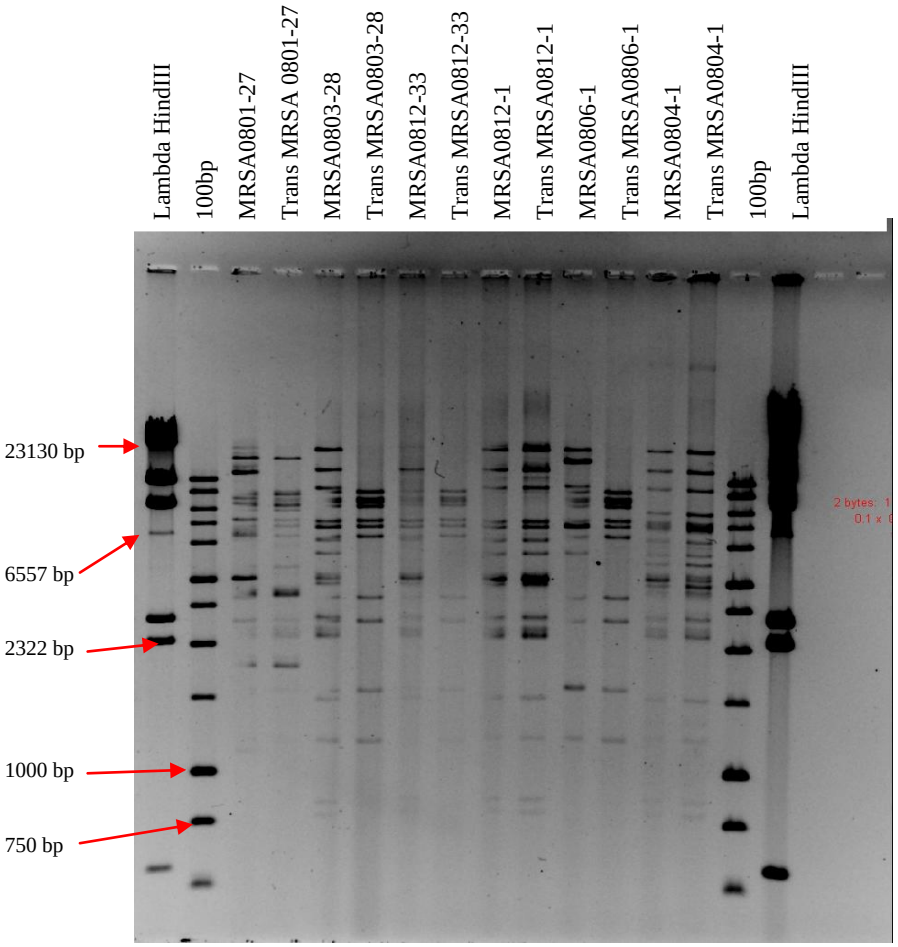


Figure 4.7 Agarose gel of *EcoRI*-digested plasmids extracted from MRSA strains and its ATCC29213 transformants.

Table 4.5: Transformation efficiencies, the size of *EcoR1* digested plasmid, resistant gene transfer and the MIC for the selected MRSA strains and their transformants

Strains no	Transformation efficiency	Size of plasmid in donor strains (kb)	Size of plasmid in transformant (kb)	Resistant gene detected in transformant	MIC for Ery detected in transformant
MRSA0801-27	2.3 X 10^8	15.4, 11.5, 7.0, 6.4, 5.2, 4.2, 3.0, 2.5, 1.8	15.5, 7.0, 6.2, 5.2, 4.1, 3.3, 2.5, 1.9	*ermC*	128 µg/ml
MRSA0803-28	2.4 X 10^8	12.3, 8.4, 6.6, 6.2, 4.5, 4.0, 3.7, 3.1, 2.5, 1.9, 1.1	8.0, 6.7, 6.2, 5.0, 4.5, 4.0, 2.5, 1.9, 1.1	*ermC*	64 µg/ml
MRSA0812-33	2.5 X 10^9	12.0, 8.2, 7.0, 6.4, 4.7, 4.1, 3.2, 2.8, 1.8	8.2, 7.0, 6.4, 5.2, 4.7, 2.7, 2.1	*ermC*	50 µg/ml
MRSA0812-1	9.6 X 10^8	18.5, 12.6, 10.7, 8.6, 5.2, 4.6, 4.1, 3.8, 3.2, 2.5, 2.0, 1.3, 1.0	18.5, 12.6, 10.7, 8.6, 5.2, 4.6, 4.1, 3.8, 3.2, 2.5, 2.0, 1.3, 1.0	*ermC*	128 µg/ml
MRSA0806-1	3.0 X 10^6	18.3, 14.7, 8.8, 8.2, 6.9, 6.4, 4.8, 4.2, 3.8, 2.0, 2.5, 1.4	8.1, 6.8, 6.4, 5.2, 4.8, 4.2, 3.0, 2.5, 1.4, 1.1	*ermC*	256 µg/ml
MRSA0804-1	8 X 10^8	17.8, 12.4. 8.5, 5.8, 4.9, 4.1, 3.8, 3.6, 3.3, 2.5, 2.1	17.8, 12.4. 8.5, 5.8, 4.9, 4.1, 3.8, 3.6, 3.3, 2.5, 2.1	*ermC*	256 µg/ml

4.6 Fusidic acid and rifampicin resistance determinants

4.6.1 *fusA* primers designed

PCR detection of *fusA* gene was carried out by using primers as previously described (O'Neill *et al*., 2007; McLaws *et al*., 2008; Castanheira *et al.,* 2010a; Chen *et al*., 2010). Despite repeated PCR tests for *fusA* using the published primers, no amplification was obtained. Therefore, new *fusA* primers were designed in this study.

In the *fusA* primer designed, *fusA* sequence from *S. aureus* (GenBank accession number NC_003923.1, NC_007622.1 and NC_002745.2) were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/gene?term=fusA%20staphylococcus%20aureus). *fusA*

gene sequences (~2082bp) were aligned by using Mega4 programme (The Biodesign Institute, USA).

Two pairs of primers (FusA1-F, FusA1-R, FusA3-F and FusA1,2,3-R) were designed based on the sequence using Primer 3 programme (http://frodo.wi.mit.edu/primer3). PCR amplification using primer pairs FusA-1F (5' CGG TAT CAT GGC TCA CAT TG-3') and FusA-1R (5'-AGC TGA ATC GTC TGC TTT CG-3') produced amplicon size of 832 bp whereas PCR amplification using primer pairs FusA3-F (5'-CGA AAG CAG ACG ATT CAG CT-3') and FusA1,2,3-R (5'-GTA CCG CGA CCT TGA GTG TT-3') produced amplicon size of 1099 bp. In Primer 3 programme, the primer GC was set from 50 - 60 while primer temperature ranged from 57 - 63°C.

Primers FusA1-F and FusA1,2,3-R can also be used together in order to obtain PCR amplicon with size of 1.9 kb. These primers were found to be 100% specific when tested by using in-silico PCR programme (http://insilico.ehu.es/PCR/).

4.6.2 Detection of rifampicin and fusidic acid resistance determinants by PCR

PCR amplification using *rpoB* primers gave amplicon with 432 bp in size whereas PCR amplification using FusA1 and Fus3A gave amplicons with size of 832 bp and 1099 bp, respectively. The PCR amplification using *fusE* primers gave amplicon with 505 bp in size (Figure 4.8).

Six rifampicin-resistant MRSA strains presented mutational change 484Arg/His in the cluster I of *rpoB* gene (Appendix 14 - 37). Five of these strains had non-synonymous change 517Glu/Gln in cluster II (Table 4.6). Amino acid alteration of 477Ala/Asp was observed in MRSA03008-10 with MIC 4 µg/ml whereas mutational change 481His/Asn was observed in MRSA0809-1 with MIC 2 µg/ml. Silent mutation

Ala(GCG → GCT) at 325' present in all the rifampicin resistant-strains and silent mutation Gly(GGT→GGA) at 462' was exhibited by MRSA0308-23 (Table 4.6).

Amino acid alteration of 461Leu/Lys in *fusA* gene was common among the resistant strains (n = 7, 31.33%) with a high-level of fusidic acid resistance (MIC 256 µg/ml). One strain (MRSA0812-33) which had high-level of resistance to fusidic acid (MIC 256 µg/ml) has multiple mutations at 461Leu/Lys, 596Cys/Trp, 602Glu/Lys and 317Met/Trp. Amino acid alteration 461Leu/Ser was also observed in two strains (MRSA0801-26 and MRSA0805-17) with MIC 6 µg/ml. MRSA0805-17 also exhibit mutational change at 404Pro/Leu. Six other strains with MIC 64 - 96 µg/ml showed amino acid alteration at 67Ala/Thr (n = 1), 457His/Tyr (n = 2) and combination of 67Ala/Thr and 457His/Tyr (n=3) (Table 4.6). No mutation was observed for *fusA* gene in 3 strains, each with MIC of 6 µg/ml and 16 µg/ml. Based on the Kruskal-Wallis test, there was a significant difference between the level of resistance with different types of mutations ($H = 16.39$, $df = 2$, $P < 0.05$) in *fusA* gene.

A silent mutation, Val(GTC→GTT) at 553' was present in 12 fusidic acid-resistant strains whereas silent mutation Leu(TTA→TTG) at 325' was present in two fusidic acid- resistant strains (MRSA0810-7 and MRSA0810-17). Two silent mutations, Pro(CCT→CCA) at 342' and Ala(GCT→GCG) at 325' were exhibited by MRSA0805-1.

Three fusidic acid-resistant strains (MRSA0302-4, MRSA0312-35 and MRSA0805-1) showed no mutations in *fusA* and *fusE* gene. Based on the Spearman's rank correlation coefficient test, invert correlation between rifampicin and fusidic acid resistance ($R = -0.52046$, $P < 0.05$) was observed. This indicates that strain with rifampicin resistance is most likely to be sensitive to fusidic acid.

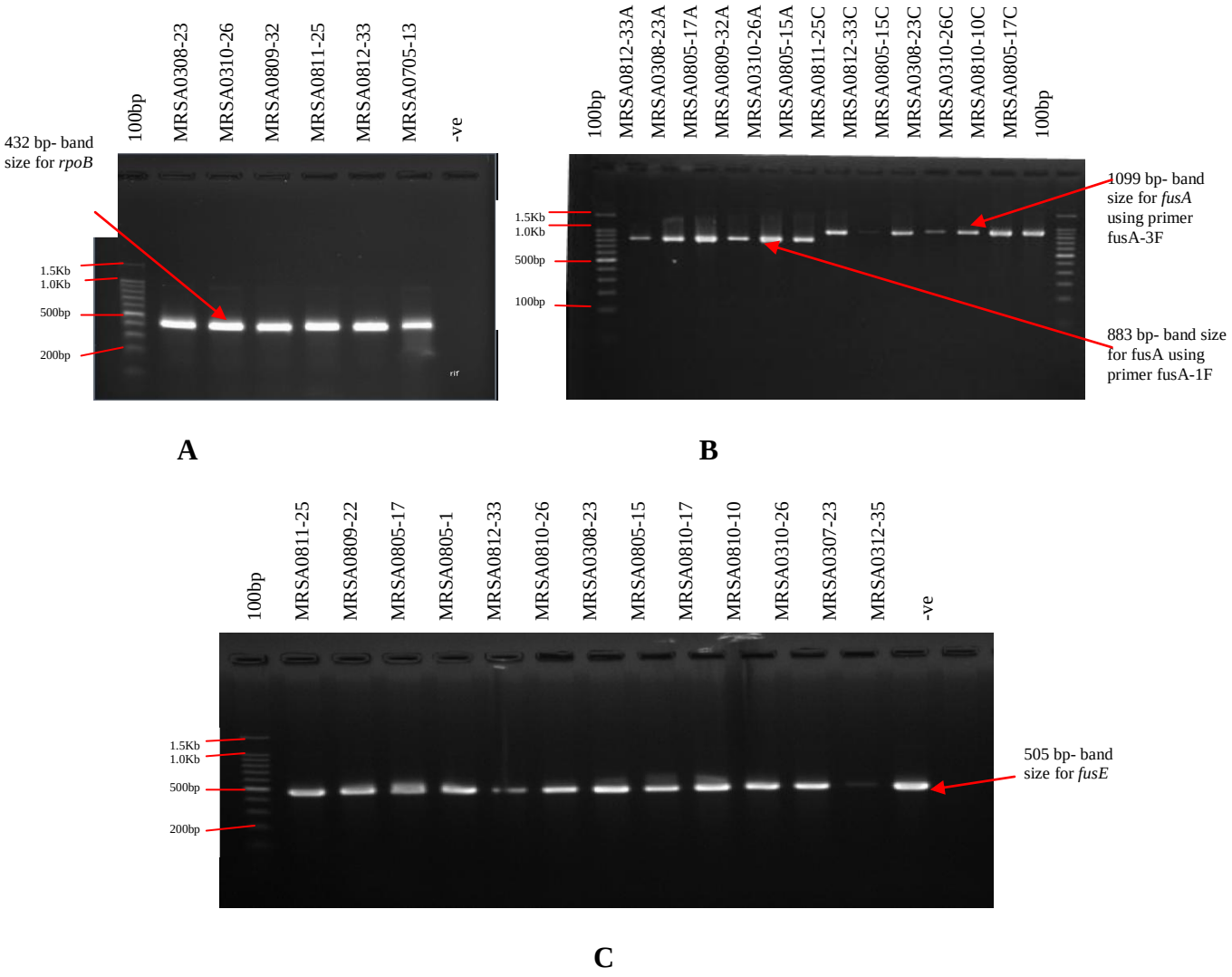


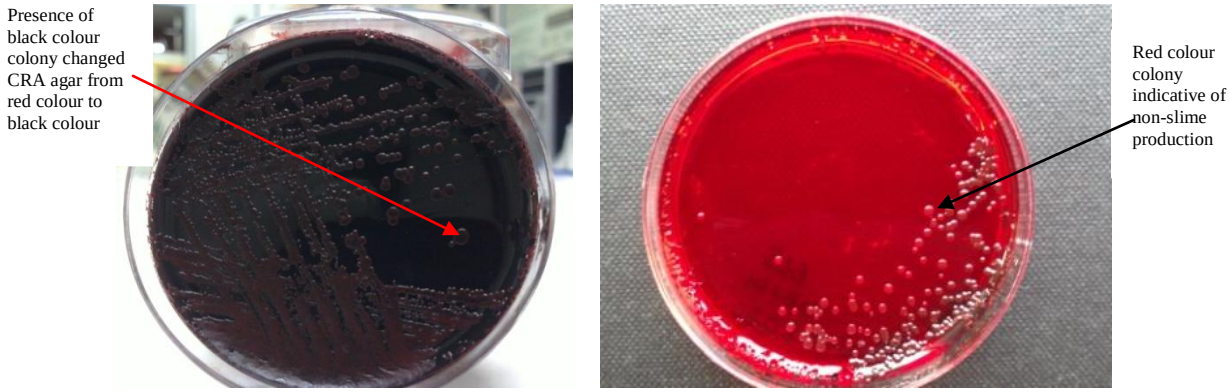
Figure 4.8 Representative agarose gels of PCR amplified products using genomic DNA as template for detection of chromosomal associated resistant genes with (A) detection of *rpoB* gene, (B) detection of *fusA* gene where capital A represent PCR amplified product using primer FUSA-1F and FUSA-1R whereas capital C represent PCR amplified product using primer FUSA-3F and FUSA1,2,3-R and (C) detection of *fusE* gene. Sterile deonised water was used as PCR control.

Table 4.6: Characteristics of rifampicin- and fusidic acid-resistant MRSA strains

Strain	Rifampicin Resistance		Fusidic acid Resistance		GenBank Accession No
	Nucleotide mutation	Amino acid substitution	Nucleotide mutation	Amino acid substitution	
MRSA0308-23	CGT-CAT GAA-CAA	484Arg→ His 517Glu→ Gln	TTA-AAA	461Leu→ Lys	JN597293, HQ914957
MRSA0310-26	CGT-CAT GAA-CAA	484Arg→ His 517Glu→ Gln	TTA-AAA	461Leu→ Lys	JN597294, HQ914958
MRSA0809-32	CGT-CAT GAA-CAA	484Arg→ His 517Glu→ Gln	TTA-AAA	461Leu→ Lys	JN597299, HQ914961
MRSA0811-25	CGT-CAT	484Arg→ His	TTA-AAA	461Leu→ Lys	JN597304, HQ914962
MRSA0812-33	CGT-CAT GAA-CAA	484Arg→ His 517Glu→ Gln	TTA-AAA TGT-TGG GAA-AAA ATG-TGG	461Leu→ Lys 596Cys→ Trp 602Glu→Lys 317Met→Trp	JN597305, HQ914963
MRSA0705-13	CGT-CAT GAA-CAA	484Arg→ His 517Glu→ Gln	-	-	HQ914959
MRSA0308-10	GCT-GAT	477Ala→ Asp	-	-	HQ914953
MRSA0809-1	CAT-AAT	481His→Asn	-	-	HQ914960
MRSA0805-15	-	-	TTA-AAA	461Leu→ Lys	JN597296
MRSA0812-30	-	-	TTA-AAA	461Leu→ Lys	JN597303
MRSA0810-6	-	-	GCA-ACA	67Ala→Thr	JN597306
MRSA0810-10	-	-	GCA-ACA CAC-TAC	67Ala→Thr 457His→Tyr	JN597301
MRSA0307-23	-	-	CAC-TAC	457His→Tyr	JN597292
MRSA0810-7	-	-	GCA-ACA CAC-TAC	67Ala→Thr 457His→Tyr	JN597300
MRSA0807-8	-	-	CAC-TAC	457His→Tyr	JN597298
MRSA0810-17	-	-	GCA-ACA CAC-TAC	67Ala→Thr 457His→Tyr	JN597302
MRSA0805-17	-	-	TTA-TCA CCA-CTA	461Leu→Ser 404Pro→Leu	JN597297
MRSA0801-26	-	-	TTA-TCA	461Leu→Ser	JN597295

4.7 Congo red agar (CRA) method for detection of biofilm formation phenotype

Using Congo red agar, biofilm formation bacteria formed black colour colonies whereas non-biofilm formation bacteria will develop red colour colonies. One hundred forty-three of 188 MRSA strains tested (76%) produced black colour colonies on congo red agar indicative of biofilm formation (Figure 4.9A; Table 4.7). The other 45 of 188 MRSA strains (24%) produced smooth and red colonies indicative of non-biofilm formations (Figure 4.9B, Table 4.7).



A B

Figure 4.9 MRSA strain in two representative plate used for detection of biofilm formation with congo red agar (A) Biofilm producer and (B) non biofilm producer.

4.8 Prevalence of virulence genes in MRSA strains

All MRSA strains were screened for the presence of adhesions (*cna*, *hlg*, *ica*, *sdrE*, *efb*, *fnbA* and *fnbB*), enterotoxins (*sea* to *see*, *seg* to *sej*, *tst*) and exfoliative-toxins (*eta*, *etb* and *etd*) genes.

Majority of the strains were positive for the adhesion genes such as extracellular fibrinogen binding protein (*efb*) (96%), fibrinogen binding protein (*fnbA*) (96%), intracellular adhesion (*ica*) (78%) while hemolysin (*hlg*) and putative adhesin (*sdrE*)

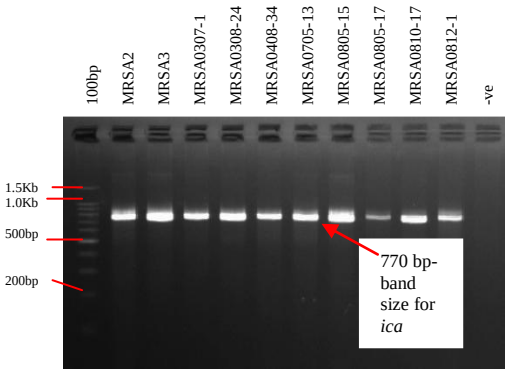
were amplified in 59% and 27%, respectively (Figure 4.10). All biofilm formation MRSA strains harboured *ica* gene. No collagen adhesin (*cna*) gene was detected. Based on Spearman's rank correlation coefficient test, correlation between *efb* and *fnb* (R=1, $P < 0.05$), *hlg* and *ica* (R= 0.326, $P < 0.05$) were observed.

A total of 101 strains were tested positive for at least one type of staphylococcal enterotoxin (SEs) while one strain was positive for exfoliative toxin (*etd*). No enterotoxins (*seb, sed, see* and *seh*) or exfoliative toxins (*eta, etb*) gene was detected. One strain (MRSA0805-10) harboured three SEs. Two strains harboured three toxin genes simultaneously whereas 30 strains harboured two toxin genes (Table 4.7, 4.8). The distribution of virulence genes is summarized in Table 4.8 (Appendix 38 - 47).

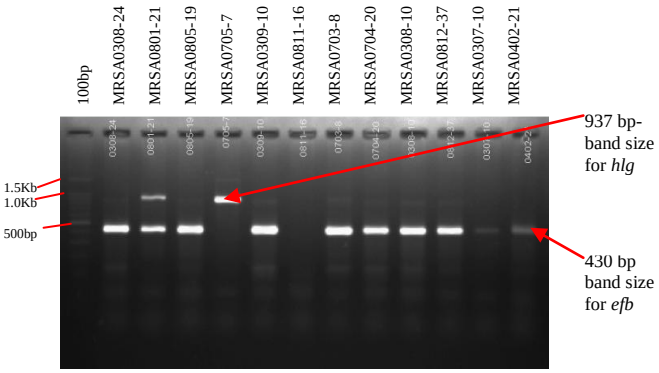
The occurrence of SEs and exfoliative genes had significantly increased between year 2003 and 2008 ($P = 0.001$). There was a significant increase in the prevalence of virulence genes, *sea* ($P = 0.003$), *sec* ($P = 0.006$) and *ica* ($P = 0.010$) in 2008 strains compared with 2003 strains. However, no significant difference in the prevalence of virulence genes in 2003 and 2008 strains for the following genes: *efb, fnbA, hlg, sdrE, seg, sei, etd* and *tst*.

sea and *sec* genes were detected in 17.5% and 6.9% MRSA strains, respectively and these strains were isolated from invasive samples (tissue, wound, blood, bone and pus). On the other hand, eight (67%) and ten (71%) of MRSA strains isolated from colonization samples (sputum and nasal swabs) were tested positive for *seg* and *sei* genes, respectively.

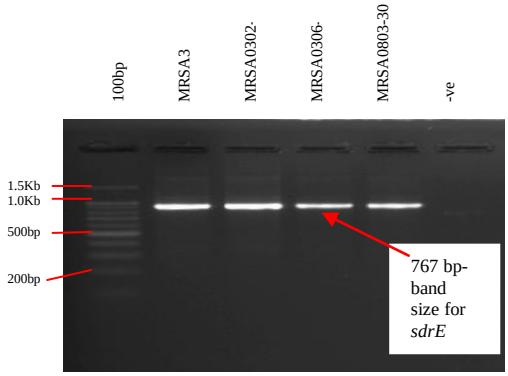
Based on Spearman's rank correlation coefficient test, correlation between intercellular adhesion (*ica*) and SEs (R = 0.046, $P < 0.05$), hemolysin (*hlg*) and SEs (R = 0.007, $P < 0.05$) were observed.


100bp
MRSA2
MRSA3
MRSA0307-1
MRSA0308-24
MRSA0408-34
MRSA0705-13
MRSA0805-15
MRSA0805-17
MRSA0810-17
MRSA0812-1
-ve
1.5Kb
1.0Kb
500bp
200bp
770 bp-band size for ica

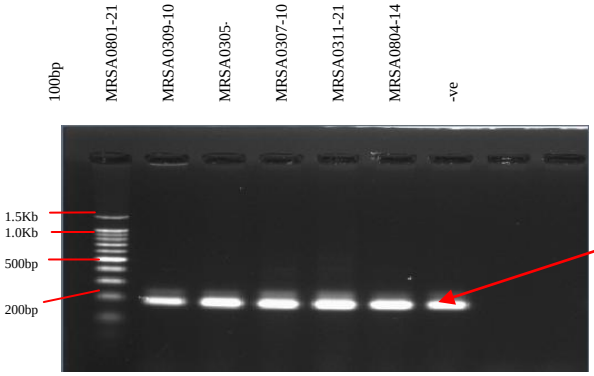
A


100bp
MRSA0308-24
MRSA0801-21
MRSA0805-19
MRSA0705-7
MRSA0309-10
MRSA0811-16
MRSA0703-8
MRSA0704-20
MRSA0308-10
MRSA0812-37
MRSA0307-10
MRSA0402-21
1.5Kb
1.0Kb
500bp
937 bp-band size for hlg
430 bp band size for efb

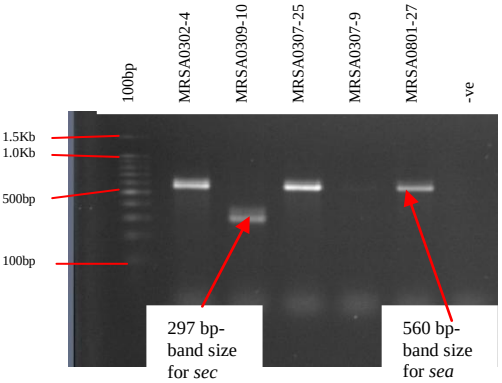
B


100bp
MRSA3
MRSA0302-
MRSA0306-
MRSA0803-30
-ve
1.5Kb
1.0Kb
500bp
200bp
767 bp-band size for sdrE

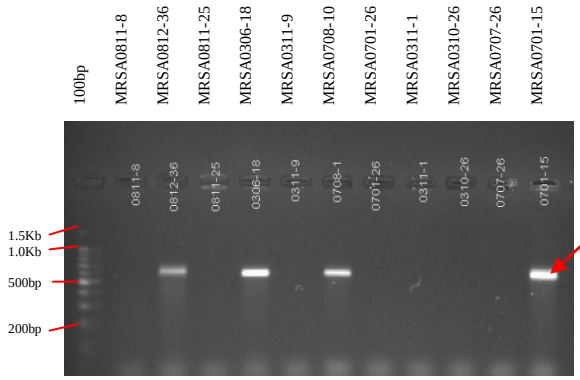
C


100bp
MRSA0801-21
MRSA0309-10
MRSA0305-
MRSA0307-10
MRSA0311-21
MRSA0804-14
-ve
1.5Kb
1.0Kb
500bp
200bp
152 bp-band size for fnbA

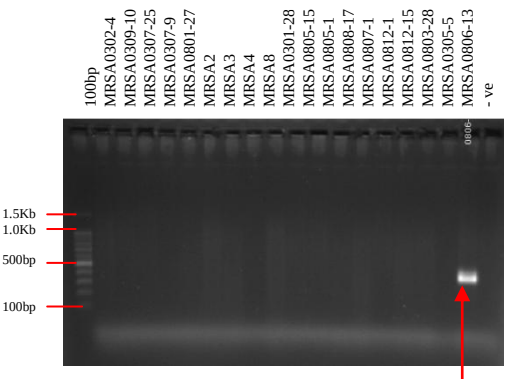
D


100bp
MRSA0302-4
MRSA0309-10
MRSA0307-25
MRSA0307-9
MRSA0801-27
-ve
1.5Kb
1.0Kb
500bp
100bp
297 bp-band size for sec
560 bp-band size for sea

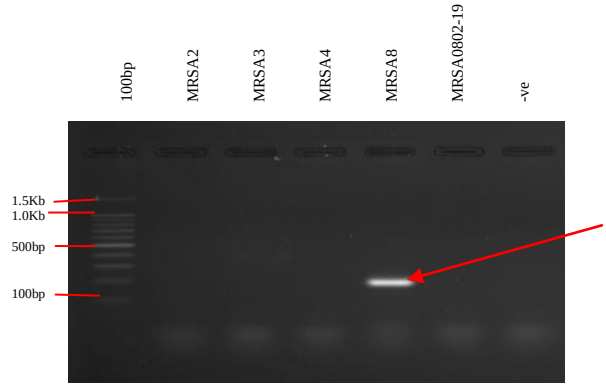
E


100bp
MRSA0811-8
MRSA0812-36
MRSA0811-25
MRSA0306-18
MRSA0311-9
MRSA0708-10
MRSA0701-26
MRSA0311-1
MRSA0310-26
MRSA0707-26
MRSA0701-15
1.5Kb
1.0Kb
500bp
200bp
642 bp-band size for seg

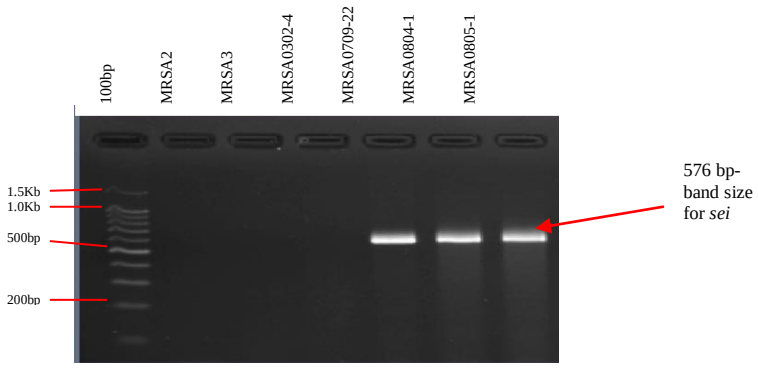
F


100bp
MRSA0302-4
MRSA0309-10
MRSA0307-25
MRSA0307-9
MRSA0801-27
MRSA2
MRSA3
MRSA4
MRSA8
MRSA0301-28
MRSA0805-15
MRSA0805-1
MRSA0808-17
MRSA0807-1
MRSA0812-1
MRSA0812-15
MRSA0803-28
MRSA0305-5
MRSA0806-13
- ve
1.5Kb
1.0Kb
500bp
100bp
376 bp-band size for etd

G


100bp
MRSA2
MRSA3
MRSA4
MRSA8
MRSA0802-19
-ve
1.5Kb
1.0Kb
500bp
100bp
180 bp-band size for tst

H



I

Figure 4.10 Representative agarose gels of PCR amplified products using primers specific for (A) *ica*, (B) *hlg* and *efb*, (C) *sdrE*, (D) *fnbA*, (E) *sea* and *sec*, (F) *seg*, (G) *etd*, (H) *tst* and (I) *sei* genes. Sterile deonised water was used as PCR control.

Table 4.7: Virulence genes detected in the 188 MRSA strains

Strain No	Virulence gene	Biofilm formation on congo red agar
MRSA2	*efb*, *fnbA*, *ica*	Black
MRSA3	*efb*, *fnbA*, *ica*, *sdrE*	Black
MRSA4	*efb*, *fnbA*	Red
MRSA8	*efb*, *fnbA*, *ica*, *hlg*	Black
MRSA0301-1	*efb*, *fnbA*	Red
MRSA0301-28	*efb*, *fnbA*, *hlg*	Red
MRSA0302-4	*efb*, *fnbA*, *ica*, *hlg*, *sdrE*, *sea*	Black
MRSA0304-16	*efb*, *fnbA*	Red
MRSA0305-10	*efb*, *fnbA*, *ica*, *hlg*	Black
MRSA0305-18	*efb*, *fnbA*, *ica*, *hlg*, *sea*	Black
MRSA0305-23	*efb*, *fnbA*, *ica*, *sec*	Black
MRSA0306-10	*efb*, *fnbA*, *hlg*, *sdrE*	Red
MRSA0306-14	*efb*, *fnbA*	Red
MRSA0306-15	*efb*, *fnbA*, *ica*, *hlg*	Black
MRSA0306-18	*efb*, *fnbA*, *ica*, *hlg*, *sea*, *seg*	Black
MRSA0306-26	*efb*, *fnbA*, *ica*, *hlg*, *sea*	Black
MRSA0306-7	*efb*, *fnbA*, *ica*, *hlg*, *sea*	Black
MRSA0307-1	*efb*, *fnbA*, *ica*, *hlg*, *sdrE*	Black
MRSA0307-10	*efb*, *fnbA*	Red
MRSA0307-14	*efb*, *fnbA*, *ica*, *hlg*, *sdrE*, *sea*	Black
MRSA0307-20	*efb*, *fnbA*	Red
MRSA0307-23	*efb*, *fnbA*, *sdrE*	Red
MRSA0307-25	*efb*, *fnbA*, *ica*, *hlg*, *sdrE*, *sea*	Black
MRSA0307-5	*efb*, *fnbA*, *ica*, *hlg*	Black
MRSA0307-9	*efb*, *fnbA*	Red
MRSA0308-1	*efb*, *fnbA*, *ica*	Black

Table 4.7 (continue)

Strain No	Virulence gene	Biofilm formation on congo red agar
MRSA0308-10	*efb, fnbA, ica, hlg*	Black
MRSA0308-22	*efb, fnbA, ica, hlg*	Black
MRSA0308-23	*efb, fnbA, ica, hlg*	Black
MRSA0308-24	*efb, fnbA, ica, sea*	Black
MRSA0308-28	*efb, fnbA, hlg*	Red
MRSA0309-10	*efb, fnbA, ica, hlg, sec*	Black
MRSA0309-11	*efb, fnbA, ica, hlg*	Black
MRSA0309-9	*efb, fnbA, sec*	Red
MRSA0310-19	*efb, fnbA, hlg, sea*	Red
MRSA0310-23	*efb, fnbA*	Red
MRSA0310-26	*efb, fnbA, sec*	Red
MRSA0310-9	*efb, fnbA, ica, hlg*	Black
MRSA0311-1	*efb, fnbA, ica, hlg, sdrE, sea*	Black
MRSA0311-21	*efb, fnbA, hlg*	Red
MRSA0311-23	*efb, fnbA, ica, hlg, sea*	Black
MRSA0311-4	*ica, sdrE*	Black
MRSA0311-7	*efb, fnbA, ica, hlg, sdrE, sea*	Black
MRSA0311-8	*efb, fnbA, ica, hlg*	Black
MRSA0311-9	*efb, fnbA, sea*	Red
MRSA0312-13	*efb, fnbA, ica, hlg, sdrE*	Black
MRSA0312-15	*efb, fnbA, sea*	Red
MRSA0312-17	*efb, fnbA, sea*	Red
MRSA0312-2	*efb, fnbA*	Red
MRSA0312-3	*efb, fnbA, ica, hlg, sdrE*	Black
MRSA0312-30	*efb, fnbA, ica, hlg*	Black
MRSA0312-35	-	Red
MRSA0401-13	*efb, fnbA, ica*	Black
MRSA0402-21	*efb, fnbA, sea*	Red
MRSA0402-8	*efb, fnbA, sea*	Red
MRSA0403-20	*efb, fnbA, ica, hlg*	Black
MRSA0405-20	*efb, fnbA, ica, hlg, sdrE, sea*	Black
MRSA0406-8	*efb, fnbA, ica*	Black
MRSA0408-33	*efb, fnbA, ica*	Black
MRSA0408-34	*efb, fnbA, ica, hlg, sdrE, sea*	Black
MRSA0409-17	*efb, fnbA*	Red
MRSA0701-15	*efb, fnbA, ica, hlg, sdrE, sea, seg*	Black
MRSA0701-26	*efb, fnbA, ica, sdrE, sea*	Black
MRSA0703-8	*efb, fnbA, ica*	Black
MRSA0704-15	*efb, fnbA, ica, hlg, sea*	Black
MRSA0704-18	*efb, fnbA, ica*	Black
MRSA0704-20	*efb, fnbA, ica, sea*	Black
MRSA0704-3	*efb, fnbA, ica, sdrE, sea*	Black
MRSA0705-13	*efb, fnbA, ica, hlg, sdrE, sea*	Black
MRSA0705-17	*ica, hlg, sdrE, sec, seg*	Black
MRSA0705-7	*ica, hlg, sdrE, seg*	Black
MRSA0705-8	*efb, fnbA, ica, sea*	Black
MRSA0707-17	*efb, fnbA, ica, hlg, sdrE, sea*	Black
MRSA0707-26	*efb, fnbA, ica, sdrE, sea*	Black

Table 4.7 (continue)

Strain No	Virulence gene	Biofilm formation on congo red agar
MRSA0708-1	*ica, hlg, sec, seg*	Black
MRSA0708-10	*ica, hlg, sdrE, sec, seg*	Black
MRSA0709-22	*ica, hlg, sdrE, sei*	Black
MRSA0801-1	*efb, fnbA, ica, hlg, sea, sec*	Black
MRSA0801-13	*efb, fnbA, sea*	Red
MRSA0801-16	*efb, fnbA, ica, hlg*	Black
MRSA0801-2	*efb, fnbA, ica*	Black
MRSA0801-21	*efb, fnbA, ica, hlg, sdrE, sec*	Black
MRSA0801-26	*efb, fnbA, ica, sdrE, sea, sec*	Black
MRSA0801-27	*efb, fnbA, ica, hlg, sea, sec*	Black
MRSA0801-30	*efb, fnbA, ica, hlg*	Black
MRSA0801-4	*efb, fnbA, ica, hlg*	Black
MRSA0801-9	*efb, fnbA, ica, sdrE*	Black
MRSA0802-14	*efb, fnbA, ica*	Black
MRSA0802-19	*efb, fnbA, ica, sdrE, tst*	Black
MRSA0802-2	*efb, fnbA, ica, hlg*	Black
MRSA0802-3	*efb, fnbA, ica, hlg*	Black
MRSA0803-28	*efb, fnbA, ica, sec, sei*	Black
MRSA0803-29	*efb, fnbA, ica*	Black
MRSA0803-30	*efb, fnbA, ica, hlg, sdrE*	Black
MRSA003-35	*efb, fnbA, ica, hlg*	Black
MRSA0804-1	*efb, fnbA, ica, hlg, sdrE, sec, sei*	Black
MRSA0804-14	*efb, fnbA, ica, sea*	Black
MRSA0804-20	*efb, fnbA, ica, hlg, sea, sec*	Black
MRSA0804-24	*efb, fnbA, ica, hlg, sea*	Black
MRSA0805-1	*efb, fnbA, ica, hlg, sdrE, sei*	Black
MRSA0805-10	*efb, fnbA, hlg, sec, seg, sei*	Red
MRSA0805-11	*efb, fnbA, ica, hlg, sdrE*	Black
MRSA0805-15	*efb, fnbA, ica*	Black
MRSA0805-17	*efb, fnbA, ica, hlg*	Black
MRSA0805-19	*efb, fnbA, ica, sea*	Black
MRSA0805-20	*efb, fnbA, ica, hlg, sdrE, sec*	Black
MRSA0805-21	*efb, fnbA, ica, hlg*	Black
MRSA0805-22	*efb, fnbA*	Red
MRSA0805-23	*efb, fnbA, ica, hlg*	Black
MRSA0805-24	*efb, fnbA, ica*	Black
MRSA0805-3	*efb, fnbA, ica, hlg*	Black
MRSA0805-4	*efb, fnbA, sea, sec*	Red
MRSA0805-5	*efb, fnbA, ica, sea*	Black
MRSA0805-6	*efb, fnbA, ica, hlg*	Black
MRSA0805-9	*efb, fnbA, ica, hlg, sea, sec, sei*	Black
MRSA0806-1	*efb, fnbA, ica, hlg, sdrE, sea, sec*	Black
MRSA0806-11	*efb, fnbA, ica, hlg, sdrE, sea, sec*	Black
MRSA0806-13	*efb, fnbA, ica, hlg, sdrE, etd*	Black
MRSA0806-14	*efb, fnbA, ica, hlg, sdrE, seg*	Black
MRSA0806-18	*efb, fnbA, ica, hlg*	Black
MRSA0806-21	*efb, fnbA, ica, hlg, seg*	Black
MRSA0806-22	*efb, fnbA, ica, hlg*	Black

Table 4.7 (continue)

Strain No	Virulence gene	Biofilm formation on congo red agar
MRSA0806-26	*efb, fnbA,hlg*	Red
MRSA0806-33	*efb, fnbA, ica, hlg, sea*	Black
MRSA0807-1	*efb, fnbA, ica, hlg, sea*	Black
MRSA0807-13	*efb, fnbA, ica*	Black
MRSA0807-14	*efb, fnbA, sdrE, sea*	Red
MRSA0807-19	*efb, fnbA, ica, sei*	Black
MRSA0807-7	*efb, fnbA, ica, hlg, sdrE, sea*	Black
MRSA0807-8	*efb, fnbA, ica, hlg, sea*	Black
MRSA0808-17	*efb, fnbA, ica, hlg, sea, sei*	Black
MRSA0808-19	*ica, sea*	Black
MRSA0808-21	*efb, fnbA, hlg, sea*	Red
MRSA0808-24	*efb, fnbA, ica, sdrE, sea*	Black
MRSA0808-25	*efb, fnbA, ica, sdrE*	Black
MRSA0808-26	*efb, fnbA, ica, hlg, sea*	Black
MRSA0808-35	*efb, fnbA, ica, hlg, sea, sec*	Black
MRSA0809-1	*efb, fnbA, ica, sea*	Black
MRSA0809-10	*efb, fnbA, ica, hlg*	Black
MRSA0809-14	*efb, fnbA*	Red
MRSA0809-15	*efb, fnbA, hlg, sea*	Red
MRSA0809-24	*efb, fnbA, ica, sea, sec*	Black
MRSA0809-25	*efb, fnbA, ica, hlg*	Black
MRSA0809-27	*efb, fnbA, hlg*	Red
MRSA0809-30	*efb, fnbA, ica, hlg*	Black
MRSA0809-32	*efb, fnbA, hlg*	Red
MRSA0809-33	*efb, fnbA, ica, hlg, sea*	Black
MRSA0809-36	*efb, fnbA, sea*	Red
MRSA0809-38	*efb, fnbA, ica, hlg, sea*	Black
MRSA0810-10	*efb, fnbA, ica, hlg, sec, sei*	Black
MRSA0810-13	*efb, fnbA, ica, sea*	Black
MRSA0810-15	*efb, fnbA, ica, hlg, sea*	Black
MRSA0810-16	*efb, fnbA, ica, sdrE, sec*	Black
MRSA0810-17	*efb, fnbA, ica, hlg, sec, sei*	Black
MRSA0810-18	*efb, fnbA, ica, hlg*	Black
MRSA0810-2	*efb, fnbA, sea, sei*	Red
MRSA0810-22	*efb, fnbA, ica, hlg, sec, sei*	Black
MRSA0810-23	*efb, fnbA, ica, hlg, sea, sec*	Black
MRSA0810-6	*efb, fnbA, sec*	Red
MRSA0810-7	*efb, fnbA, ica, sea*	Black
MRSA0810-9	*efb, fnbA, ica, hlg, sdrE*	Black
MRSA0811-10	*efb, fnbA, ica*	Black
MRSA0811-11	*efb, fnbA, ica, hlg, sea, sec*	Black
MRSA0811-13	*efb, fnbA, ica, hlg*	Black
MRSA0811-16	*efb, fnbA, sec, seg*	Red
MRSA0811-2	*efb, fnbA, ica, hlg*	Black
MRSA0811-22	*efb, fnbA, ica, hlg, sec, sei*	Black
MRSA0811-24	*efb, fnbA, ica, hlg, sdrE, sea*	Black
MRSA0811-25	*efb, fnbA, ica, sea, sec*	Black
MRSA0811-26	*efb, fnbA, ica, hlg*	Black

Table 4.7 (continue)

Strain No	Virulence gene	Biofilm formation on congo red agar
MRSA0811-28	*efb, fnbA, ica, hlg*	Black
MRSA0811-30	*efb, fnbA, ica, sdrE, seg*	Black
MRSA0811-5	*efb, fnbA*	Red
MRSA0811-8	*efb, fnbA, ica, hlg, sdrE, sea*	Black
MRSA0812-1	*efb, fnbA, ica, hlg*	Black
MRSA0812-11	*efb, fnbA, ica, hlg, sdrE, sea*	Black
MRSA0812-15	*efb, fnbA, ica, sea*	Black
MRSA0812-17	*efb, fnbA, ica, sdrE*	Black
MRSA0812-2	*efb, fnbA, ica, hlg*	Black
MRSA0812-22	*efb, fnbA, ica, hlg, sdrE*	Black
MRSA0812-23	*efb, fnbA, ica, sdrE, sec, sei*	Black
MRSA0812-27	*efb, fnbA, sea, sec*	Red
MRSA0812-30	*efb, fnbA, ica*	Black
MRSA0812-31	*efb, fnbA, hlg, sdrE*	Red
MRSA0812-33	*efb, fnbA, ica*	Black
MRSA0812-35	*efb, fnbA*	Red
MRSA0812-36	*efb, fnbA, sec, seg*	Red
MRSA0812-37	*efb, fnbA, ica, sea, sec*	Black

Table 4.8: Prevalence of genes encoding virulence determinants in Malaysian MRSA strains in 2003, 2004, 2007 and 2008

Gene	No of strains		*P* value	Gene combination	No of strains	
	2003 to 2004	2007 to 2008			2003 to 2004	2007 to 2008
sea	19	54	0.152	*sec + seg + sei*		1
sec	4	32	0.003	*sea + sec + sei*		1
seg	1	11	0.107	*sea + sec*		14
sei	0	14	0.006	*sec + sei*		7
etd	0	1	1.000	*sec + seg*		5
tst	0	1	1.000	*sea + sei*		2
efb	59	121	1.000	*sea + seg*	1	1
fnbA	59	121	1.000	*sea*	18	36
hlg	34	75	0.753	*sec*	3	4
ica	39	104	0.010	*seg*		4
sdrE	14	37	0.484	*sei*		3
				etd		1
				tst		1
				Total	22	80

4.9 Prevalence of *pvl* gene in MRSA strains

PCR amplification was carried out on genomic DNA isolated from the 188 MRSA strains to determine if *pvl* gene was present among the strains. *pvl* gene was detected from five MRSA strains (2 from year 2007 and 3 from year 2008) (Figure 4.11; Appendix 4.8) and these strains were isolated from three invasive samples (2 wound and 1 tissue) and two colonization samples (1 tracheal secretion and 1 nasal swabs). There was no significant difference in the prevalence of *pvl* gene in 2003 and 2008 strains ($P > 0.05$).

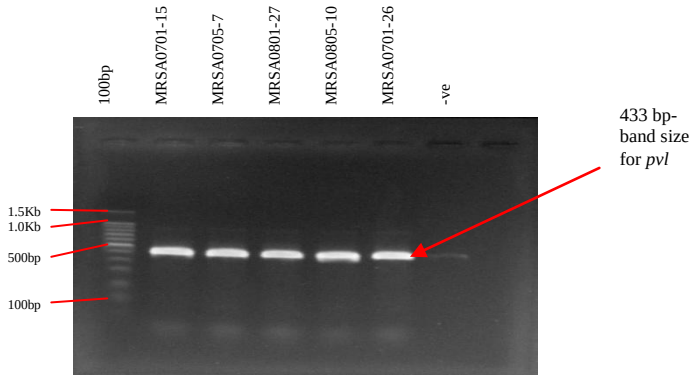


Figure 4.11 Representative agarose gel of PCR amplified product using primers specific for *pvl* gene. Sterile deionised water water used as PCR control.

4.10 *agr* grouping of MRSA strains

Three *agr* genotypes were observed among the MRSA strains: *agr* type I (97%; 51 strains from 2003, 9 from 2004, 15 from 2007 and 108 from 2008), *agr* type II (1.6%; 1 from 2007 and 2 from 2008) and *agr* type III (0.5%; 1 from year 2008) (Figure 4.12; Table 4.10; Appendix 49-51). No *agr* type IV was observed. One strain (MRSA0312-35) did not belong to any *agr* group. Moreover, this MRSA0312-35 also did not harbour any virulence gene.

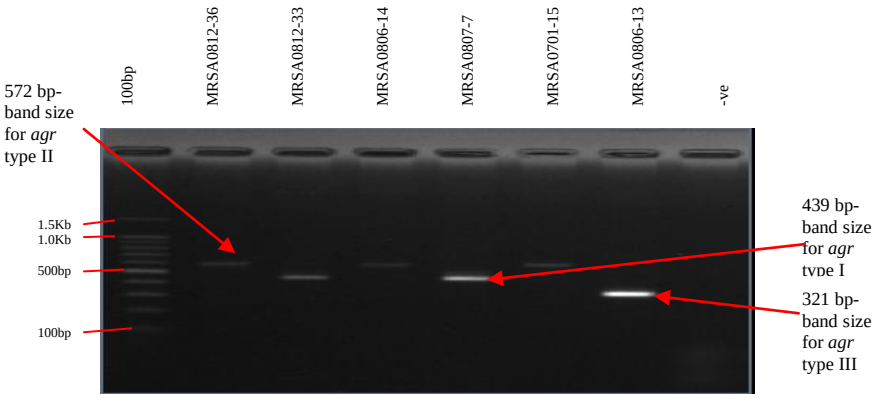


Figure 4.12 Representative agarose gel of PCR amplified products using primers specific for *agr* grouping for MRSA strains. Sterile deonised water was used as PCR control.

4.11 SCC*mec* types

Three SCC*mec* types were observed: SCC*mec* type III (86%, n=162), SCC*mec* type IV (13%, n=25) and SCC*mec* type V (1%, n=1) (Figure 4.13; Table 4.10). Thirteen SCC*mec* type IV isolates were further subtyped as SCC*mec* type IVa (Figure 4.14). The subsequent 12 SCC*mec* type IV isolates could not be furthered subgrouped using the available primers used in this study.

SCC*mec* type III was observed among MRSA strains isolated from year 2003, 2004, 2007 and 2008 while SCC*mec* type IV was observed among MRSA strains isolated from year 2007 to 2008. The only SCC*mec* type V MRSA strain was isolated in year 2008.

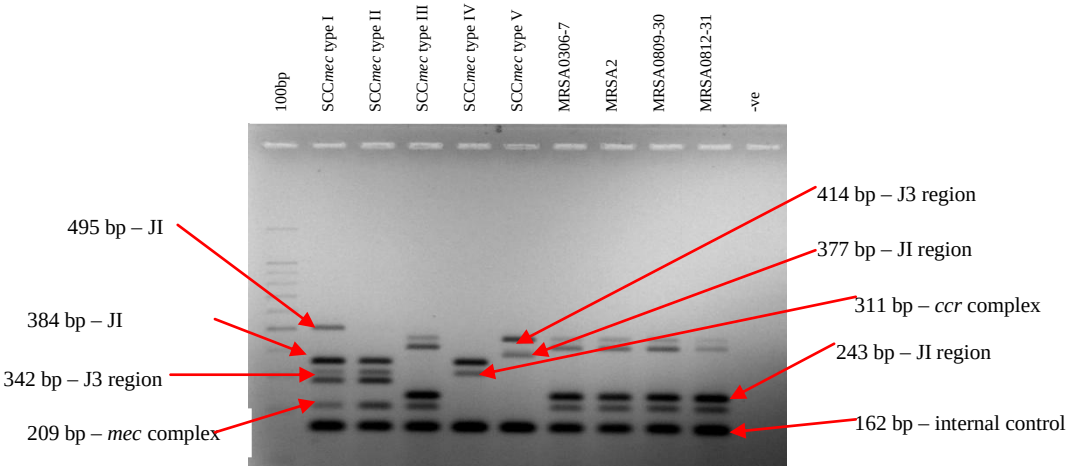


Figure 4.13 Representative agarose gel of PCR-amplified products for SCC*mec* typing. Sterile deionised water was used as PCR control.

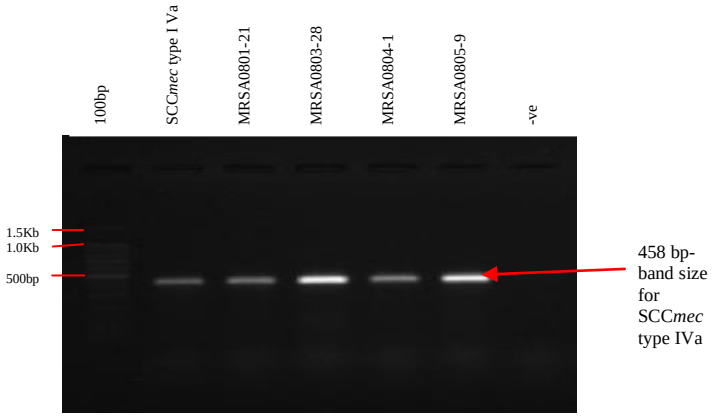


Figure 4.14 Representative agarose gel of PCR-amplified products for SCC*mec* IVa. JCSC4744 was used as positive control while sterile deionised water was used as PCR control.

4.12 Genomic Diversity of *S. aureus* strains

4.12.1 Genotypes of MRSA based on PCR-RFLP of *coa* gene

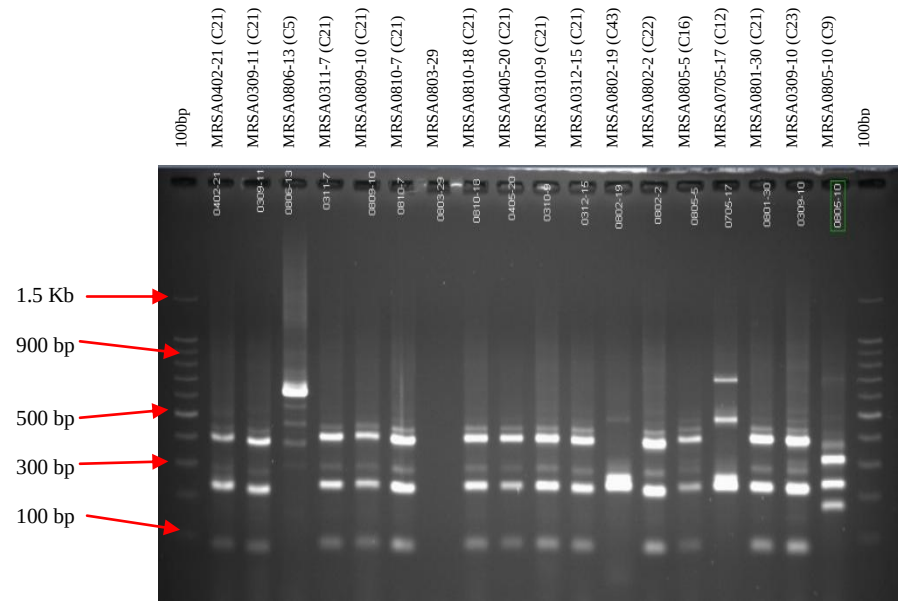
Digestion of *coa* positive PCR amplicons with *Alu*I enzyme yielded 47 different restriction profiles (F = 0.24 - 1.0) (Figure 4.15 – 4.16; Table 4.10). Four strains (MRSA0312-35, MRSA0803-29, MRSA0707-26 and MRSA0802-14) could not be typed by *coa*-RFLP typing despite, repeated attempts. Seventy two MRSA strains with *coa*-RFLP profiles C12 to C14 were found to be clonally related as they shared more than 80% similarity. Among them, 37 strains shared identical *coa*-RFLP profiles (*coa*-RFLP profile C12), even though they were cultured from different occasions and sources (Figure 4.16). MRSA strains with *coa*-RFLP profile C12 were cultured from both invasive (n=17; including bone, tissue, blood, pus) and colonization samples (n=20; including nasal swabs, tracheal secretions; nasopharyngeal secretion; sputum) samples obtained from different patients wards.

Ten MRSA strains (MRSA0309-11, MRSA0310-9, MRSA0311-7, MRSA0312-15, MRSA0402-21, MRSA0405-20, MRSA0801-30, MRSA0809-10, MRSA0810-18 and MRSA0810-7) which were isolated from three different years (year 2003, 2004 and 2008) shared similar *coa*-RFLP profiles, C21.

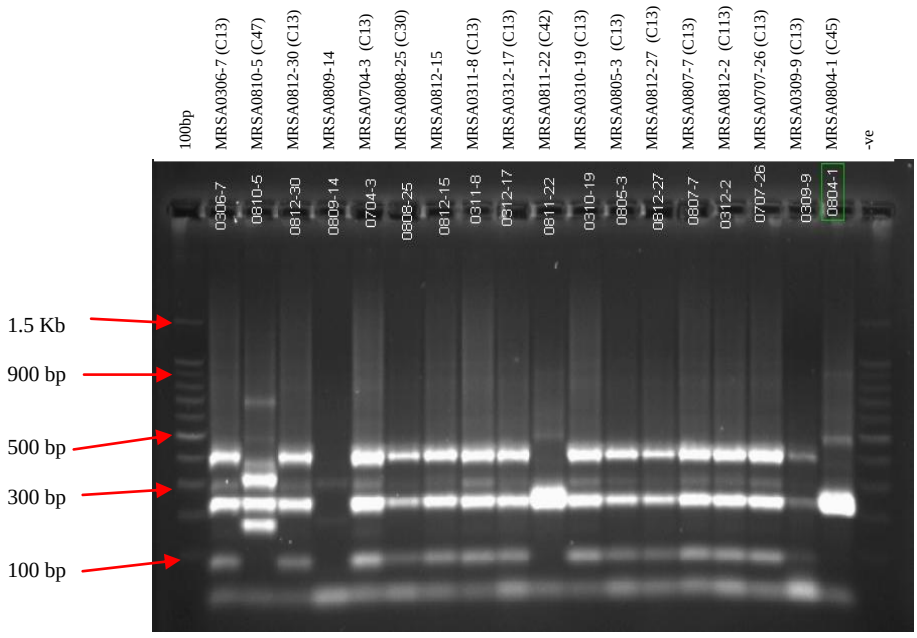
Two MRSA strains (MRSA2 and MRSA8) obtained from two staff nurses shared identical *coa*-RFLP profiles (C13) with 31 clinical MRSA strains, although they were cultured from different sources (tracheal secretions, slough, pus, blood, wound swab, urine, tip, sputum) and time period (year 2003, 2004, 2007 and 2008).

One MRSA strain, MRSA0805-10 which harboured three enterotoxin genes (*sec*, *seg* and *sei*) was found to be clonally related to four other strains, MRSA0704-15, MRSA0705-8, MRSA0807-13 and MRSA0811-25 (*coa*-RFLP profile, C8) as they shared 90.9% similarity in their *coa*-RFLP profiles (Figure 4.16). Three out of four MRSA strains with C8, *coa*-RFLP profiles (MRSA0704-15, MRSA0705-8 and

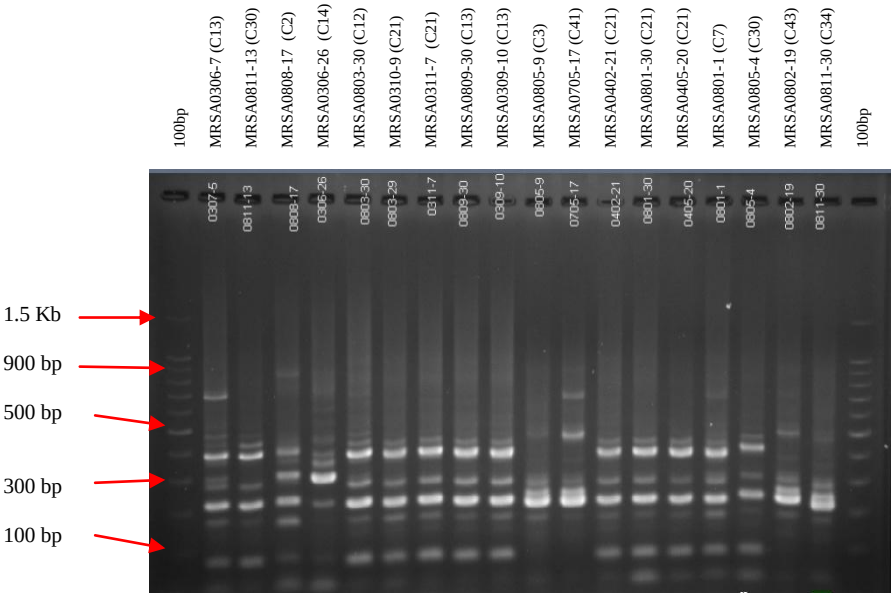
MRSA0811-25) harboured *sea* enterotoxin genes even though they were cultured from different sources (nasal swabs, blood and tips) and occasion (year 2007 and 2008).



A



B



C

Figure 4.15 Three representative agarose gels of PCR-RFLP of *coa* gene for MRSA strains. PCR-RFLP profiles are indicates in the brackets.

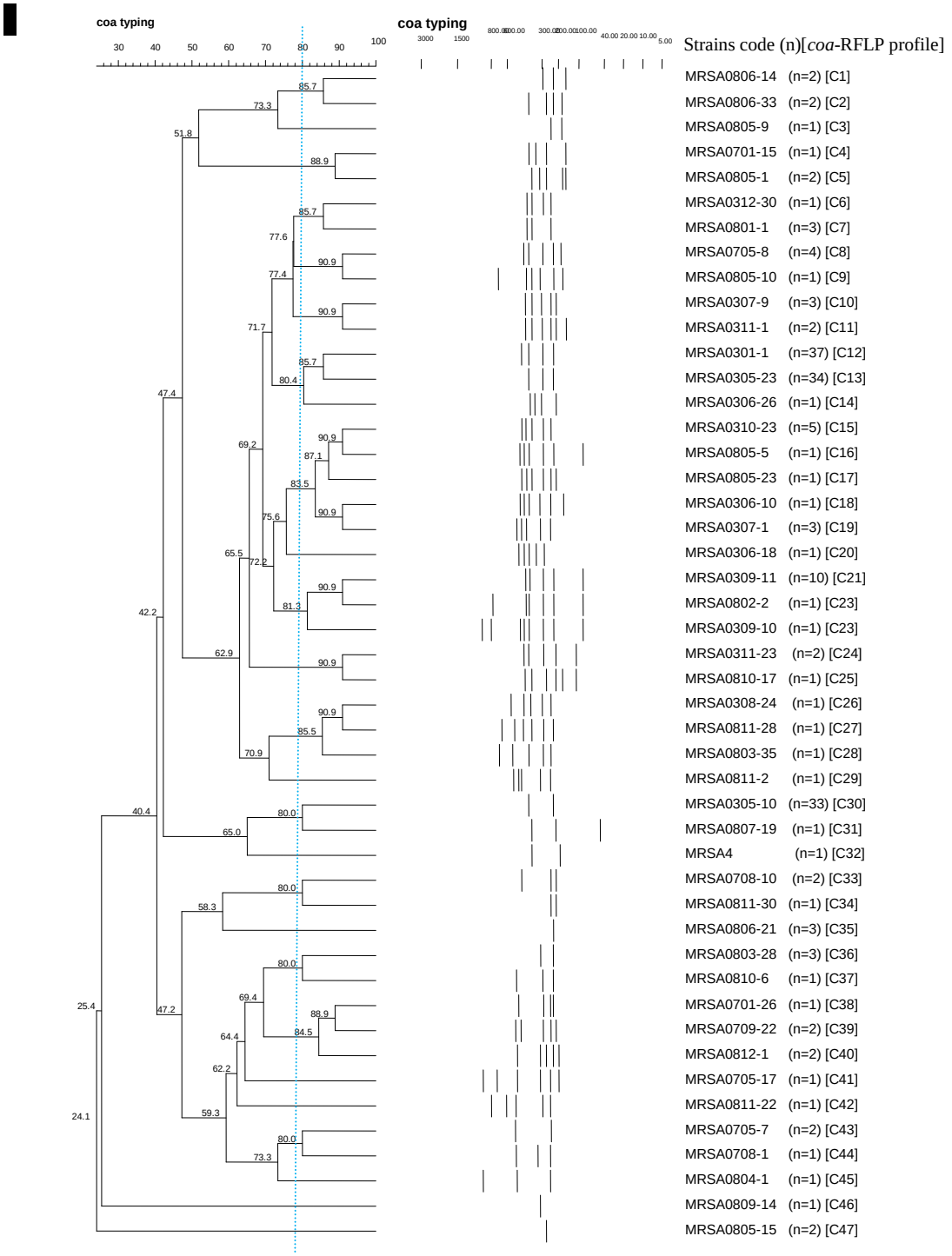


Figure 4.16 Dendrogram of PCR-RFLP of *coa* gene of MRSA strains. The dotted vertical line indicates 80% similarity level.

4.12.2 Genotypes of MRSA based on Pulsed-field Gel Electrophoresis (PFGE)

Genomic DNA from the 188 MRSA strains were digested with *SmaI* and this resulted in 85 distinct pulsed-field profiles (PFPs; F = 0.57-1.0) comprising of 10 – 18 restriction fragments that ranged in size from approximately 23 Kb to 755 Kb (Figure 4.17 and Table 4.10).

Based on 80% similarity, nine clusters were observed, namely Cluster 1 to Cluster 9. Cluster 1 consists of 48 MRSA strains; Cluster 2 consists of 11 strains; Cluster 3 consists of 46 strains; Cluster 4 consists of 30 strains; Cluster 5, 6 and 9 consists of 5 strains; and Cluster 7 and 8 consists of 9 strains. Twenty MRSA strains were not grouped under any clusters (Figure 4.18).

MRSA strains within Clusters 1, 5 and 7 were isolated from three different years including year 2003, 2007 and 2008 while MRSA strains from Cluster 3 and 4 were isolated from year 2003, 2004 and 2008. Cluster 6 consists of MRSA strains from year 2003 and 2008 whereas Cluster 8 consists of MRSA strains isolated from year 2007 to 2008. Finally, Cluster 2 consists of MRSA strains isolated from year 2003, 2004, 2007 and 2008 while Cluster 9 consists of MRSA strains from year 2008. All the MRSA strains from Cluster 1 to Cluster 7 shared a common band with size of 28 kB.

Thirty-six MRSA from year 2003, 2007 and 2008 shared similar PFGE profile; SM7 even though they were cultured from different sources such as nasal swabs, tissues, urine, wound, tracheal secretions, chest tube, sputum, tips, nasopharyngeal secretions and pus. Interestingly, the only *coa*-negative MRSA strain (MRSA0312-35) was also found to exhibit the same PFGE profile; SM7.

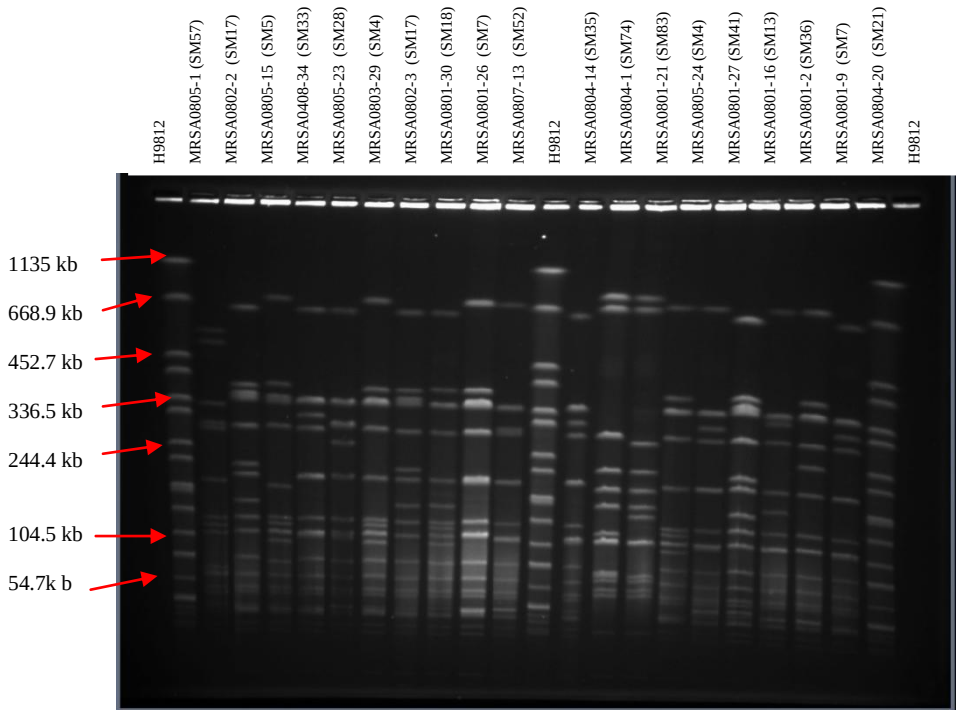
Furthermore, some MRSA strains with PFGE profile SM7 were also found to harbour different types of virulence genes with five harboured *sea* and *sec* enterotoxin genes together whereas one strain harboured *sea* and *pvl* toxin's genes. Eight other strains with PFGE profile; SM7 harboured *sea* gene whereas two harboured *sec* gene.

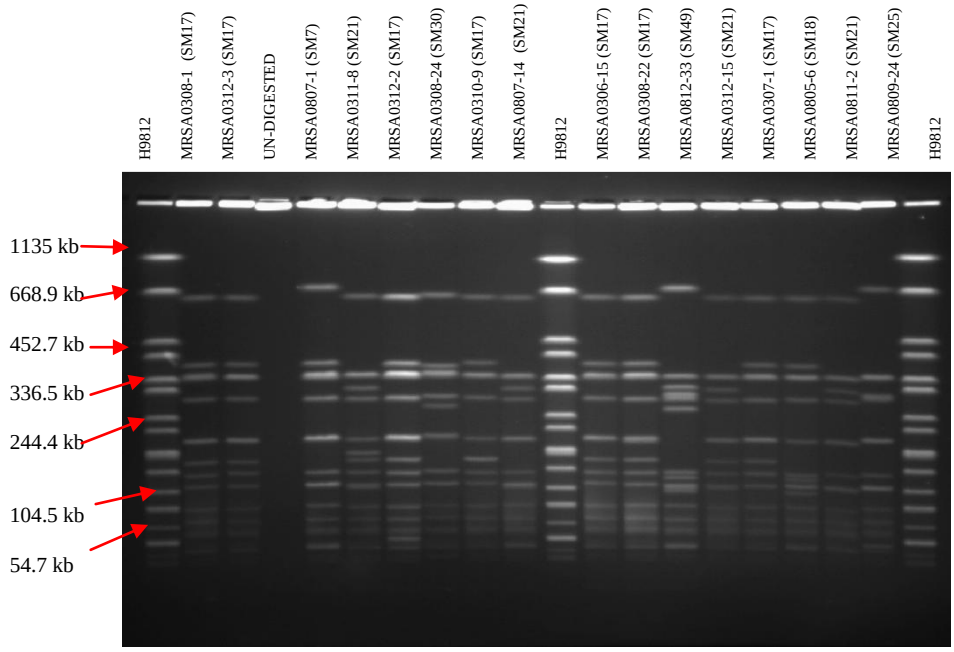
The remaining 20 MRSA strains with this similar PFGE profile; SM7 did not harbour any virulence gene. All MRSA strains with PFGE profile; SM7 was resistant to erythromycin and oxacillin. Except for MRSA0301-28 and MRSA0805-22 which of SCC*mec* type IV, all the others 34 MRSA strains with PFGE profile SM7 belong to SCC*mec* type III.

On the other hand, 21 MRSA strains (MRSA0306-15, MRSA0306-7, MRSA0307-1, MRSA0307-14, MRSA0307-20, MRSA0307-23, MRSA0308-1, MRSA0308-22, MRSA0309-11, MRSA0310-9, MRSA0311-23, MRSA0311-7, MRSA0311-9, MRSA0312-2, MRSA0312-3. MRSA0802-2, MRSA0802-3, MRSA0809-10, MRSA0809-27, MRSA0810-18 and MRSA0810-2) were indistinguishable by PFGE, although they were isolated from year 2003 and 2008. Similar to PFGE profile SM7, these 21 MRSA strains with PFGE profile SM17 were also being cultured from different sources such as pus, wound swabs, tracheal secretions, blood, tissue, slough and nasal swab).

On the other hand, 15 out of 25 SCC*mec* type IV strains with PFGE profiles SM73 to SM78 and SM80 to SM85 were grouped together, and they shared 73.4% similarity. Among them, eight MRSA strains with PFGE profiles SM73 to SM78 were found to be clonally related to one SCC*mec* type III strain (MRSA0802-19).

The MRSA strain (MRSA0805-10) which harboured three enterotoxin genes (*sec*, *seg* and *sei*) shared the same pulsed-field profiles, SM21 with 16 MRSA strains isolated from year 2003 and 2008. These 17 MRSA strains were also being isolated from different sources including nasal swabs, pus, blood, tracheal secretions, wound swabs, urine and sputum.


H9812
MRSA0805-1 (SM57)
MRSA0802-2 (SM17)
MRSA0805-15 (SM5)
MRSA0408-34 (SM33)
MRSA0805-23 (SM28)
MRSA0803-29 (SM4)
MRSA0802-3 (SM17)
MRSA0801-30 (SM18)
MRSA0801-26 (SM7)
MRSA0807-13 (SM52)
H9812
MRSA0804-14 (SM35)
MRSA0804-1 (SM74)
MRSA0801-21 (SM83)
MRSA0805-24 (SM4)
MRSA0801-27 (SM41)
MRSA0801-16 (SM13)
MRSA0801-2 (SM36)
MRSA0801-9 (SM7)
MRSA0804-20 (SM21)
H9812
1135 kb
668.9 kb
452.7 kb
336.5 kb
244.4 kb
104.5 kb
54.7k b


H9812
MRSA0308-1 (SM17)
MRSA0312-3 (SM17)
UN-DIGESTED
MRSA0807-1 (SM7)
MRSA0311-8 (SM21)
MRSA0312-2 (SM17)
MRSA0308-24 (SM30)
MRSA0310-9 (SM17)
MRSA0807-14 (SM21)
H9812
MRSA0306-15 (SM17)
MRSA0308-22 (SM17)
MRSA0812-33 (SM49)
MRSA0312-15 (SM21)
MRSA0307-1 (SM17)
MRSA0805-6 (SM18)
MRSA0811-2 (SM21)
MRSA0809-24 (SM25)
H9812
1135 kb
668.9 kb
452.7 kb
336.5 kb
244.4 kb
104.5 kb
54.7 kb

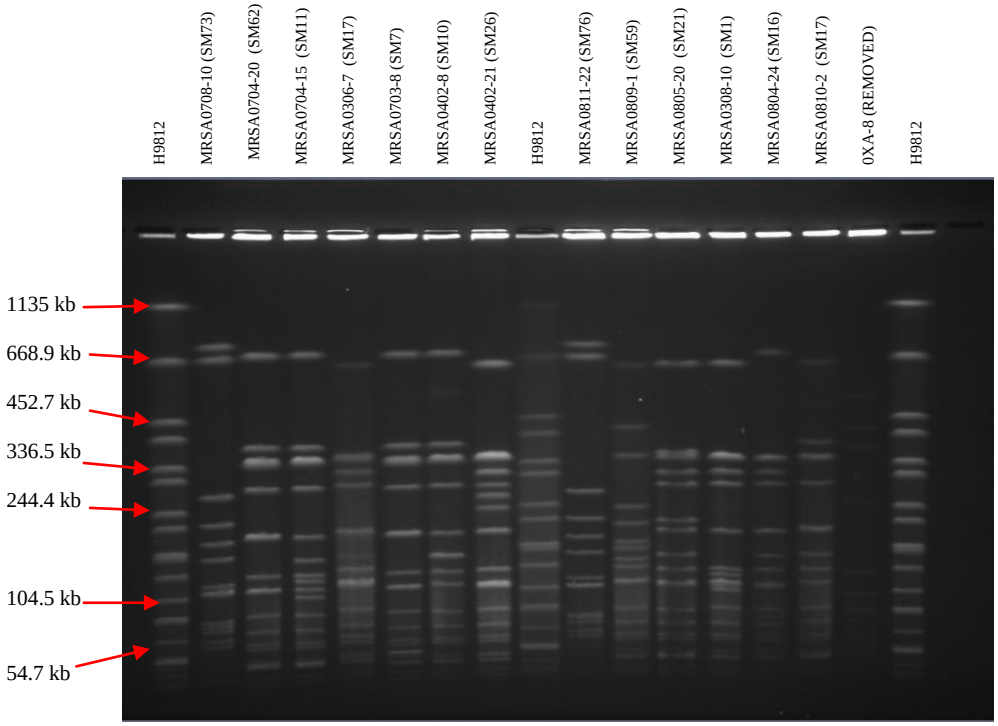
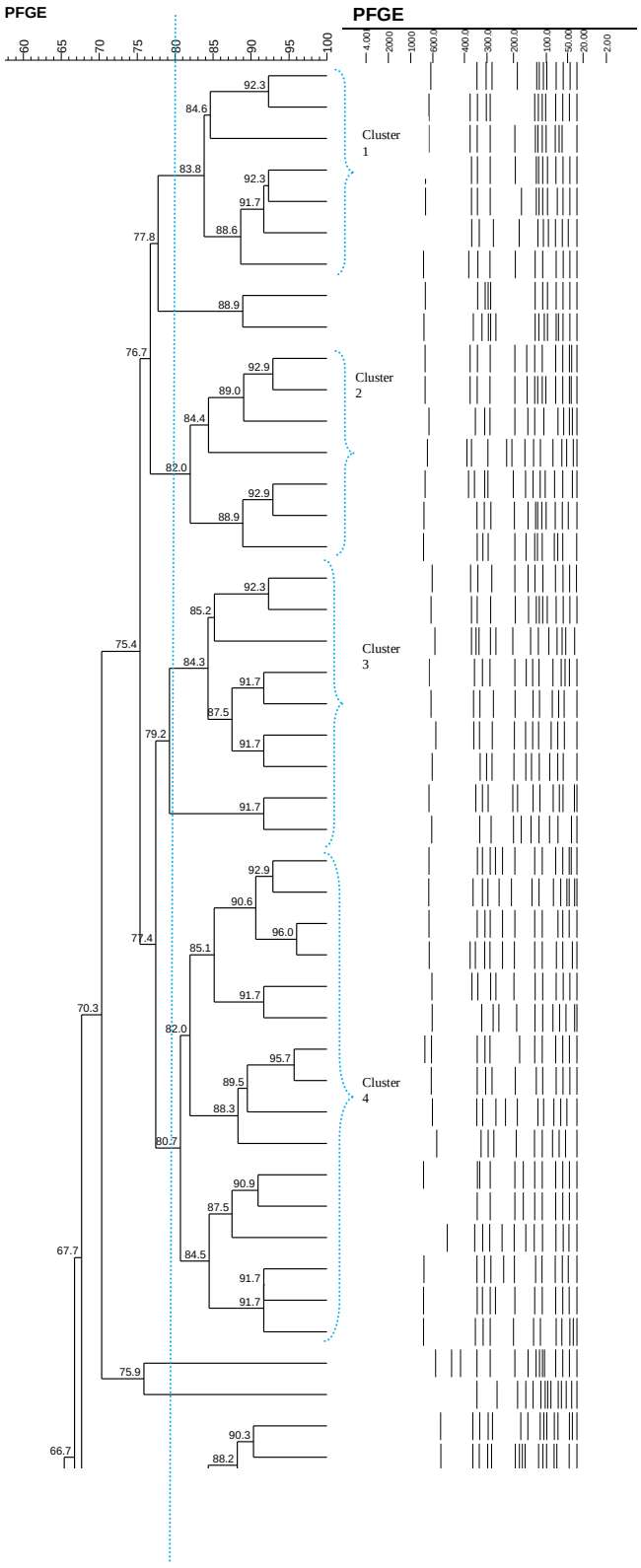


Figure 4.17 Representative pulsed-field gels of *Sma*I-digested genomic DNA from MRSA strains. PFGE profiles are indicates in brackets. *Salmonella* Braenderup H9812 was used as DNA marker.



Strains code (n)[PFGE profile]

MRSA0308-10 (n=3) [SM1]
MRSA0805-17 (n=1) [SM2]
MRSA0803-30 (n=1) [SM3]
MRSA0803-29 (n=3) [SM4]
MRSA0805-15 (n=1) [SM5]
MRSA0305-23 (n=1) [SM6]
MRSA0805-22 (n=36)[SM7]
MRSA0310-23 (n=1) [SM8]
MRSA0811-25 (n=1) [SM9]
MRSA0402-8 (n=1) [SM10]
MRSA0704-15 (n=1) [SM11]
MRSA0403-20 (n=1) [SM12]
MRSA0801-16 (n=2) [SM13]
MRSA0306-10 (n=1) [SM14]
MRSA0805-5 (n=2) [SM15]
MRSA0804-24 (n=3) [SM16]
MRSA0309-11 (n=21)[SM17]
MRSA0801-30 (n=2) [SM18]
MRSA0312-30 (n=1) [SM19]
MRSA0405-20 (n=1) [SM20]
MRSA0805-10 (n=17) [SM21]
MRSA0306-14 (n=1) [SM22]
MRSA0306-18 (n=1) [SM23]
MRSA0801-1 (n=1) [SM24]
MRSA0809-24 (n=1) [SM25]
MRSA0402-21 (n=1) [SM26]
MRSA0409-17 (n=1) [SM27]
MRSA0309-9 (n=2) [SM28]
MRSA0401-13 (n=1) [SM29]
MRSA0308-24 (n=2) [SM30]
MRSA0811-13 (n=1) [SM31]
MRSA0308-23 (n=1) [SM32]
MRSA0408-34 (n=1) [SM33]
MRSA0809-15 (n=1) [SM34]
MRSA0804-14 (n=1) [SM35]
MRSA0801-4 (n=10) [SM36]
MRSA0806-18 (n=1) [SM37]
MRSA0307-5 (n=1) [SM38]
MRSA0801-13 (n=1) [SM39]
MRSA0809-30 (n=4) [SM40]
MRSA0801-27 (n=1) [SM41]
MRSA0311-21 (n=1) [SM42]
MRSA0809-14 (n=1) [SM43]
MRSA0707-17 (n=1) [SM44]
MRSA0812-11 (n=1) [SM45]

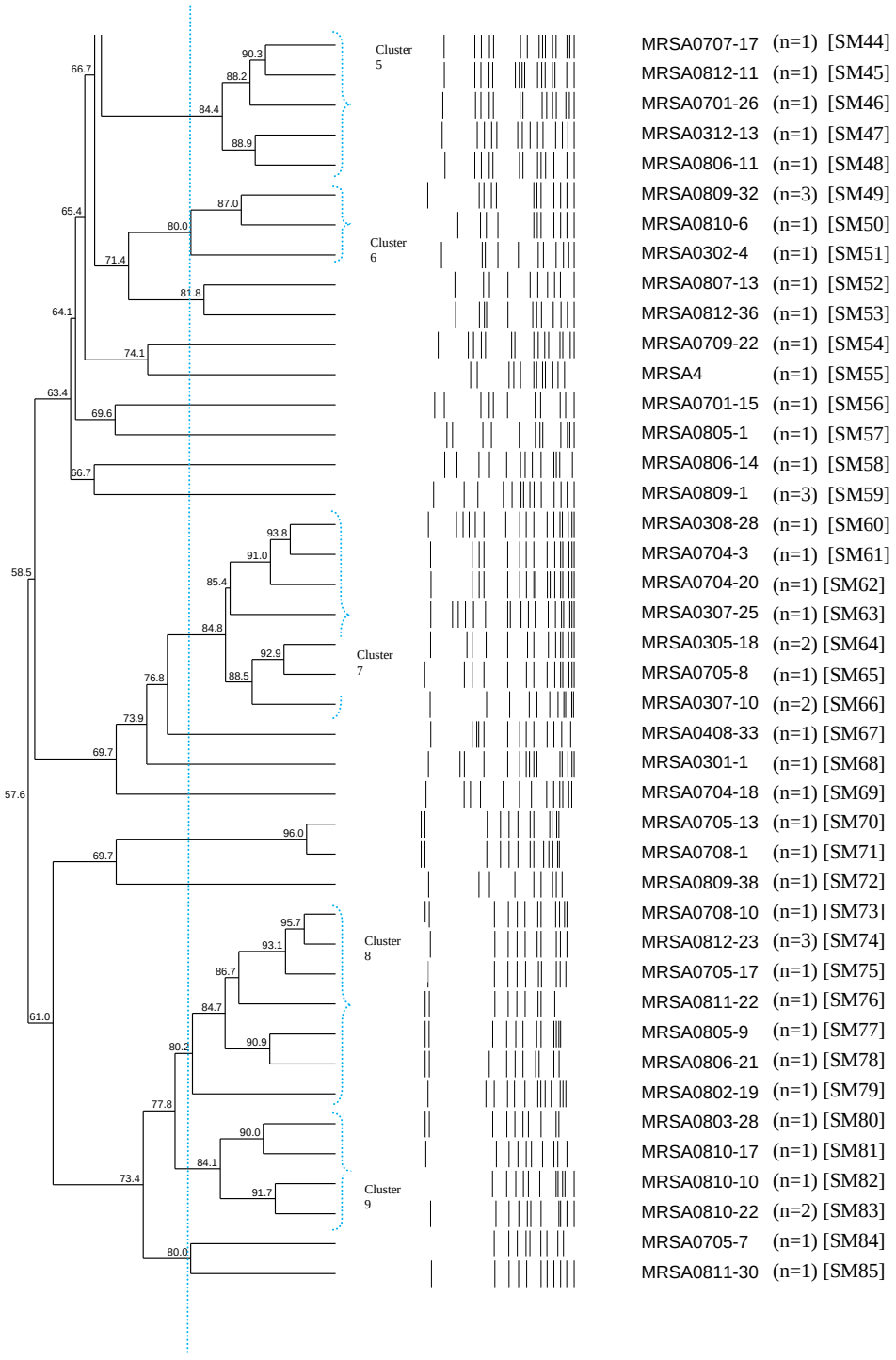


Figure 4.18 Dendrogram of PFGE of MRSA strains. The dotted vertical line indicates 80% similarity level.

4.12.3 Genotypes of MRSA based on *spa* types

PCR amplification of *spa* gene using primers 1095F and 1517R gave amplicons that ranged from 300 bp to 600 bp (Figure 4.19). Sequence analysis of *spa* amplicons from 188 MRSA strains gave 17 distinct *spa* types. One novel *spa* type (t6405) was detected, and deposited to ridom *spa* server (http://www.spaserver.ridom.de/) (Appendix 52). This t6405 *spa* type was only present among MRSA strains from year 2008. The most frequent *spa* type was t037 (66.50%). Repeats among other *spa* types varied from one strain (t002, t363, t458, t657, t860, t1544, t2029, t4150 and t4152), two strains (t1107), three strains (t1378), four strains (t304, t4184 and t6405), 12 strains (t032) and finally to 25 strains (t421) (Table 4.10 – 4.11). The discriminatory power of *spa* typing was 0.53.

Three *spa* types t037 (n=50), t421 (n=1) and t1544 (n=1) were present among strains isolated in year 2003 while five *spa* types (t304, t4184, t002, t860 and t032) were introduced in year 2007 and another nine *spa* types (t1378, t6405, t4150, t1107, t458, t4152, t657, t2029 and t363) emerged in year 2008.

The minimum spanning tree shows two *spa* clonal complexes (*spa*CC) arbitrarily named *spa*CC1 and *spa*CC2 (Figure 4.20). Strains which shared more than 98.5% similarity were grouped in the same *spa* clonal complex (*spa*CC). *spa*CC1 comprised of 156 strains from five *spa* types (t037, t6405, t421, t363 and t2029) (Figure 4.20). Among them, 56% (88/156) were from year 2008. *spa*CC2 contains 19 strains from three *spa* types (t4184, t1378 and t032). The other 9 *spa* types (t860, t4150, t1544, t458, t1107, t002, t657, t304 and t4152) were not grouped in any *spa* clonal complexes as they only shared 87.1% similarity.

All MRSA within s*pa*CC2 belongs to SCC*mec* type IV. Similarly, strains from *spa* type t002, t1107 and t304 were also from SCC*me*c type IV. Among them, *spa* type

t002 and t1107 were sharing 95.5% similarity All SCC*mec* type IV strains with *spa* types t4184, t1378, t032, t002, t1107 and t304 only shared 92.2% similarity.

On the other hand, most of the MRSA strains with same *spa* types were often cultured from different types of samples. For examples, the MRSA strains with *spa* type t421 were isolated from nasal swabs, blood, wound swabs, tissues, tracheal secretions, urine, sputum and pus whereas MRSA strains with *spa* type t032 were isolated from tracheal secretions, nasal swabs, pus and wound swabs.

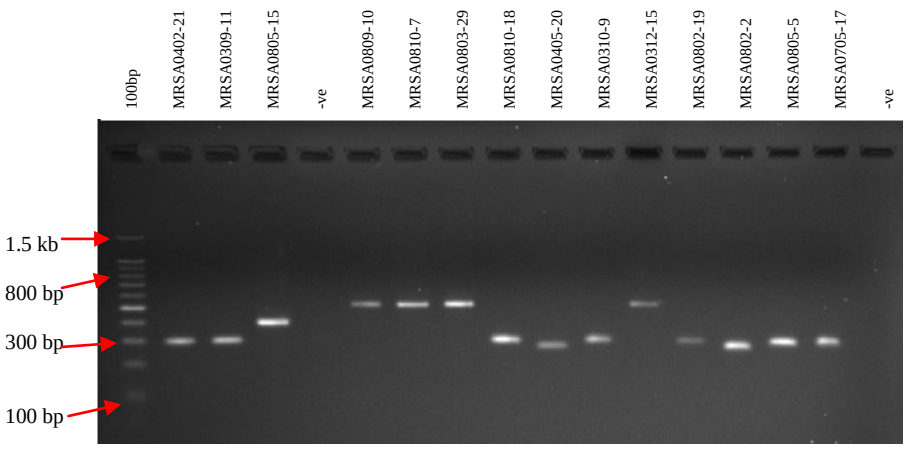


Figure 4.19 Representative agarose gel of *spa* typing for MRSA strains. Sterile deonised water was used as PCR control.

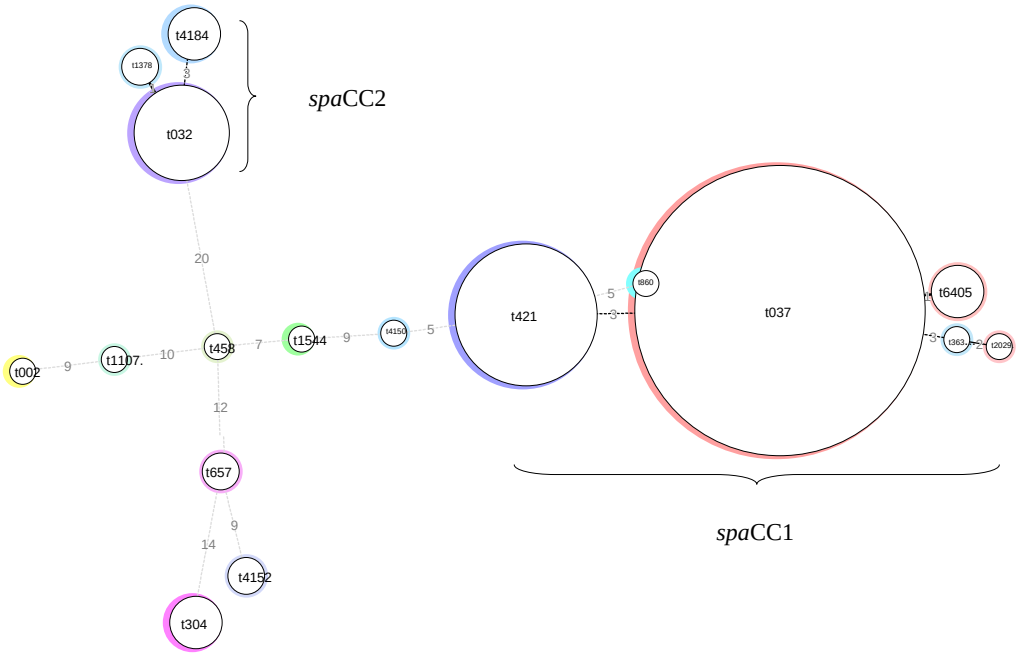


Figure 4.20 Minimal spanning tree analyses for the *spa* types of 188 MRSA strains. Clusters of related *spa* types were arbitrarily assigned clonal complexes (*spa*CC1-2). The sizes of node indicate the number of strains of each MLST types.

4.12.4 Genotypes of MRSA based on Multilocus-sequence typing (MLST) of seven housekeeping genes

All 188 MRSA strains were screened for the pandemic ST239 lineage. Out of the 188 MRSA strains, 157 (51 from 2003, all 2004 strains, 8 from 2007 and 89 from 2008) produced both DNA bands (220 bp and 480 bp), as determined via the heteroduplex PCR (Figure 4.21). This indicates that presence of ST239 lineage (CC8) and this was later confirmed by MLST.

PCR amplification of the seven house-keeping genes (*arcC, aroE, gmk, glpF, yqil, tpi and pta*) resulted in amplicons around 500 bp (Figure 4.22). Sequence analysis by using online MLST.net revealed ten different multilocus sequence types including ST22 (CC22) (n = 19), ST6 (CC6) (n = 4), ST20 (CC20) (n=1), ST5 (CC5) (n = 1), ST573 (CC1) (n = 1), ST80 (CC80) (n = 1), ST241 (CC8) (n = 1), ST772 (n = 1) (CC1), ST1178 (n = 2) (CC5) and ST239 (CC8) (Figure 4.22, Table 4.9 - 4.11;Appendix 53-54).

ST239 and ST20 were present since 2003 whereas ST5, ST6 and ST22 were introduced in year 2007. The other MLST types (ST80, ST573, ST241, ST1178 and ST772) were only present in year 2008.

A minimum spanning tree based on the degree of similarity between seven housekeeping genes of MRSA strains was generated (Figure 4.23). The analysis showed that MLST types ST239 and ST241 were closely related with only one MST distance away as they shared six alleles including *arcC, aroE, glpF, gmk, pta* and *tpi*. Similarly, both ST772 and ST573 were also closely related with only one allele difference (*pta*).

There are two allelic differences between ST5 and ST1178 as they shared five similar alleles including *arcC, glpF, gmk, pta* and *yqil*. Similarly, this ST5 was also found to share five alleles with ST6 and the only two different alleles were *arcC* and

yqiL. All the MRSA strains from these three MLST types (ST5, ST6 and ST1178) were of SCC*mec* type IV.

On the other hand, most of the MRSA strains with same MLST types were often cultured from different types of samples. For examples, the MRSA strains with MLST type ST6 were isolated from wound swabs, nasopharygeal secretions and tracheal secretions whereas MRSA strains with *spa* type t032 were isolated from tracheal secretions, nasal swabs, pus and wound swabs.

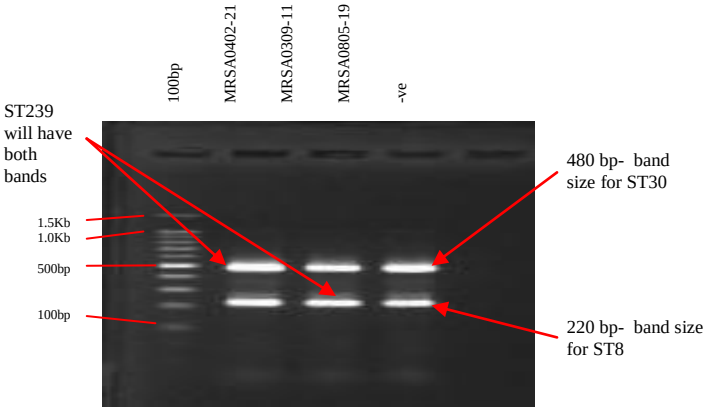


Figure 4.21 Representative agarose gel of PCR amplified products using primers specific for ST8, ST30 and ST239. Sterile deonised water was used as PCR control.

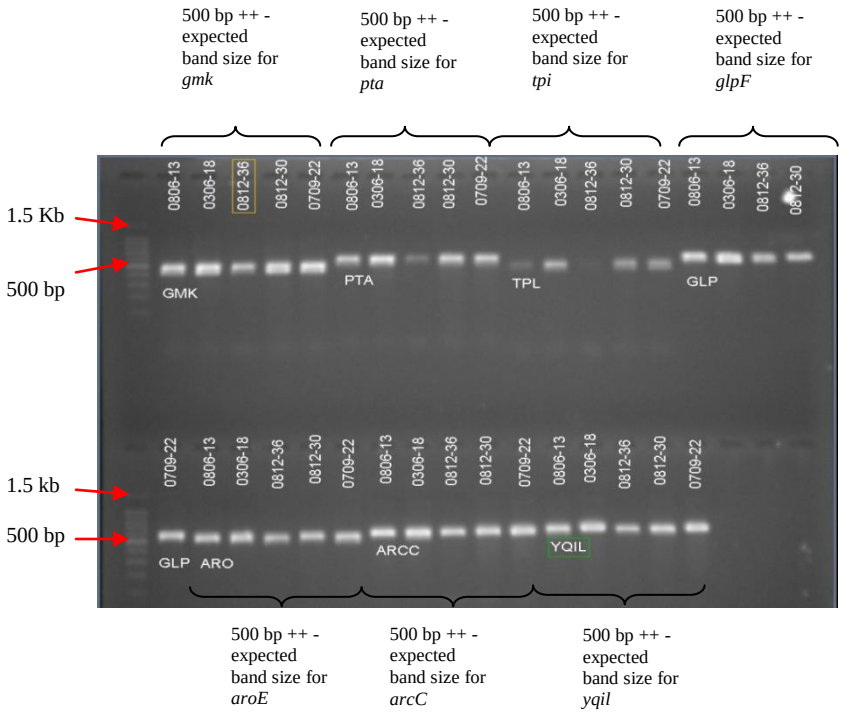


Figure 4.22 Representative agarose gel of PCR amplified products using primers specific for seven housekeeping gene (*gmk, pta, tpi, glpF, aroE, arccC* and *yqil*) in MRSA using genomic DNA as template. The labeling of strains is indicated in the gel photo.

Table 4.9: Allelic profiles of different MLST types

ST	No of MRSA strains	Allelic profile (allele no)						
		arcC	*aroE*	*glpF*	*gmk*	*pta*	*tpi*	*yqiL*
5	4	1	4	1	4	12	1	10
6	1	12	4	1	4	12	1	3
20	1	4	9	1	8	1	10	1
22	19	7	6	1	5	8	8	6
80	1	1	3	1	14	11	51	10
239	157	2	3	1	1	4	4	3
241	1	2	3	1	1	4	4	30
573	1	1	1	1	1	12	1	1
772	1	1	1	1	1	22	1	1
1178	2	1	181	1	4	12	10	10

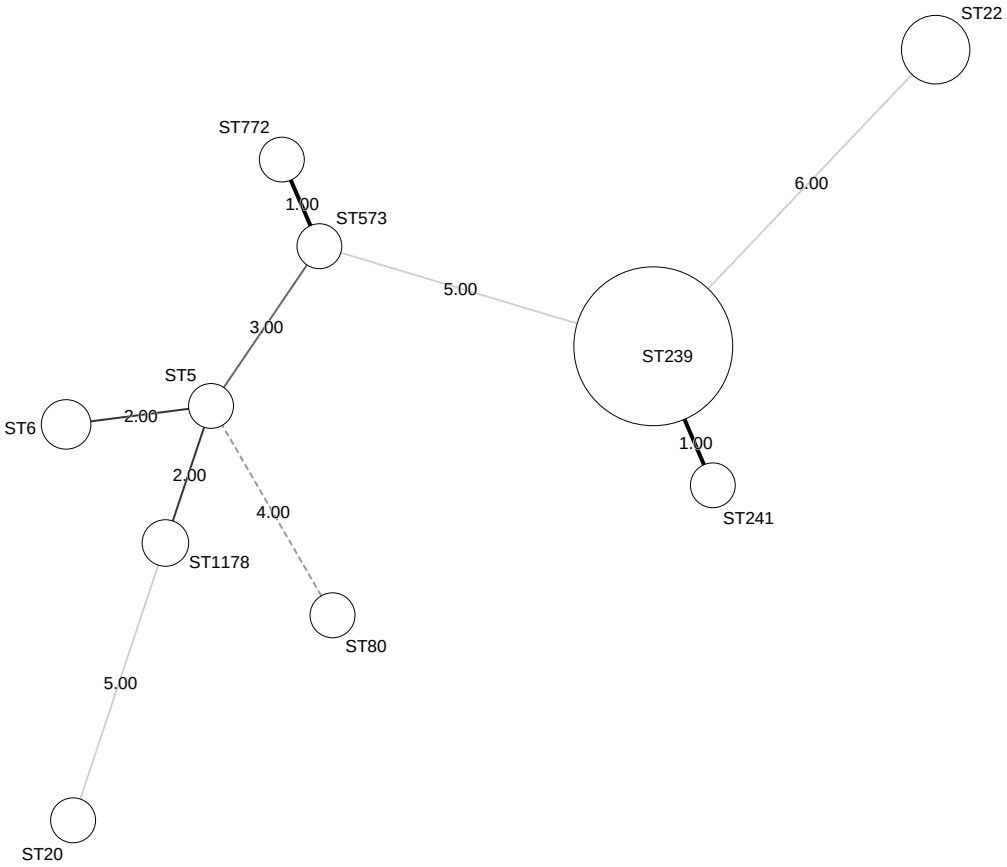


Figure 4.23 Minimum spanning tree analyses for the MLST types of 188 MRSA strains. Each sequence type is represented by a node. The distance between each node represents the number of allelic differents. The sizes of node indicate the number of strains of each MLST types.

4.12.5 Genotypes of MRSA based on *mec*-associated direct repeat unit (*dru*) types

PCR amplification of *mec* gene was performed by using primers as described earlier by Goering *et al.* (2008) resulted in *mec* amplicon that ranged from 800 bp to 1000 bp (Figure 4.24).

Sequence analysis of *mec*-associated direct repeat unit (*dru*) in 188 strains gave 30 distinct *dru* types. Eighteen novel *dru* types (dt13l, dt13m, dt11al, dt15l, dt15m, dt7v, dt13n, dt15n, dt10aw, dt13ao, dt11am, dt13p, dt11an, dt13q, dt10ax, dt12j, dt12k and dt14h) were detected (Appendix 55). The information of these novel sequences has been deposited in www.dru-typing.org.

The predominant *dru* type was dt13d (n=57, 30.32%). Repeats among other *dru* types varied from one strain (dt12k, dt12j, dt11an, dt2c, dt13p, dt7l, dt10aw, dt15n, dt13i, dt15l, dt15m, and dt14h), two strains (dt13l, dt13m, dt7v, dt10ax, and dt11am), three strains (dt11c, dt13ao, dt10ao and dt13q), four strains (dt13f), five strains (dt9w, dt13n, 11al and dt13j), six strains (dt14c), 32 strains (dt10a) and 35 strains (dt13g) (Table 4.10 - 4.11). The discriminatory power of *mec*-associated *dru* typing was 0.85.

A minimum spanning tree based on the degree of similarity between *dru* repeats successions of MRSA strains was generated (Figure 4.25). The analysis showed four *dru* clonal complexes (*dru*CC) arbitrarily named *dru*CC1 to *dru*CC4 (Figure 4.25). *dru*CC1 consists of strains from *dru* types dt13d, dt13j, dt13g, dt13n, dt13ao, dt13p, dt13m, dt13f and dt13i, whereas *dru*CC2 consists of strains from *dru* types dt15n, dt15l and dt15m (Figure 4.25). dt9w and dt10ax were grouped in *dru*CC3 and dt11al and dt13q were grouped in *dru*CC4 (Figure 4.25). Fourteen *dru* types (dt11an, dt12j, dt14h, dt12k, dt14c, dt11am, dt7v, dt13l, dt7l, dt2c, dt10ao, dt11c, dt10a and dt10aw) were not grouped in any *dru* clonal complexes as they only shared 91.6% similarity.

Twenty-three different *dru* types (dt11an, dt12j, dt12k, dt15n, dt15l, dt15m, dt9w, dt10ax, dt11am, dt13l, dt11al, dt13q, dt14h, dt14c, dt13j, dt13g, dt13n, dt13ao,

dt13p, dt13m, dt13f, dt13i and dt13d) including all strains within *dru* clonal complexes druCC1 to druCC4 were found to share 95% similarity.

MRSA strains from *dru* type dt13d were cultures from different years (2003, 2004, 2007 and 2008) and sources such as nasal swabs, wound swabs, tissue, urine, nasopharygeal secretions, pus, tracheal secretions, blood, sputum, shoulder swab and bone.

On the other hand, MRSA strains from *dru* type dt10a were isolated from three different years (2003, 2007 and 2008) and from different specimens including wound swabs, sputum, tracheal secretions, nasopharygeal secretions, nasal swabs, pus and blood.

Twenty-one out of 32 MRSA strains with *dru* type dt10a belong to SCC*mec* type IV. The remaining four SCC*mec* type IV strains were from *dru* type dt13ao (2 strains), dt10aw (1 strain), and dt13d (one strain).

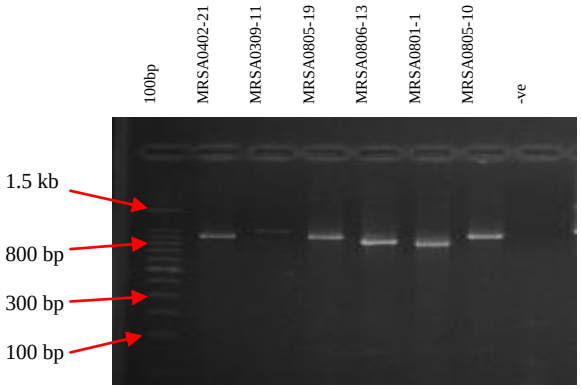


Figure 4.24 Representative agarose gel of *mec*-associated *dru* typing for MRSA strains. Sterile deonised water was used as PCR control.

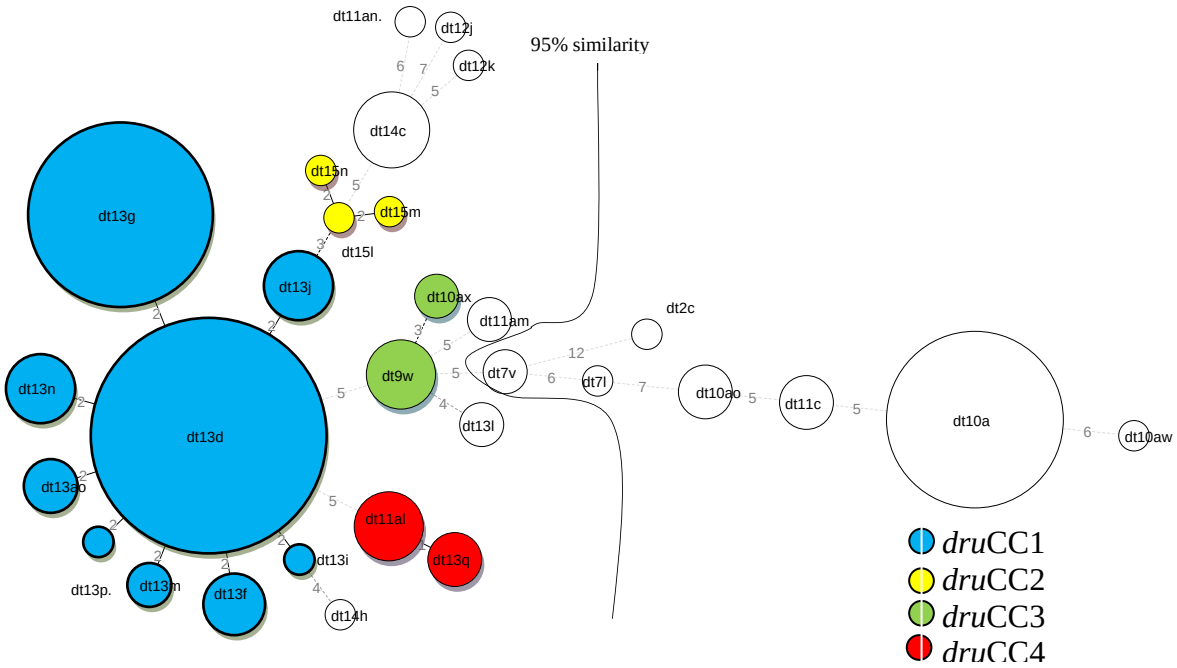


Figure 4.25 Minimal spanning tree analyses for the *dru* types of 188 MRSA strains. Clusters of related *dru* types were arbitrarily assigned clonal complexes (*dru*CC1-4). Each sequence type is represented by a node. The sizes of node indicate the number of strains of each *dru* types.

Table 4.10: SCC*mec* and *agr*, *spa*, MLST and *dru types*, coa-RFLP and PFGE profiles of the 188 MRSA strains.

Strain No	SCC*mec* type	*agr* type	*spa* type	MLST type	*dru* type	*coa*-RFLP profile	*Sma*1-PFGE profile
MRSA2	III	I	t037	ST239	dt13d	C13	SM7
MRSA3	III	I	t037	ST239	dt13d	C30	SM40
MRSA4	III	I	t037	ST239	dt13d	C32	SM55
MRSA8	III	I	t037	ST239	dt14h	C13	SM15
MRSA0301-1	III	I	t037	ST239	dt13d	C12	SM68
MRSA0301-28	III	I	t037	ST239	dt13d	C12	SM7
MRSA0302-4	III	I	t037	ST239	dt14c	C12	SM51
MRSA0304-16	III	I	t037	ST239	dt13d	C12	SM13
MRSA0305-10	III	I	t037	ST239	dt13d	C30	SM7
MRSA0305-18	III	I	t037	ST239	dt13i	C12	SM64
MRSA0305-23	III	I	t037	ST239	dt13l	C13	SM6
MRSA0306-10	III	I	t037	ST239	dt13d	C18	SM14
MRSA0306-14	III	I	t037	ST239	dt13g	C12	SM22
MRSA0306-15	III	I	t037	ST239	dt13d	C13	SM17
MRSA0306-18	III	I	t1544	ST20	dt14c	C20	SM23
MRSA0306-26	III	I	t037	ST239	dt13g	C14	SM7
MRSA0306-7	III	I	t037	ST239	dt13d	C13	SM17
MRSA0307-1	III	I	t037	ST239	dt9w	C19	SM17
MRSA0307-10	III	I	t037	ST239	dt13m	C12	SM66
MRSA0307-14	III	I	t037	ST239	dt13d	C12	SM17
MRSA0307-20	III	I	t037	ST239	dt10a	C13	SM17
MRSA0307-23	III	I	t037	ST239	dt10a	C12	SM17
MRSA0307-25	III	I	t037	ST239	dt13g	C12	SM63
MRSA0307-5	III	I	t037	ST239	dt11al	C13	SM38
MRSA0307-9	III	I	t421	ST239	dt13d	C10	SM21
MRSA0308-1	III	I	t037	ST239	dt13d	C30	SM17
MRSA0308-10	III	I	t037	ST239	dt14c	C12	SM1
MRSA0308-22	III	I	t037	ST239	dt13d	C13	SM17
MRSA0308-23	III	I	t037	ST239	dt15l	C12	SM32
MRSA0308-24	III	I	t037	ST239	dt13g	C26	SM30
MRSA0308-28	III	I	t037	ST239	dt13d	C30	SM60
MRSA0309-10	III	I	t037	ST239	dt13g	C23	SM7
MRSA0309-11	III	I	t037	ST239	dt13g	C21	SM17
MRSA0309-9	III	I	t037	ST239	dt13d	C30	SM28
MRSA0310-19	III	I	t037	ST239	dt13d	C13	SM30
MRSA0310-23	III	I	t037	ST239	dt13d	C15	SM8
MRSA0310-26	III	I	t037	ST239	dt15m	C30	SM36
MRSA0310-9	III	I	t037	ST239	dt9w	C21	SM17
MRSA0311-1	III	I	t037	ST239	dt13m	C11	SM66
MRSA0311-21	III	I	t037	ST239	dt13d	C12	SM42
MRSA0311-23	III	I	t037	ST239	dt13d	C24	SM17
MRSA0311-4	III	I	t037	ST239	dt11al	C30	SM21
MRSA0311-7	III	I	t037	ST239	dt13d	C21	SM17
MRSA0311-8	III	I	t037	ST239	dt10a	C13	SM21
MRSA0311-9	III	I	t037	ST239	dt13d	C12	SM17
MRSA0312-13	III	I	t037	ST239	dt13d	C12	SM47

Table 4.10 (continue)

Strain No	SCC*mec* type	*agr* type	*spa* type	MLST type	*dru* type	*coa*-RFLP profile	*Sma*1-PFGE profile
MRSA0312-15	III	I	t037	ST239	dt11al	C21	SM21
MRSA0312-17	III	I	t037	ST239	dt11al	C13	SM7
MRSA0312-2	III	I	t037	ST239	dt9w	C13	SM17
MRSA0312-3	III	I	t037	ST239	dt13d	C13	SM17
MRSA0312-30	III	I	t037	ST239	dt11c	C6	SM19
MRSA0312-35	III	ND	t037	ST239	dt13l	ND	SM7
MRSA0401-13	III	I	t037	ST239	dt13d	C12	SM29
MRSA0402-21	III	I	t037	ST239	dt7v	C21	SM26
MRSA0402-8	III	I	t037	ST239	dt13d	C19	SM10
MRSA0403-20	III	I	t037	ST239	dt11al	C12	SM12
MRSA0405-20	III	I	t421	ST239	dt13d	C21	SM20
MRSA0406-8	III	I	t037	ST239	dt13d	C12	SM64
MRSA0408-33	III	I	t037	ST239	dt7v	C13	SM67
MRSA0408-34	III	I	t037	ST239	dt13n	C15	SM33
MRSA0409-17	III	I	t037	ST239	dt13d	C11	SM27
MRSA0701-15	IV	II	t002	ST5	dt10a	C4	SM56
MRSA0701-26	IV	I	t304	ST6	dt10a	C38	SM46
MRSA0703-8	III	I	t421	ST239	dt13d	C30	SM7
MRSA0704-15	III	I	t037	ST239	dt13ao	C8	SM11
MRSA0704-18	III	I	t860	ST239	dt13d	C30	SM69
MRSA0704-20	III	I	t421	ST239	dt13d	C30	SM62
MRSA0704-3	III	I	t037	ST239	dt13d	C13	SM61
MRSA0705-13	III	I	t037	ST239	dt15n	C24	SM70
MRSA0705-17	IV	I	t032	ST22	dt10a	C41	SM75
MRSA0705-7	IV	I	t032	ST22	dt10aw	C43	SM84
MRSA0705-8	III	I	t421	ST239	dt13ao	C8	SM65
MRSA0707-17	IV	I	t304	ST6	dt10a	C30	SM44
MRSA0707-26	III	I	t037	ST239	dt13a	ND	SM59
MRSA0708-1	IV	I	t4184	ST22	dt13d	C44	SM71
MRSA0708-10	IV	I	t032	ST22	dt10a	C33	SM73
MRSA0709-22	IV	I	t032	ST22	dt10a	C39	SM54
MRSA0801-1	III	I	t037	ST239	dt11am	C7	SM24
MRSA0801-13	III	I	t421	ST239	dt13d	C13	SM39
MRSA0801-16	III	I	t6405	ST239	dt13j	C30	SM13
MRSA0801-2	III	I	t037	ST239	dt13g	C12	SM36
MRSA0801-21	IV	I	t037	ST22	dt10a	C33	SM83
MRSA0801-26	III	I	t037	ST239	dt13d	C12	SM7
MRSA0801-27	III	I	t421	ST239	dt13f	C7	SM41
MRSA0801-30	III	I	t037	ST239	dt13f	C21	SM18
MRSA0801-4	III	I	t037	ST239	dt13g	C10	SM36
MRSA0801-9	III	I	t037	ST239	dt13d	C30	SM7
MRSA0802-14	III	I	t037	ST239	dt13p	ND	SM1
MRSA0802-19	III	I	t037	ST239	dt10a	C43	SM79
MRSA0802-2	III	I	t6405	ST239	dt13j	C22	SM17
MRSA0802-3	III	I	t6405	ST239	dt13j	C39	SM17
MRSA0803-28	IV	I	t4184	ST22	dt10a	C36	SM80
MRSA0803-29	III	I	t037	ST239	dt14c	ND	SM4
MRSA0803-30	III	I	t037	ST239	dt14c	C12	SM3

Table 4.10 (continue)

Strain No	SCC*mec* type	*agr* type	*spa* type	MLST type	*dru* type	*coa*-RFLP profile	*Sma*1-PFGE profile
MRSA0803-35	III	I	t421	ST239	dt13d	C28	SM21
MRSA0804-1	IV	I	t4184	ST22	dt10a	C45	SM74
MRSA0804-14	III	I	t037	ST239	dt13d	C30	SM35
MRSA0804-20	III	I	t421	ST239	dt13j	C30	SM21
MRSA0804-24	III	I	t037	ST239	dt13d	C15	SM16
MRSA0805-1	IV	I	t1107	ST1178	dt13ao	C5	SM57
MRSA0805-10	IV	I	t1107	ST1178	dt13ao	C9	SM21
MRSA0805-11	III	I	t037	ST239	dt13g	C12	SM40
MRSA0805-15	III	I	t363	ST241	dt7l	C47	SM5
MRSA0805-17	III	I	t037	ST239	dt13d	C12	SM2
MRSA0805-19	III	I	t037	ST239	dt13g	C12	SM36
MRSA0805-20	III	I	t6405	ST239	dt11an	C13	SM21
MRSA0805-21	III	I	t4152	ST239	dt13g	C13	SM7
MRSA0805-22	III	I	t037	ST239	dt14c	C12	SM7
MRSA0805-23	III	I	t037	ST239	dt13g	C17	SM28
MRSA0805-24	III	I	t037	ST239	dt13ao	C30	SM4
MRSA0805-3	III	I	t037	ST239	dt13g	C13	SM7
MRSA0805-4	III	I	t037	ST239	dt13d	C30	SM7
MRSA0805-5	III	I	t037	ST239	dt13d	C16	SM15
MRSA0805-6	III	I	t037	ST239	dt13j	C12	SM18
MRSA0805-9	IV	I	t032	ST22	dt10a	C3	SM77
MRSA0806-1	III	I	t421	ST239	dt13f	C10	SM21
MRSA0806-11	IV	I	t304	ST6	dt10a	C40	SM48
MRSA0806-13	III	III	t037	ST80	dt10a	C5	SM36
MRSA0806-14	III	II	t458	ST573	dt2c	C1	SM58
MRSA0806-18	III	I	t037	ST239	dt11am	C12	SM37
MRSA0806-21	IV	I	t4184	ST22	dt10a	C35	SM78
MRSA0806-22	III	I	t037	ST239	dt13g	C12	SM36
MRSA0806-26	III	I	t037	ST239	dt13g	C12	SM7
MRSA0806-33	III	I	t037	ST239	dt13d	C2	SM7
MRSA0807-1	III	I	t037	ST239	dt10a	C13	SM36
MRSA0807-13	III	I	t037	ST239	dt13g	C8	SM52
MRSA0807-14	III	I	t421	ST239	dt13n	C12	SM21
MRSA0807-19	III	I	t037	ST239	dt13d	C31	SM7
MRSA0807-7	III	I	t421	ST239	dt13q	C12	SM7
MRSA0807-8	III	I	t037	ST239	dt13q	C13	SM7
MRSA0808-17	III	I	t421	ST239	dt13f	C2	SM21
MRSA0808-19	III	I	t421	ST239	dt13g	C30	SM7
MRSA0808-21	III	I	t037	ST239	dt13g	C13	SM36
MRSA0808-24	III	I	t421	ST239	dt13d	C15	SM16
MRSA0808-25	III	I	t037	ST239	dt13g	C30	SM36
MRSA0808-26	III	I	t421	ST239	dt13d	C30	SM21
MRSA0808-35	III	I	t037	ST239	dt13q	C13	SM16
MRSA0809-1	III	I	t037	ST239	dt11c	C12	SM59
MRSA0809-10	III	I	t037	ST239	dt13g	C21	SM17
MRSA0809-14	III	I	t421	ST239	dt10ax	C46	SM43
MRSA0809-15	III	I	t037	ST239	dt13d	C13	SM34

Table 4.10 (continue)

Strain No	SCC*mec* type	*agr* type	*spa* type	MLST type	*dru* type	*coa*-RFLP profile	*Sma*1-PFGE profile
MRSA0809-24	III	I	t037	ST239	dt13d	C13	SM25
MRSA0809-25	III	I	t4150	ST239	dt13d	C30	SM7
MRSA0809-27	III	I	t037	ST239	dt13d	C30	SM17
MRSA0809-30	III	I	t421	ST239	dt10ax	C13	SM40
MRSA0809-32	III	I	t037	ST239	dt12j	C15	SM49
MRSA0809-33	III	I	t037	ST239	dt13g	C12	SM7
MRSA0809-36	III	I	t421	ST239	dt13n	C12	SM21
MRSA0809-38	III	I	t037	ST239	dt13g	C12	SM72
MRSA0810-10	IV	I	t1378	ST22	dt10a	C36	SM82
MRSA0810-13	III	I	t037	ST239	dt13d	C7	SM1
MRSA0810-15	III	I	t421	ST239	dt13d	C13	SM21
MRSA0810-16	III	I	t037	ST239	dt13n	C12	SM40
MRSA0810-17	IV	I	t1378	ST22	dt10a	C25	SM81
MRSA0810-18	III	I	t421	ST239	dt13d	C21	SM17
MRSA0810-2	III	I	t037	ST239	dt13g	C30	SM17
MRSA0810-22	IV	I	t032	ST22	dt10a	C35	SM83
MRSA0810-23	III	I	t037	ST239	dt13g	C13	SM7
MRSA0810-6	III	I	t1378	ST22	dt10a	C37	SM50
MRSA0810-7	III	I	t421	ST239	dt13d	C21	SM21
MRSA0810-9	III	I	t421	ST239	dt13d	C13	SM7
MRSA0811-10	III	I	t037	ST239	dt13d	C30	SM7
MRSA0811-11	III	I	t037	ST239	dt13d	C19	SM21
MRSA0811-13	III	I	t037	ST239	dt13g	C30	SM31
MRSA0811-16	III	I	t032	ST22	dt10a	C30	SM7
MRSA0811-2	III	I	t037	ST239	dt13d	C29	SM21
MRSA0811-22	IV	I	t032	ST22	dt10a	C42	SM76
MRSA0811-24	III	I	t037	ST239	dt13g	C30	SM7
MRSA0811-25	III	I	t037	ST239	dt13g	C8	SM9
MRSA0811-26	III	I	t037	ST239	dt13g	C13	SM7
MRSA0811-28	III	I	t037	ST239	dt13g	C27	SM7
MRSA0811-30	IV	I	t032	ST22	dt10a	C34	SM85
MRSA0811-5	III	I	t037	ST239	dt9w	C30	SM4
MRSA0811-8	III	I	t037	ST239	dt10a	C13	SM7
MRSA0812-1	IV	I	t032	ST22	dt10a	C40	SM74
MRSA0812-11	III	I	t304	ST6	dt10a	C35	SM45
MRSA0812-15	III	I	t037	ST239	dt13g	C13	SM7
MRSA0812-17	III	I	t037	ST239	dt13g	C12	SM36
MRSA0812-2	III	I	t037	ST239	dt11c	C30	SM59
MRSA0812-22	III	I	t037	ST239	dt13g	C13	SM7
MRSA0812-23	IV	I	t032	ST22	dt10a	C40	SM74
MRSA0812-27	III	I	t037	ST239	dt13g	C30	SM7
MRSA0812-30	III	I	t2029	ST239	dt12k	C13	SM49
MRSA0812-31	III	I	t037	ST239	dt13g	C30	SM7
MRSA0812-33	III	I	t037	ST239	dt10a	C36	SM49
MRSA0812-35	III	I	t037	ST239	dt13g	C30	SM7
MRSA0812-36	V	II	t657	ST772	dt10ao	C1	SM53
MRSA0812-37	III	I	t037	ST239	dt9w	C30	SM7

4.13 Genomic changes of MRSA based on three different sequence typing methods.

4.13.1 Genomic changes of MRSA based on *spa* typing

Three *spa* types (i.e. t037, t1544 and t421) were detected in year 2003. Five *spa* types (t032, t4184, t002, t304 and t860) were introduced in UMMC in year 2007 and nine *spa* types (t1378, t6405, t4150, t1107, t458, t4152, t657, t363 and t2029) were only found in 2008 strains (Figure 4.26; Table 4.11).

t037 was found to be closely related with *spa* type (t421) with differences in only one mutation and both *spa* types shared the same MLST type ST239, even though they were obtained from four years apart.

Five *spa* types (t4184, t032, t1378, t002 and t6405) which were introduced between year 2007 and 2008 were related with *spa* type t037 with differences of five to 25 mutations (Figure 4.26). *spa* type t304 was also related with *spa* t037 with differences of nine mutations (Figure 4.26).

On the other hand, eight *spa* types (t4150, t1107, t458, t4152, t657, t860, t2029 and t363) which were also being introduced in year 2007 to 2008 were related with *spa* type 421 with differences of three to 12 mutations (Figure 4.26). All eight *spa* types which were introduced in year 2007 to 2008 was isolated from tissue, wound swab, nasopharyngeal secretions, nasal swab, sputum and tracheal secretions obtained from patients who stayed in orthopaedic, paediatric and medical wards. Strain with *spa* type t421 was isolated from nasal swab, blood, wound swabs, tissue, tracheal secretions, urine, sputum, pus obtained from patients who stayed in CICU, medical, surgical, ICU, dialysis, orthopaedic, dialysis, gynecology and obstetrics.

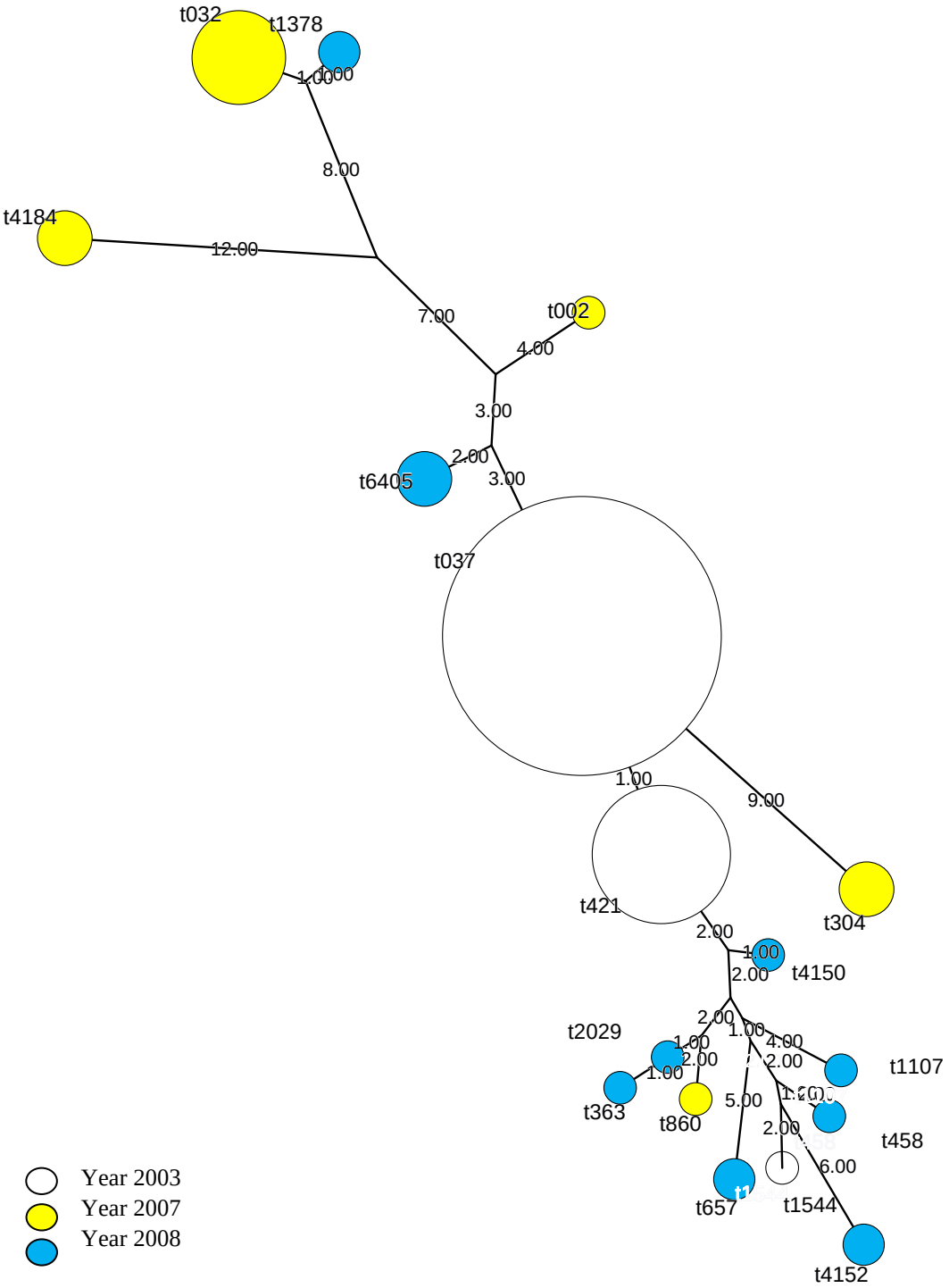


Figure 4.26 Maximum Parsimony Tree derived from *spa* types of MRSA strains in 2003-04 and 2007-08 from UMMC, Malaysia. The clustering was performed by using the BioNumerics 6.0. The numerals on the line indicate the genetic distance between each *spa* types. The sizes of node indicate the number of strains of each *spa* types.

4.13.2 Genomic changes of MRSA based on MLST

Two MLST types (i.e. ST20 and ST239) were detected in year 2003. Three MLST types (ST5, ST6 and ST22) were introduced in UMMC in year 2007 and five MLST types (ST80, ST241, ST573, ST772 and ST1178) were only found in 2008 strains (Figure 4.27; Table 4.11).

MLST type ST241 which was present in year 2008 was found to be closely related with MLST type ST239 that was present since year 2003. Both MLST types shared six similar allele including *arcC, aroE, glpF, gmk, pta* and *tpi*.

Seven other MLST types including ST22, ST573, ST772, ST6, ST5, ST1178 and ST80 which were introduced between year 2007 and 2008 was found to be related with MLST type, ST239 with differences of six to 11 mutations (Figure 4.27). It was noted that ST772 which was also SCC*mec* type V was closely related with ST572 with difference of one mutation.

ST1178 was found to be related with ST5 (from clonal complex CC5) with differences of two mutations (Figure 4.27). ST1178 was introduced in May 2008, 14 months after ST5 was introduced (Jan, 2007)

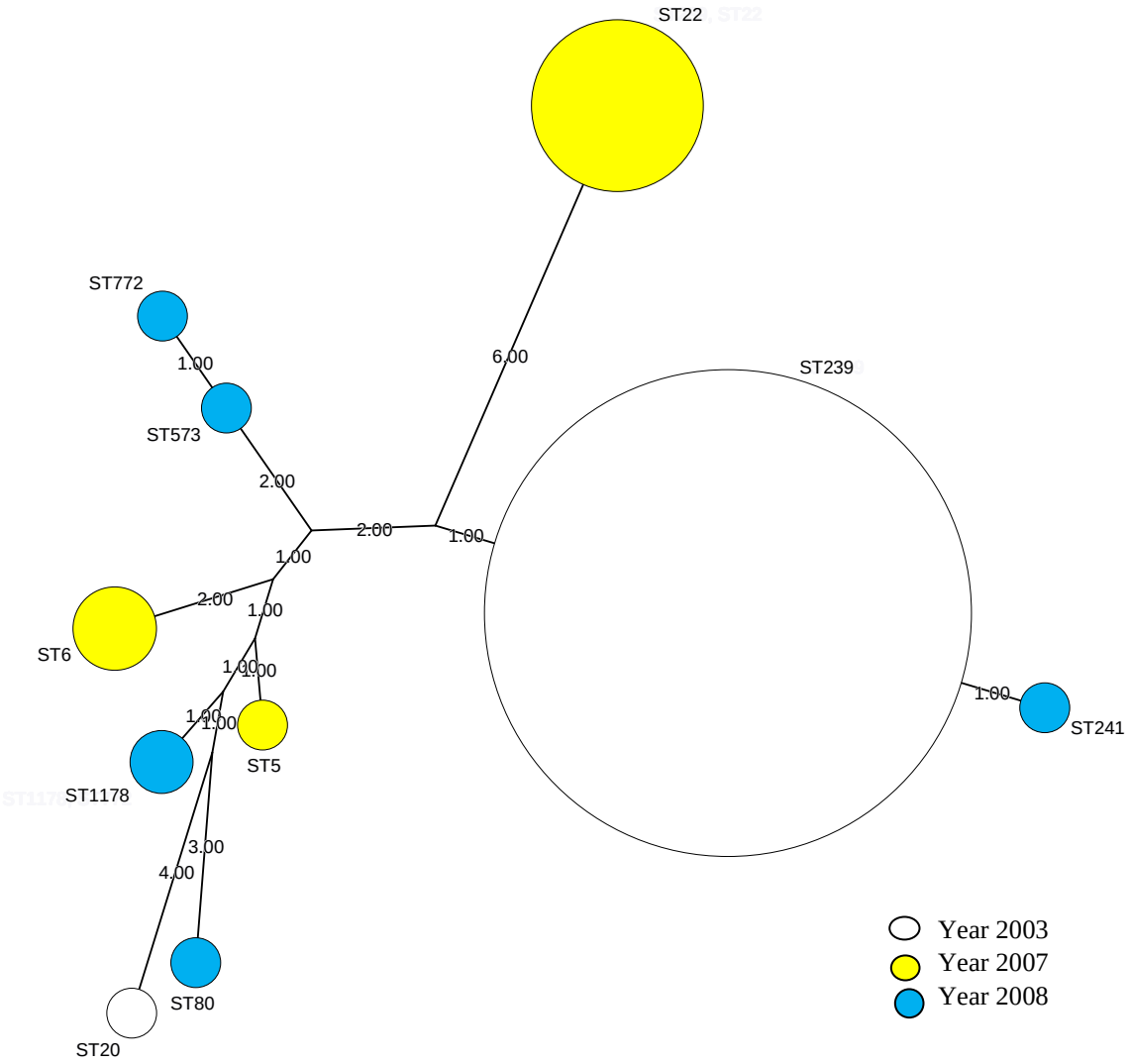


Figure 4.27 Maximum Parsimony Tree derived from MLST types of MRSA strains in 2003-04 and 2007-08 from UMMC, Malaysia. The clustering was performed by using the BioNumerics 6.0. The numerals on the line indicate the genetic distance between each MLST types. The sizes of node indicate the number of strains of each MLST types.

4.13.3 Genomic changes in MRSA based on *mec*-associated *dru* typing

Thirteen *dru* types (i.e. dt9w, dt13i, dt13m, dt13g, dt13d, dt13l, dt15m, dt15l, dt14h, dt14c, dt11al, dt10a and dt11c) were detected in year 2003. Two *dru* types (dt13n and dt7v) were introduced in UMMC in year 2004, three *dru* types (dt10aw, dt15n and dt13ao) in year 2007 and 12 *dru* types (dt11am, dt12k, dt13q, dt13j, dt13p, dt13f, dt11an, dt10ax, dt12j, dt7l, dt10ao and dt2c) were only found in 2008 strains (Figure 4.28; Table 4.11).

dt13d was found to be closely related with eight other dt types (i.e dt13f, dt13ao, dt13m, dt13i, dt13g, dt13n, dt13j and dt13p) with differences in only one mutation and all nine *dru* types shared the same MLST type ST239, even though they were obtained four years apart. These eight *dru* types (dt13f, dt13ao, dt13m, dt13i, dt13g, dt13n, dt13j and 13p) were closely related to dt13d as there is only one MST distance away from dt13d (shared more than 98.5%) similarity.

dt10a which consists of strains from three different years (2003, 2007 and 2008) belong to five different MLST types, including ST5, ST6, ST22, ST80 and ST239. dt10aw was closely related to dt10a with only three mutations differences.

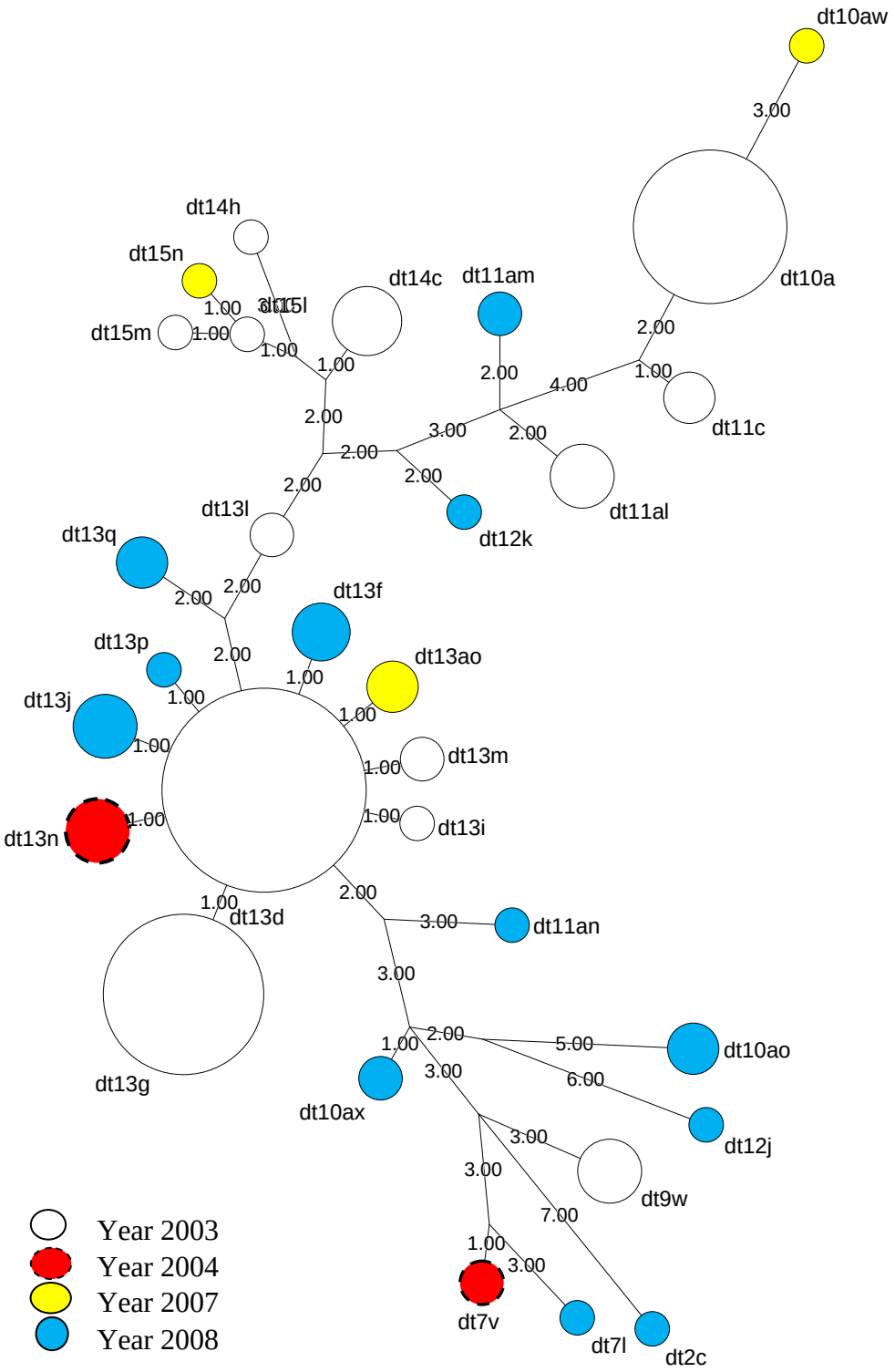


Figure 4.28 Maximum Parsimony Tree derived from *dru* types of MRSA strains in 2003-04 and 2007-08 from UMMC, Malaysia. The clustering was performed by using the BioNumerics 6.0. The numerals on the line indicate the genetic distance between each *dru* types. The sizes of node indicate the number of strains of each *dru* types.

Table 4.11: Summary of MLST, *dru* and *spa* types among 188 MRSA strains

Sequence typing	Year				Cluster complexes	Total
	2003	2004	2007	2008		
***spa* type**						
t037	50	8	4	63	*Spa*CC1	125
t1544	1	-	-	-	ND	1
t421	1	1	3	20	*Spa*CC1	25
t002	-	-	1	-	ND	1
t304	-	-	2	2	ND	4
t4184	-	-	1	3	*Spa*CC2	4
t032	-	-	4	8	*Spa*CC2	12
t1378	-	-	-	3	*Spa*CC2	3
t6405	-	-	-	4	*Spa*CC1	4
t4150	-	-	-	1	ND	1
t1107	-	-	-	2	ND	2
t458	-	-	-	1	ND	1
t4152	-	-	-	1	ND	1
t657	-	-	-	1	ND	1
t2029	-	-	-	1	*Spa*CC1	1
t860	-	-	1	-	ND	1
t363	-	-	-	1	*Spa*CC1	1
MLST type						
ST239	51	9	8	89	CC8	157
ST20	1	-	-	-	CC20	1
ST5	-	-	1	-	CC5	1
ST6	-	-	2	2	CC6	4
ST22	-	-	5	14	CC22	19
ST772	-	-	-	1	CC1	1
ST1178	-	-	-	2	CC5	2
ST573	-	-	-	1	CC1	1
ST80	-	-	-	1	CC80	1
ST241	-	-	-	1	CC8	1
***dru* type**					ND	
dt2c	-	-	-	1	ND	1
dt7l	-	-	-	1	ND	1
dt7v	-	2	-	-	ND	2
dt9w	3	-	-	2	*dru*CC3	5
dt10a	3	-	8	21	ND	32
dt10ao	-	-	-	3	ND	3
dt10aw	-	-	1	-	ND	1
dt10ax	-	-	-	2	*dru*CC3	2
dt11al	4	1	-	-	*dru*CC4	5
dt11am	-	-	-	2	ND	2
dt11an	-	-	-	1	ND	1
dt11c	1	-	-	2	ND	3
dt12j	-	-	-	1	ND	1
dt12k	-	-	-	1	ND	1
dt13d	24	5	4	24	*dru*CC1	57
dt13g	6			29	*dru*CC1	35
dt13f				4	*dru*CC1	4

Table 4.11 (continued)

Sequence typing	Year				Cluster complexes	Total
	2003	2004	2007	2008		
dt13i	1				*dru*CC1	1
dt13j				5	*dru*CC1	5
dt13l	2				ND	2
dt13m	2				*dru*CC1	2
dt13n		1		4	*dru*CC1	5
dt13ao			2	1	*dru*CC1	3
dt13p				1	*dru*CC1	1
dt13q				3	*dru*CC4	3
dt14c	3			3	ND	6
dt14h	1				ND	1
dt15l	1				*dru*CC2	1
dt15m	1				*dru*CC2	1
dt15n			1		*dru*CC2	1

4.13.4 Clonal evolution of Malaysian MRSA from year 2003, 2004, 2007 and 2008

Although MRSA clone ST239 remained as the predominant clone in UMMC over the years (51/52 in year 2003 and 89/111 in year 2008), the combined analysis by MLST-*spa* types showed genotypic changes of MRSA clones ST239-t037 and ST239-t421 between year 2003 and 2008 where (i) the predominant ST239-t037 clone in year 2003 (96%) has decreased in year 2008 (56.8%) while (ii) the ST239-t421 (1.9% in year 2003) has increased in year 2008 (18.0%). It is observed that ST239-t421 clone (from year 2003) that was from ICU might have spread to other wards; i.e medical, dialysis, surgical and orthopaedic in year 2008. Both ST239-t037 and ST239-t421 clones cover 98.0% of strains from year 2003 and 79.8% of strains from year 2008.

Four new MLST-*spa* clones (ST22-t032, ST5-t002, ST6-t304, ST22-t4184) were introduced in year 2007 and another four MLST-*spa* clones (ST241-t363, ST80-t037, ST573-t1378) in year 2008. All four MLST-*spa* clones which were introduced in year 2007 was cultured from medical and surgical wards whereas newly introduced clones in year 2008 was cultured from orthopaedic, dialysis and medical wards.

Further combined analysis by MLST-*spa-dru* can increase the discriminatory power in subtyping of the MRSA strains. Forty-seven different MRSA clones were present in UMMC over the study period (2003, 2004, 2007 and 2008) where (i) ST239-t037-dt13d clone in year 2003 (44.2%) and decreased in year 2008 (13.5%), (ii) ST239-t037-dt14h, ST239-t037-dt13i, ST239-t037-dt13l, ST239-t037-dt13m, ST239-t037-dt15l, ST239-t037-dt15m, ST20-t1544-dt14c clones that only present in year 2003 (17.4%), (iii) ST239-t421-dt13d introduced in year 2003 and slightly increased in year 2008 (7.2%), (iv) the ST239-t037-dt13g clone in year 2003 (11.5%) that were culture from orthopaedic and ICU wards had spread to six others wards including orthopaedic, ICU, surgical, medical, cardiac care unit and pediatric and become more dominant in year 2008 (23.4%), (v) ST22-t032-dt10a, ST22-t4184-dt10a, ST6-t304-dt10a, ST239-t037-dt13ao clones introduced in year 2007 and existed at low prevalence in year 2008, (vi) ST239-t037-dt13n and ST239-t037-dt7v clones introduced in year 2004 (vii) ST5-t002-dt10a, ST22-t4184-dt10a, ST239-t860-dt13d, ST239-t037-dt15n, ST22-t032-dt10aw and ST239-t421-dt13ao clones introduced in year 2007 and (viii) 23 new clones introduced in year 2008 and they were cultured from five different wards including dialysis, medical, orthopaedic, surgical and ICU (Figure 4.29).

Distribution of different MLST-*spa-dru* types of MRSA strains from years 2003, 2004, 2007 and 2008 in the UMMC, Kuala Lumpur, Malaysia

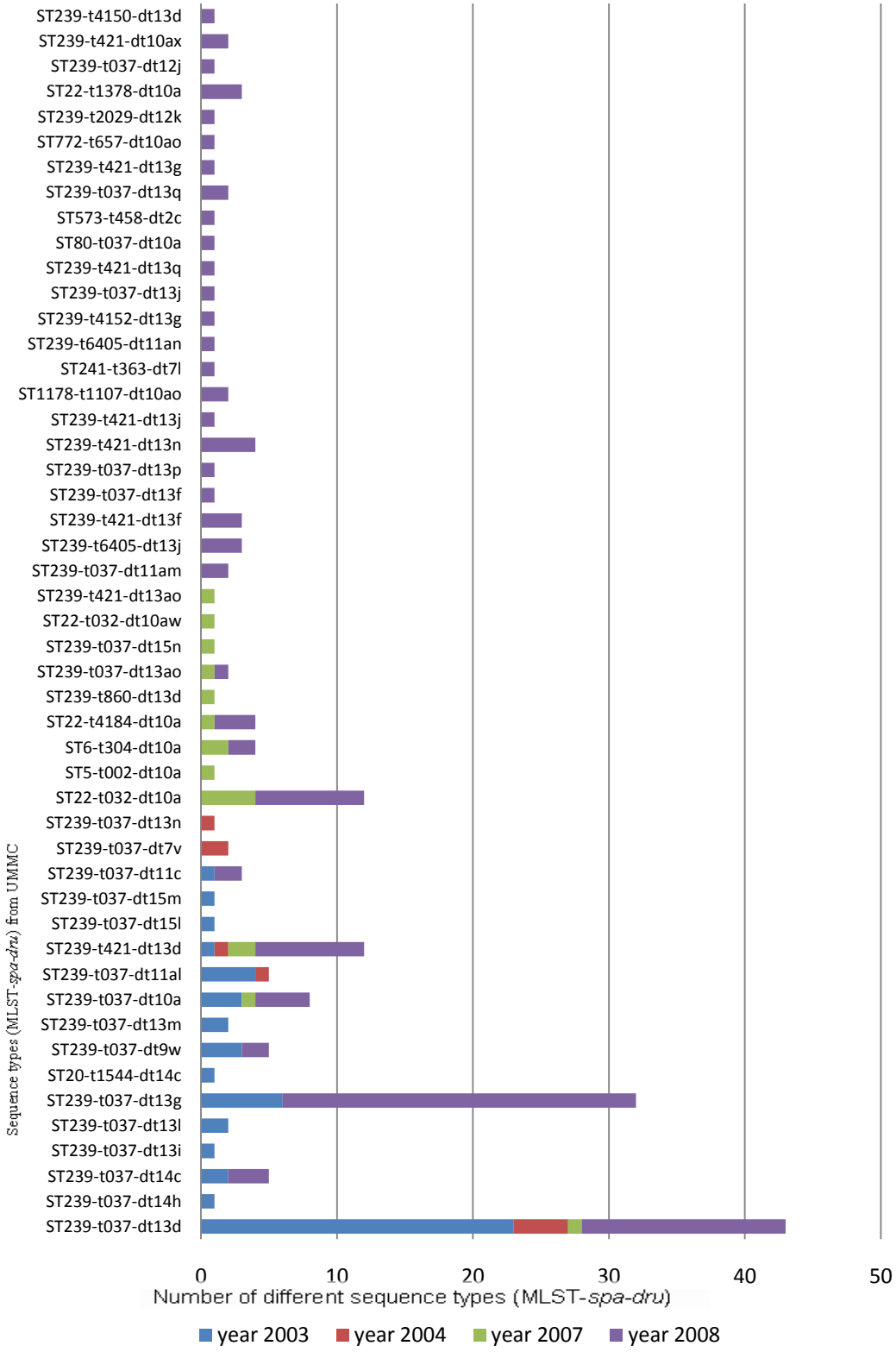


Figure 4.29 Distribution of different MLST-*spa-dru* types of MRSA strains from year 2003, 2004, 2007 and 2008 in the UMMC, Kuala Lumpur, Malaysia.

CHAPTER 5

DISCUSSION

5.1 Antibiograms of MRSA strains from a Malaysian hospital

Infections caused by MRSA continue to be a problem in Malaysian hospitals. Although several studies have documented the antimicrobial resistance trends of MRSA in other countries (Udo *et al*., 2006; Klein *et al*., 2007), reports comparing resistance trends between two periods of time in Malaysia are scanty. This study shows the antimicrobial resistance trends of MRSA isolated in UMMC between year 2003 to 2004 and 2007 to 2008.

All the 188 MRSA strains obtained from UMMC remain susceptible to vancomycin. This is concordant with previous reports (Rohani *et al*., 2000; Neela *et al*., 2008; Al-Talib *et al*., 2010) from Malaysia. This information is relevant and important as vancomycin is known to be one of the few remaining antibiotics left for the treatment of MRSA infections (Ahmad *et al*., 2010).

Elsewhere, vancomycin-resistant *S. aureus* (VRSA) has been reported in-hospital settings in Japan (Hiramatsu, 2001) and India (Saha *et al*., 2008). Similarly, presence of MRSA strains with reduced susceptibility to vancomycin and vancomycin-intermediate *S. aureus* (VISA) have also been reported in other Asia countries such as Thailand (Trakulsomboon *et al*., 2001), Singapore (Fong *et al*., 2009), Japan (Song *et al*., 2004), Korea (Kim *et al*., 2000), Vietnam (Song *et al*., 2004), Taiwan (Hsueh *et al*., 2010) and Philippines (Song *et al*., 2004).

Although MRSA strains in Malaysia are still susceptible to vancomycin, report on vancomycin treatment failure in vancomycin-susceptible MRSA strains has been reported in Malaysia (Norazah *et al*., 2009). Furthermore, Ahmad *et al.* (2010) also reported of an increase of vancomycin MIC values among MRSA strains in Malaysia.

Therefore, the use of vancomycin for treatment of MRSA should be treated with cautions in order to avoid the emergence of VISA or VRSA in Malaysia and to minimize the occurrence of treatment failure.

On the other hand, Neela *et al.* (2008) reported that linezolid and teicoplanin were highly effective against MRSA infection, and in this study a majority of the MRSA strains remained sensitive to both linezolid and teicoplanin. Linezolid is known to be effective against MRSA strains with reduced susceptibility to vancomycin (Yanagihara *et al*., 2002). As teicoplanin is one of the glycopeptides antibiotics (Matthews *et al*., 2007), it is not suitable to be used to treat MRSA infectious with decreased susceptibility of vancomycin.

The rifampicin-resistance rate for MRSA was lower than the 5% rate previously reported by Norazah *et al*. (2002), 15.6% (5 out of 32 MRSA isolates) by Neela *et al*. (2008) and 12.1% from the 2008 Malaysian National Surveillance on Antibiotic Resistance Report (Ministry of Health Malaysia, 2008). Similarly, MRSA rifampicin-resistance rate in Malaysia (12.1% reported in 2008 Malaysian National Surveillance of Antibiotic Resistance Report) is relatively lower when compared to earlier studies reported in South Africa between year 2001-2003 (Shittu and Lin, 2006) and Italy between year 2000-2007 (Campanile *et al*., 2009). The low rifampicin resistance rate reported in UMMC might possibility due to limited usage of this drug in the hospital. Furthermore, rifampicin is often used along with fusidic acid or vancomycin and gentamicin or cloxacillin and gentamicin to treat infections due to MRSA in order to minimize the development of rifampicin-resistant MRSA (Norazah *et al*., 2002; National Antibiotic Report Malaysia, 2008).

Tetracycline-resistant MRSA had significantly increased over the six-year periods ($P < 0.01$), possibility because of an increased usage of tetracycline or doxycyline in the hospital (unpublished hospital records). Thong *et al*. (2009) reported

tetracycline-resistance rate of MRSA at 32% in year 2003. The increase in tetracycline-resistance rate has also been reported in another Malaysian hospital (Neela *et al*., 2008).

There was a significant increase in resistance rates towards trimethoprim-sulfamethoxazole from year 2003 to 2008. This was probably due to an increased usage of trimethoprim-sulfamethoxazole in the hospital (unpublished hospital records). Similar high resistance rates of trimethoprim-sulfamethoxazole were reported by Neela *et al*. (2008) (75%) and Thong *et al*. (2009) (73%).

There was a sharp increase in netilmicin-resistance rates between year 2003 and 2008. Thong *et al*. (2009) reported an increase in netilmicin-resistance rate from 16% in 2003 to 41% in year 2004. However, netilmicin-resistance rates (42%) noted in this study was lower when compared to the 68.7% from year 2006 - 2007 reported by Neela *et al*. (2008).

The resistance rates of clindamycin had increased slightly from 94% (in year 2003) to 96% (in year 2008) over six years. This was much higher than that previously reported in Malaysia by Neela *et al*. (2008) (0%), Ahmad *et al.* (2009) (0%) and Al-Talib *et al*. (2010) (6%). Similarly, the mupirocin-resistance rate was relatively higher than 1.25% reported in Malaysia (Norazah *et al*., 2001) and 2% reported in United States (Wolk *et al*., 2009). Although the mupirocin-resistance rate in UMMC had increased (5% in year 2008) over the years, this antibiotic is still of limited use in UMMC as it is only recommended for outpatients and not for in-patients (Lim *et al*., 2010). This antibiotic was also being used for the treatment of MRSA colonization in medical staff for a period of five days and then re-tested on the seventh day to ensure clearance of MRSA (Lim *et al*., 2010).

On the other hand, the rates of resistance to erythromycin reported in this study remained high (96%) in year 2003 and 2008. This is concordant with previously reported erythromycin-resistance rates from 81.2 - 96.0% (Neela *et al*., 2008; Ahmad *et*

al., 2009; Thong *et al*., 2009). Sam *et al*. (2008) reported that MRSA from UMMC is often resistant to erythromycin, gentamicin and ciprofloxacin. Similar observation was reported here as most of the MRSA strains isolated from year 2003, 2004, 2007 and 2008 from UMMC were also resistant to erythromycin, gentamicin and ciprofloxacin.

Besides, the significant increase of high-level tetracycline (MIC (≥ 256 μg/ml) and erythromycin (MIC (≥ 128 μg/ml) resistant strains between year 2003 and 2008 along with the co-resistance between erythromycin, ciprofloxacin and tetracycline is worrisome because choices of antimicrobial agents for treatment of life-threatening cases will be limited as use of tetracycline, ciprofloxacin and erythromycin is still common in Malaysian hospitals for treatment of respiratory tract and other nosocomial infections (National Antibiotic Guideline Malaysia, 2008).

Both inducible and constitutive macrolide, lincosamide and streptogramin (MLSB) phenotypes were observed among erythromycin-resistant strains with the majority (96%) showing inducible macrolide, lincosamide and streptogramin (i-MLSB) phenotype. The prevalence of i-MLSB isolates (92%) was much higher when compared to 63% reported in Korea (in 2004) (Lim *et al*., 2006).

5.2 β-lactam, mupirocin, gentamicin, vancomycin, linezolid, erythromycin, tetracycline and fusidic acid resistance genotypes in MRSA strains

Genotypic detection of β-lactam resistance genes indicated that all MRSA harboured *blaZ* gene. This is not surprising as penicillin resistance is due to the production of β-lactamases, which is encoded by *blaZ* gene (Olsen *et al*., 2006; Vali *et al*., 2008). A similar result was reported by Vali *et al*. (2008) as 97.5% (n=117) where their MRSA strains also harboured the *blaZ* gene.

On the other hand, genotypic detection of resistance genes also pointed out that erythromycin, tetracycline, gentamicin and mupirocin resistance were always attributed to the presence of resistance genes. The most prevalent resistance gene reported in erythromycin-resistant strains was *ermA* (85%) followed by *ermC* (21%) and *msrA* (2%). Similar observation was reported by Sekiguchi *et al.* (2004) where all their erythromycin-resistant strains harboured *ermA* gene. However, this differs from the report by Spiliopoulou *et al*. (2004) and Schmitz *et al.* (2000) where most of their erythromycin-resistant strains harboured *ermC* gene. In addition, the presence of different erythromycin resistance genes (*ermA, ermC* and *msrA*) were not directly related to higher MIC values (based on Kruskal-Wallis test). For example, 31% of erythromycin-resistant strains that harboured *ermA* or *ermC* gene was associated with MIC 8 - 64 µg/ml. This is concordant with the previous report by Sekiguchi *et al*. (2004) as all their *ermA*-positive strains were associated with low-level of erythromycin resistance (MIC > 4 µg/ml). Chavez-Bueno *et al*. (2005) reported that all the non-inducible MRSA strains harboured *msrA* gene, and a similar observation was reported here as all four *msrA*-positive strains showed negative results in the D-zone test (indication of non-inducible MRSA strains.

A majority of tetracycline-resistant strains harboured *tetM* (97%) followed by *tetK* (41%) gene. This is in-contrast to reports by Jones *et al*. (2006) and El-Mahdy *et al*. (2010) where *tetK* gene was the predominant gene in tetracycline-resistant strains. Spearman's rank correlation tests showed that a strain which harboured *tetK* gene also harboured *tetM* gene. This is in agreement with Schmitz *et al.* (2001) as their MRSA strain also harbours both *tetK* and *tetM*. Schmitz *et al.* (2001) also reported that strains with both *tetK* and *tetM* genes often display higher MIC values than strains containing a single gene. In contrast, this study showed the combination of *tetK* and *tetM* genes did

not display higher MIC values as over 78% of the strains that harboured both *tetK* and *tetM* together were associated with MIC value of 16 - 64 µg/ml.

All ten mupirocin-resistant strains harboured *ileS2* gene with two MRSA strains (MRSA0406-8 and MRSA0801-27) that display higher MIC of 256 µg/ml harboured additional *mupA* gene. This is in-contrast with the earlier reports by Yun *et al.* (2003) and Perez-Roth *et al.* (2006) which showed that presence of *ileS2* gene was associated with high-level mupirocin resistance. The association of *ileS2* gene with low mupirocin-resistance in UMMC strains could possibly due to lack of *ileS2* gene expression among the bacterial colonies as similar observation have been reported earlier by Anthony *et al.* (1999).

aac(6')-aph(2") gene that encodes for enzyme AAC(6')/APH(2") which inactivates a broad range of aminoglycoside, including gentamicin and netilmicin (Rouch *et al*., 1987; Hodel-Christian *et al*., 1991; Martineau *et al*., 2000;) was detected among all gentamicin- and netilmicin-resistant strains in this study. This showed a correlation between the presence of *aac(6')-aph(2")* gene with gentamicin-resistance, and similar observation was also being reported by Martineau *et al*. (2000).

No glycopeptides (vancomycin and teicoplanin) and linezolid resistance genes were detected in any of the MRSA strains. The absence of genes encoding for glycopeptides and linezolid resistance is important as these antimicrobial agents are the drug of choice to treat patients with MRSA infections (National Antibiotic Guideline Malaysia, 2008).

Although fusidic acid resistance is often associated with the presence of acquired-fusidic acid resistance genes (*fusB, fusC* or *fusD*) or mutation in *fusA* gene (Lannergard *et al*., 2009), no *fusB, fusC or fusD* gene was detected among fusidic acid-resistant strains in this study despite repeated experiments were carried out by using three different sets of published primers. Absence of *fusB* and *fusC* genes among fusidic

acid-resistant strain is uncommon as previous reports in Taiwan showed that 73.5% of fusidic acid-resistant strains harboured *fusC* gene (Chen *et al*., 2011) and more than 10% of fusidic acid resistant-strains in 13 European countries harboured either *fusB* or *fusC* gene (Castanheira *et al*., 2010b). Fusidic acid-resistant strains reported in this study might be different from those in different countries as none of these strains harboured *fusB, fusC* or *fusD* gene. The reduced-susceptibility of fusidic acid in UMMC could be due to mutational change in *fusA* or *fusE* genes.

5.3 Detection of tetracycline and gentamicin transposon-associated genes

Tn*5801*-like integrase was detected in majority (85%) of the *tetM*-positive strains suggesting that *tetM* gene is located on Tn*5801*-like conjugative transposons. The other *tetM* gene (15%) could be located on other transposon such as Tn*1545*. Association between *tetM* gene and Tn*1545* has been reported in other gram positive bacterium including *Listeria monocytogenes, Enterococcus* sp and *Streptococcus pneumoniae* (Bertrand *et al*., 2005; Agerso *et al*., 2006; Varaldo *et al*., 2009). In addition, Doucet-populaire *et al*. (1991) have indicated the transfer of conjugative transposon Tn*1545* from *E. faecalis* to *L. monocytogenes.*

Although Chow *et al.* (2007) reported that high-level gentamicin resistance is mediated by a Tn*4001*-like transposon, none of the gentamicin-resistant strains from this study harboured Tn*4001*-like transposon. The gentamicin-resistant strains from UMMC could be mediated by other transposons such as Tn*5281*. This Tn*5281* transposon is known as the main distributor of *aac(6')-aph(2")* gene among *E. faecalis* strains in Tehran hospitals (Feizabadi *et al*., 2008).

5.4 Transferability of erythromycin and tetracycline resistance determinants by transformation

Transformation experiments were carried out to determine if erythromycin and tetracycline phenotype could be transmissible to other *S. aureus* strains. The recipient strain used in this study was *S. aureus* ATCC29213, which harboured *blaZ* gene (resistant to penicillin).

The result from transformation experiments yielded success in six out of 30 MRSA strains where only erythromycin-resistance phenotype was transferable, and no tetracycline-resistance phenotype was transferred. Further analysis showed that only *ermC* was transmissible.

Although Monecke *et al*. (2009) reported that *tetK* gene was plasmid-borne and transmissible; none of the *tetK* gene was transferable in this study. Similarly, no *tetM* gene was transferable. Discrepancy observed might be due to the inherent property of the recipient *S. aureus* ATCC29213 as Schenk and Laddaga (1992) reported that this strain ATCC29213 has lower transformation efficiency when compared to another recipient strain, RN4220.

Size of plasmid carrying *ermC* gene reported in this study was similar to the plasmid size (2.5 kb) reported by Westh *et al*. (1995). Based on the *EcoR1* restriction profiles obtained, size of plasmids that were isolated from the transformants (~ 1.1 – 18.5 kB) was slightly smaller than its donor (~ 1.4 – 18.5 kB). This was because the donor strains harboured more than a single type of plasmids.

5.5 Mutations analysis of *rpoB* (rifampicin) and *fusA* (fusidic acid) resistance determinants

All rifampicin and fusidic acid-resistant strains were subjected for mutational analysis. The mutation identification and analysis of the rifampicin resistance was based

on the published cluster I (amino acid number 462 – 488) and II (amino acid number 515 – 530) of *rpoB* gene (Mick *et al*., 2010) whereas mutation analysis of fusidic acid resistance was based on *fusA* and *fusE* genes (Castanheira *et al*., 2010a, b).

Wichelhaus *et al.* (2002) reported that the presence of mutational change 484Arg/His or 477Ala/Asp in *rpoB* gene is capable of conferring high-level rifampicin resistance (MIC 256 µg/ml). However, in this study, the presence of the same mutational change was associated with MIC of 4 - 8 µg/ml. Mutational change 517Glu/Gln in five Malaysian MRSA strains with MIC 8 µg/ml is new and has not been reported elsewhere. Although Wichelhaus *et al.* (2002) reported that double mutations in *rpoB* gene can cause high resistance in rifampicin, this study showed that the double mutations in *rpoB* gene of five MRSA strains resulted in rifampicin resistance at MIC 8 µg/ml.

This study indicated that different types of mutations in *fusA* gene were associated with different levels of resistance (based on Kruskal-Wallis Test) in fusidic acid. For example, seven MRSA strains with high-level fusidic acid-resistance (MIC 256 µg/ml) were associated with mutational change 461Leu/Lys in *fusA* gene while non-synonymous change 461Leu/Ser in *fusA* gene confers to low-level resistance (MIC 4-8 µg/ml). Besier *et al*. (2003) and Canstanheira *et al.* (2010b) also showed that substitution of 461Leu/Lys in *fusA* gene is often linked to higher fusidic acid resistance (MIC 256 µg/ml) when compared to other alterations (461Leu/Ser) at the same amino acid 461'.

Besier *et al.* (2005) reported that mutation change 67Ala/Thr in *fusA* gene did not contribute to fusidic acid resistance, and that mutational change 67Ala/Thr is unable to compensate for the fitness loss due to resistance-mediating amino acid. However in this study, the presence of similar mutational change (67Ala/Thr) in *fusA* gene was associated with MIC values from 64 to 96 µg/ml.

One strain (MRSA0812-33) which had high-level resistance to fusidic acid (MIC 256µg/ml) has multiple mutations at 461Leu/Lys, 596Cys/Trp, 602Glu/Lys and 317Met/Trp. The role of three novels mutational change (317Met/Trp, 596Cys/Trp and 602Glu/Lys) remain unknown as mutational change 461Leu/Lys itself is associated with high-level of fusidic acid resistance.

The absence of *fusE* mutation is not surprising as this mutational change in *fusE* gene is rare and was only reported in one strain from Ireland (Castanheira *et al.,* 2010b).

Three fusidic acid-resistant strains (MRSA0302-4, MRSA0312-35 and MRSA0805-1) that were tested negative for the presence of acquired fusidic acid resistance genes that is *fusB, fusC* and *fusD* also showed no mutation in *fusA* and *fusE* genes. Similar observation was reported by Castanheira *et al.* (2010a) as mutational change was only seen in one out of five U.S isolates that did not carry acquired fusidic acid resistance genes.

Spearman's rank correlation tests showed that rifampicin-resistant strains were mostly sensitive to fusidic acid. This is in agreement with Chen *et al*. (2011) where majority of their fusidic acid resistant-strains were sensitive to rifampicin (MIC 0.5 µg/ml). This is important as combination therapy of rifampicin and fusidic acid with or without linezolid are used to treat patients with serious infections caused by MRSA with reduced vancomycin susceptibility (Howden *et al*., 2004).

5.6 Biofilm formation phenotype by congo red agar (CRA)

One hundred forty-three MRSA strains were categorized as biofilm formation bacteria using congo red agar (CRA) methods designed by Freeman *et al*. (1989). Detection of biofilm formation bacteria based on phenotypic method is important as

formation of biofilm itself is an example of the phenotypic changes in *S. aureus* (Khan *et al*., 2011).

The present study showed that invasive MRSA strains (80%) are often associated with biofilm formation when compared to colonization MRSA strains (75%). This is a cause of concern as the ability of *S.aureus* to form biofilm played an essential role in the virulence of the bacterium leading to the development of device-related infections (El-Din *et al*., 2011).

There is no correlation between biofilm formation and antimicrobial resistance ($P > 0.05$).

5.7 Virulence genes determinants by PCR

In this study, the majority of the strains were positive for the adhesion genes such as extracellular fibrinogen binding protein (*efb*) (96%), fibrinogen binding protein (*fnbA*) (96%), intracellular adhesion (*ica*) (78%) while hemolysin (*hlg*) and putative adhesin (*sdrE*) were amplified in 59% and 27%, respectively. Further analysis by using Spearman's rank correlation showed that all *efb* positive strains were tested positive for *fnbA* gene. Similarly, correlation between *hlg* and *ica* gene was observed. This is important as both *efb* and *fnb* genes are involved in the adherence of *S. aureus* strains to the host tissue (Ferry *et al*., 2005). *ica* gene is involved in the biofilm formation whereas *hlg* promotes host cell lysis, which results in injury of the alveolar-capillary barrier in the host cell (Ferry *et al*., 2005).

Overall, 54% of the MRSA strains harboured at least one type of enterotoxins (SEs) gene. The percentage of 2007 and 2008 strains harbouring SEs (62%) were higher when compared to 2003 and 2004 strains (36%) but lower when compared to a study done by Ghaznavi-Rad *et al*. (2010). The increase in the number of strains bearing SEs

genes could be caused by horizontal gene transfer between the strains as SEs genes are known to be carried by mobile genetic elements such as plasmids, pathogenicity islands, *SCCmec* and prophages (Hu *et al*., 2008). This is a cause for concern as SEs genes are often associated with food borne poisoning, toxin shock syndrome and other toxin mediated disease (Ferry *et al*., 2005; Ortega *et al*., 2010).

sea gene was the most common SEs gene present among the MRSA strains (31% of 2003 to 2004 strains and 43% of 2007 to 2008 strains). This concurred with the reports by Ghaznavi-Rad *et al*. (2010) in another tertiary hospital in Kuala Lumpur and Pourmand *et al*. (2009) in Tehran, Iran. However, this differed from the report by Sauer *et al.* (2008) as MRSA harbouring *seg* and *sej* genes was common in a University Hospital located in Czech Republic. In this study, 39% of MRSA strains isolated from invasive samples (tissue, wound, pus, blood and bone) and lower respiratory samples (tracheal secretion, nasopharyngeal secretion and sputum) were tested positive for *sea* gene. The incidence of *sea* (39%) and *sec* (19%) genes detected in this study was much higher than the result reported by Sauer *et al.* (2008). The frequency of *sea* gene reported in this study was also higher than 36.5% reported by Ghasemzadeh-Moghaddam *et al.* (2011). Similarly, the frequency of *sea* (30%) and *sec* (16%) gene obtained from nasal swabs was also higher when compared to study reported by Collery *et al.* (2008). However, it is lower when compared to study reported by Wang *et al.* (2010) where they showed that strains bearing *sea* were found to be associated with lower respiratory infections. Hu *et al.* (2008) indicated that *sec* and *tst* genes were associated with mobile genetic element type I vSa4. This study shows that the presence of *sea* gene was mainly associated with infections such as bloodstream infections, soft-tissue infections, bone infections and lower respiratory infections.

Although both *seg* and *sei* genes are located in the same operon, only one strain (MRSA0805-10) harboured *sec*, *seg* and *sei* genes. Similarly, the Spearman correlation

test showed that strains with *seg* gene most likely do not harbour *sei* gene (R= -0.923, $P < 0.05$). This concurred with the result reported by Collery *et al.* (2008). However, it differs from the observation shown by Sauer *et al.* (2008) where they indicated that *seg* virulence determinant was always associated with the *sei* gene.

Exfoliative toxin (encoded by *etd*) that can cause an inflammatory response of the skin was detected in MRSA0806-13 strain, and this strain belonged to *agr* type III. In contrast to the finding of Ghasemzadeh-Moghaddam *et al.* (2011) on SEB and ETA production among Malaysian *S. aureus* strains, no strain in this study possessed *eta, etb* and *seb* genes. Both *eta* and *etb* are important for superficial skin infections such as bullous impetigo (Mertz *et al*., 2007) whereas *seb* gene is often associated with food poisoning (Ferry *et al*. 2005; Ortega *et al*. 2010).

In this study, the number of MRSA strains (27%) harbouring *sdrE* genes was lower than the 89.5% reported in Netherland by Sabat *et al.* (2006). This is not surprising as the strains might harbour other *sdr* genes such as *sdrC* or *sdrD*. Eighteen invasive (22%) strains harboured *sdrE* gene. This is lower than the 56% reported earlier by Peacock *et al*. (2002). Although Sabat *et al*. (2006) reported that the absence of *sdrE* and *sdrD* gene in *S. aureus* will result in decreased potential in infecting bone, the result from this study showed that indigenous *S. aureus* in this tertiary hospital can cause bone infections even without the presence of *sdrE* gene. This infection could have involved other alleles of the *sdr* gene.

Fifty (27%) invasive strains were found to harbour hemolysin gene (*hlg*). This is not surprising as the previous report by Peacock *et al.* (2002) indicated that virulence factors such as *fnbA, cna, sdrE, hlg, sej, eta* and *ica* were significantly more common in invasive strains, and they contributed independently to virulence.

Fifty four (29%) of the invasive strains (blood, bone, pus, wound and tissue) were tested positive for *ica* gene. This *ica* gene plays an important role in the biofilm

formation (Martin-Lopez *et al*., 2002). Significance increase in the occurrence of *ica, sec* and *sei* genes among 2008 strains when compared to 2003 strains was observed. Furthermore, Spearman's rank correlation tests also showed that MRSA strains with *ica* and *hlg* genes showed a higher virulence potential as these strains also harboured SEs, exfoliative toxin and *tst* gene. This is a cause for concern as the biofilm associated bacteria is normally resistant to host immune systems and antimicrobial, and the presence of SEs will further weaken the host immune systems (Plata *et al*., 2009). SEs is associated with food poisoning whereas *tst* is the gene responsible for the toxic shock syndrome (Plata *et al*., 2009). The presence of *ica* and SEs genes will often complicate treatment.

There is no correlation ($P > 0.05$) between toxin genes profiles with antimicrobial resistance as strains with identical virulotypes frequently harboured different types of resistance genes.

5.8 Distribution of *pvl* genotypes among Malaysian MRSA strains

PVL toxin (encoded by *pvl* gene) that causes necrotic suppurative skin lesions (Hussain *et al*., 2007) was detected in five MRSA strains (2 in year 2007 and 3 in year 2008) cultured from wounds, tissue, tracheal secretion and nasal swabs. Among the five PVL positive strains, three were from invasive strains whereas two were from colonization strains. The prevalence of *pvl* gene among MRSA strains in this tertiary hospital (2.9% of invasive strains and 2.4% of colonization strains) were lower than 5% of invasive strains and 4.5% of carriage strains reported by Neela *et al.* (2008). However, the prevalence of *pvl* gene reported in this study was higher than 2% rate reported in ICU of 14 medial centres (2 University Hospital and 12 referral hospitals) located in The Netherland (Rijnders *et al*., 2009).

5.9 Distribution of *agr* types among Malaysian MRSA strains

In this study, the majority of the strains were of *agr* type I. This is consistent with previous reports by Peerayeh *et al*. (2009) and Ghasemzadeh-Moghaddam *et al*. (2011). Although Collery *et al*. (2008) reported that strains possessing *tst* gene are often associated with *agr* type III, only *tst* positive strain (MRSA0802-19) was associated with *agr* type I.

Both MRSA strains (MRSA0701-15 and MRSA0806-14) that were associated with *agr* type II harboured *seg* gene, and this differed from the result reported by Afroz *et al*. (2008) as their *agr* type II-MRSA strains harboured *pvl* gene.

Afroz *et al*. (2008) indicated that *pvl* positive strains were grouped into *agr* type II or III, however all the *pvl* positive MRSA strains in this study were associated with *agr* type I.

5.10 Distribution of SCC*mec* types among Malaysian MRSA strains

The predominant SCC*mec* type in this study was SCC*mec* type III (87%), and this SCC*mec* type III is also common in other Malaysian hospitals (Ahmad *et al*., 2009; Ghaznavi-Rad *et al*., 2010) as well as in other Asia countries, including Singapore, Thailand, Indonesia and Taiwan (Chongtrakool *et al*., 2006; Wang *et al*., 2007). In contrast, the predominant SCC*mec* types in Korea and Japan was SCC*mec* type II (Ko *et al*., 2005; Chongtrakool *et al*., 2006). The presence of different predominant SCC*mec* type in Malaysia when compared to Korea and Japan could possibly due to the presence of different endemic MRSA clones in these countries. The predominant MRSA clone in Malaysia was associated with SCC*mec* type III (ST239-III-t037) while the predominant MRSA clone in Korea and Japan was associated with SCC*mec* type II (ST5-II-t002) (Ghaznavi-Rad *et al.*, 2010; Song *et al*., 2011).

All the 25 SCC*mec* type IV strains in this study were associated with HA-MRSA. Similar observation was reported in Malaysia by Ahmad *et al.* (2009) as 11 out of 20 SCC*mec* type IV MRSA strains were associated with HA-MRSA. The presence of SCC*mec* type IV HA-MRSA has also been previously reported in Denmark (Faria *et al.*, 2005).

Besides, all SCC*mec* type IV isolates were susceptible to rifampicin, mupirocin, teicoplanin and vancomycin. This is in agreement with a previous report by Ahmad *et al.* (2009) that SCC*mec* type IV isolates are susceptible to four or more non β-lactam antibiotics. Similarly, D'Souza *et al.* (2010) also reported that 83% of their SCC*mec* type IV strains from India were susceptible to many classes of antimicrobials agents. Six out of 25 ST22 SCC*mec* type IV MRSA strains reported here were resistant to gentamicin. This differs from reports of Ahmad *et al*. (2009) (Malaysia) and Conceição *et al.* (2010) (Atlantic Azores island) where the ST22 strains were sensitive to gentamicin and tetracycline.

5.11 Genomic Diversity of MRSA based on PCR-RFLP of *coa*, PFGE, *spa*, MLST and *mec*-associated *dru* types

Genotyping by PCR-RFLP of *coa* gene using *AluI* enzyme and PFGE using *SmaI* enzyme showed that most of the MRSA strains were genetically related (shared more than 80% similarity) although they were cultured from different sources and different time periods. This is because all the MRSA strains used in this study were isolated from the same hospital, UMMC in a different time period (year 2003, 2004, 2007 and 2008). Norazah *et al*. (2003) reported that a majority of the MRSA strains isolated from eight tertiary hospitals in Malaysia from year 1997 until 1999 were

associated with three major PFGE patterns, indicating that a few predominant MRSA clones were circulating in Malaysian hospitals.

Four strains (MRSA0312-35, MRSA0803-29, MRSA0707-26 and MRSA0802-14) could not be typed by *coa*-RFLP typing despite, repeated attempts. Similar observation was reported by Sanjiv *et al.* (2008) as one out of 21 *S. aureus* strains studied did not produce any *coa* gene. Identical profiles were obtained in separate experiments using the same set of strains indicating that this subtyping method is reproducible.

Some of the MRSA strains (MRSA0305-10, MRSA0703-8, MRSA0801-9, MRSA0805-4, MRSA0808-19, MRSA0809-25, MRSA0811-10, MRSA0811-16, MRSA0811-24, MRSA0812-27, MRSA0812-31, MRSA0812-35 and MRSA0812-37) obtained from year 2003 and 2007 also shared similar *coa*-RFLP pattern (C13) and PFGE profile (SM7) with MRSA strains obtained from year 2008. This indicates the persistence of particular *coa*-RFLP and PFGE profiles in UMMC.

Some MRSA strains which were clonally related by PFGE (shared more than 80% similarity) harboured different types of enterotoxin genes. Similarly, some of the MRSA strains which were clonally related by *coa*-RFLP (shared more than 80% similarity) were also harboured different types of enterotoxin genes. This indicated that MRSA strains are able to acquire or lose enterotoxin genes as these genes are likely located on mobile genetic elements such as pathogenicity islands, plasmids and prophages (Hu *et al*., 2008).

Most of the PFGE clonally related strains (shared more than 80% similarity) shared very similar resistotypes. For example; all the strains from Cluster 1 of PFGE were resistant to erythromycin and gentamicin. Thirty-nine out of 48 MRSA strains from this Cluster 1 were resistant to ciprofloxacin, and 32 MRSA strains were also resistant to tetracycline and trimethoprim-sulfamethoxazole. In another example, all five

MRSA strains from Cluster 5 were resistant to oxacillin with one MRSA strain (MRSA0312-13) also resistant to erythromycin and gentamicin. This suggests that several clones (bacterial isolates that have been cultured independently from different time and sources but shared similar pulsed-field profiles) (van Belkum *et al*., 2007) of MRSA strains were circulating in this tertiary hospital during the study period. Some other MRSA strains from year 2007 and 2008 (MRSA0703-8, MRSA0805-21, MRSA0805-4, MRSA0812-22, MRSA0812-31 and MRSA0812-37) also shared similar resistotypes (resistant to oxacillin, erythromycin, gentamicin, ciprofloxacin, tetracycline, netilmicin and trimethoprim-sulfamethoxazole) as well as PFGE profiles (SM7) and this further implies the persistence of this clone within the hospital environment. MRSA strains with PFGE profile SM17 which were cultured from year 2003 and 2008 but from different wards further supports the notion of the circulation of a particular clone in the hospital.

Although PCR-RFLP of *coa* gene has been described as an excellent tool for rapid, reliable and in-expensive subtyping method for MRSA during outbreak and known to provide a reasonable result for subtyping of epidemiological un-related strains (Stranden *et al*., 2003; Himabindu *et al*., 2009), PFGE was found to be more discriminative than PCR-RFLP of *coa* gene in subtyping Malaysian MRSA strains. PFGE can differentiate strains, which are indistinguishable by PCR-RFLP of *coa* gene. In addition, PFGE showed higher reproducibility rate than PCR-RFLP of *coa* gene and able to subtype strains, which cannot by type by PCR-RFLP of *coa* gene.

Further analysis was carried out by using sequence based-typing such as *spa* typing, MLST and *mec*-associated *dru* typing. The cluster analysis based on *spa* types showed that most of the MRSA strains (98.5%) were closely related, grouped in *spa* CC1 and they shared the same *spa*-repeat succession (02-25-17) while cluster analysis

based on *dru* types indicated that over 60.1% of the MRSA strains studied were closely related with two *dru* types, dt13d and dt13g being the predominant clones.

Both *spa* and *dru* typing results concurred with the result from PCR-RFLP of *coa* gene as majority of the MRSA strains which were clonally related (share more than 80% similarity) were from *spa* types t037 and t421 and *dru* types within the *dru*CC1. Similarly, majority of the MRSA which were found to be clonally related by PFGE were also from *spa* type t037, t421, t6405, t363 and t2029 and *dru* types dt13d, dt13g, dt13n, dt13j, dt13ao, dt13p, dt13m, dt13f, dt13i.

In addition, 15 SCC*mec* type IV strains (with PFGE profiles SM73 - SM78 and ST80 - SM85) which were grouped to together with 73.4% similarity in PFGE were closely related by *spa* typing (shared 98.5% similarity) as they were from *spa* types t032, t1378 and t4184 and *dru* type dt10a. However, these 15 strains only shared 40.4% similarity in PCR-RFLP of *coa* gene with *coa*-RFLP profiles C3, C25, C33 to C36 and C40 to C43. This indicates that PFGE is an useful subtyping tool for determining SCC*mec* type IV strains.

Over 66.5% of the strains studied were of *spa* type t037, and it was present in year 2003, 2004, 2007 and 2008. This implies the persistence of these *spa* types within the hospital environment. In addition, Ghaznavi-Rad *et al.* (2009) also reported that over 90% of MRSA strains isolated between year 2007 and 2008 in another tertiary hospital (HKL) in Malaysia belonged to a single *spa* type t037. This indicates that *spa* type t037 could be the predominant *spa* type among Malaysian MRSA strains.

Two *spa* types (t6405 and t4150) which were introduced in 2008 were unique in Malaysia and have not been reported in other places (Ghaznavi-Rad *et al*., 2010; Lim *et al*., 2010). *spa* types t860, t4152 and t2029 which were also introduced in 2007 or 2008 were new in Malaysia even though they have previously been reported in Portugal, Sweden and Austria, respectively (Amorim *et al*., 2007; Ridom *spa* server).

Ghaznavi-Rad *et al.* (2011) reported the occurrence of six *dru* types (dt10a, dt14c, dt13d, dt13i, dt9w and dt13g) among strains from a tertiary hospital (HKL) in Malaysia during 2007 - 2008. However, this study indicated that these six *dru* types might have disseminated in Malaysia earlier than expected as these six *dru* types were detected in MRSA strains in UMMC since year 2003.

The 36 MRSA strains which were indistinguishable by PFGE (PFGE profile SM7) were differentiated into eight different *dru* types (dt13d, dt13g, dt11al, dt13l, dt14c, dt13q, dt10a and dt9w) and five different *spa* types (t037, t421, t4152, t4150 and t032). Besides, 21 MRSA strains which were indistinguishable by PFGE (PFGE profile SM17) were further differentiated into five *dru* types (dt13d, dt92, dt10a, dt13g and dt13j) and three *spa* types (t037, t421 and t6405). This indicated that both *spa* and *dru* typing were more discriminative than PFGE in subtyping MRSA strains from this tertiary hospital.

MLST analysis also showed that the majority (157/188; 83.5%) of MRSA belongs to the pandemic clone MLST type ST239. This indicates that ST239 was the predominant clone in UMMC, and similar results were reported in other tertiary hospitals in Malaysia (Ghaznavi-Rad *et al*., 2010), China (Xu *et al*., 2009), Germany (Wisplinghoff *et al*., 2005) and Russia (Baranovich *et al*., 2009). This Brazilian/Hungarian MRSA clone has advantageous genetic properties that enhance the ability of biofilm formation as well as the ability to adhere and invade human airway cells (Amaral *et al*., 2005). Similar observation was indicated in this study as over 77% of the biofilm-producer strains were from the pandemic clone ST239.

Both ST239 and ST241 are known to be evolved from ST8, within clonal complex 8 and they differed by a single-point mutation in the *yqil* locus. Although MLST ST241 is new in Malaysia, this genotype has been reported earlier in Germany

(Deurenburg *et al.*, 2006) and India (Gadepalli *et al.*, 2009). In this study, ST241 was also found to be associated with *spa* type t363.

The ST5 clone which was introduced in year 2007 was characterized by *spa* type t002, *dru* type dt10a and SCC*mec* type IV. The presence of ST5 with SCC*mec* type IV was also reported by Aires-de-Sousa *et al.* (2008) in Portugal. However, this MRSA ST5 which is also known as Rhinne Hesse epidemic strain is also associated with SCC*mec* type II and has been reported in German, Korean and Japan (Ko *et al*., 2005; Monecke *et al*., 2009).

ST573 clone reported in year 2008 is a single-locus variant of ST1 with a single-point mutation in the *pta* locus. Similarly, this clone was also related to another MRSA clone (ST188) reported in year 2007-2008 from another tertiary hospital (HKL) (Ghaznavi-Rad *et al*., 2010, 2011). Both clones were from the same clonal cluster, CC1 and they differed by three-point mutations in the *arcc, gmk* and *pta* locus.

Even though most of the strains in this study were closely related by MLST, some of the strains have greater diversity than MRSA strains isolated from another tertiary hospital in Malaysia. Seven clonal clusters, which are CC5 (ST5 and ST1178), CC6 (ST6), CC8 (ST239, ST241), CC1 (ST573 and ST772), CC20 (ST20), CC22 (ST22), CC80 (ST80) were observed among UMMC strains when compared to only four clonal clusters [CC1 (ST1 and ST188), CC7 (ST7), CC8 (ST239 and ST1283) and CC22 (ST22)] reported in another tertiary hospital (Hospital Kuala Lumpur) (Ghaznavi-Rad *et al*. 2010, 2011).

Among the ten major clones of MRSA (ST5, ST8, ST22, ST25, ST36, ST45, ST228, ST239, ST247 and ST250) reported worldwide (Deurenburg and Stobberigh, 2008), three MRSA clones (ST5, ST22 and ST239) were present in this hospital and other tertiary hospitals in Malaysia (Ahmad *et al*., 2009; Ghaznavi-Rad *et al*., 2010).

Ahmad *et al.* (2009) reported the presence of the Berlin clone (ST45-SCC*mec* type IV) among HA-MRSA between year 2006 and 2008 from Malaysian hospitals.

The higher resolution and greater discriminatory ability of *spa* and *mec*-associated *dru* typing methods could further differentiate ST239 clone into seven *spa* types (t037, t421, t6405, t4150, t4152, t2029 and t860) and 26 different *dru* types. In another study, Ghaznavi-Rad *et al.* (2011) also showed that *mec*-associated *dru* typing is useful to enhance the epidemiological discrimination of ST239. This suggest that combination of *spa* and *mec*-associated *dru* typing together might be useful in subtyping MRSA as both spa and *mec*-associated *dru* typing are more rapid, less laborious and relatively cheaper than MLST.

5.12 Evolutionary changes in MRSA based on *spa* typing, MLST and *mec*-associated *dru* typing

In this study, three different typing methods (*spa* typing, MLST and *mec*-associated *dru* typing) were used for the evolutionary study of MRSA strains isolated from four different years (2003, 2004, 2007 and 2008).

Maximum parsimony tree based on *spa* typing indicated that three *spa* types (t037, t421 and t1544) were detected since year 2003 with *spa* types' t037 and t421 associated with ST239 whereas *spa* type t1544 was associated with ST20. Five *spa* types (t032, t4184, t002, t304 and t860) were introduced in year 2007 with *spa* types' t032 and t4184 associated with ST22, *spa* type t002 associated with ST5, *spa* type t304 associated with ST6 and *spa* type t860 associated with ST239. Nine other *spa* types (t1378, t6405, t4150, t1107, t458, t4152, t657, t2029 and t363) were introduced in year 2008. Among the nine *spa* types, four *spa* types (t6405, t4150, t4152 and t2029) were associated with ST239.

The present data based on the maximum parsimony tree derived from *spa* types indicated that *spa* types t6405, t304, t002, t4184, t1378 and t032 might have originated from *spa* type t037 with 5 - 25 mutations differences whereas *spa* types' t4150, t1107, t458, t4152, t657, t860, t2029 and t363 might have originated from *spa* type t421 with 3 – 10 mutations differences. The data showed that 22 to 25 mutations and 3 - 4 generations gaps are required for *spa* type t1378, t032 and t4184 which are associated with ST22 (CC22) to evolve from t037 which were associated with ST239 (CC8).

Although both *spa* types t002 and t1107 were associated with MLST CC5, *spa* type t002 evolved from *spa* type t037 (MLST CC8) by 10 mutations whereas t1107 evolved from *spa* type t421 (MLST CC8) by 8 mutations and both t002 and t1107 were differed by 19 mutations. This indicates that *spa* typing has higher discriminatory power than MLST. In addition, s*pa* typing is also reported to have discriminatory power lies between PFGE and MLST (Malachowa *et al*., 2005).

On the other hand, the maximum parsimony tree derived from MLST showed that ST241 was evolved from ST239 by one mutation. This is expected as both ST239 and ST241 were originated from ST8 (CC8) and they shared six similar alleles including *arcC, gmk, glpF, aroE, pta* and *tpi.* ST772 was evolved from ST573 by one mutation different in *pta* locus.

It is noted that ST22 from clonal cluster C22 was evolved from ST239 (CC8) with one generation gap and seven mutations differences. ST6 (CC6) which was detected in year 2007 was evolved from ST239 (CC8) by three generation gaps and six mutations differences. The ST5 (CC5) which was detected in year 2007 evolved from ST239 (CC8) with 6 mutations differences and four generation gaps and ST1178 (CC5) (detected in year 2008) was evolved from ST5 with differences in two mutations in *aroE* and *tpi* locus.

Even though MLST is proven as an excellent method to study molecular evolution in MRSA, the present data support that *mec*-associated *dru* typing has a greater discriminatory ability than MLST for the analysis of highly clonal MRSA strains such as ST239. The present data based on the maximum parsimony tree indicated that 13 different *dru* types were detected since year 2003, and they were associated with ST239. Ten *dru* types (dt11am, dt15n, dt13q, dt13p, dt13j, dt13n, dt13f, dt13ao, dt12j, dt7v) which are associated with ST239 and *spa* type t037 have been introduced in year 2004, 2007 and 2008 with five *dru* types (dt13ao, dt13f, dt13p, dt13j and dt13n) evolved from dt13d by one mutation suggesting that some strains from these *dru* types might have originated from dt13d. The other five *dru* types (dt11am, dt15n, dt13q, dt12j and dt7v) were evolved from dt13d by 4 – 13 mutation differences and 1 – 5 generation gaps.

dru type dt10ax which is associated with *spa* type t421 and MLST ST239 was evolved from *dru* type dt13d with 6 mutations differences and 2 generation gaps. The *dru* type 11an which is associated with *spa* type t6405 and MLST ST239 was evolved from *dru* type dt13d with 5 mutations differences and 1 generation gap. On the other hand, dt10aw which is associated with ST22 might have evolved from dt10 with only three mutation differences.

However, *mec*-associated *dru* typing method cannot be applied on MSSA strains as they lack *mec*A gene. Another disadvantage of this *mec*-associated *dru* typing was it is unable to differentiate different lineages as dt10a consists of MRSA strains from five different lineages (CC5, CC6, CC8, CC22 and CC80). Therefore, *mec*-associated *dru* typing should be used in combination with another sequence typing method (i.e *spa* typing) to characterize MRSA strains.

5.13 Clonal evolution of Malaysian MRSA from year 2003, 2004, 2007 and 2008

This study demonstrated that the predominant ST239-t037 had decreased from 96% in 2003 to 56.8% in 2008 whereas a slight increase in the prevalence of ST239-t421 from 1.9% in 2003 to 18.0% in 2008. It is observed that ST239-t421 clone (from 2003) that was from ICU might have spread to other wards; i.e medical, dialysis, surgical and orthopaedic in 2008 suggesting changes of MRSA clones in UMMC during the study period.

Further combined analysis with MLST-*spa-dru* typing demonstrated that the prevalence rate of ST239-t037-dt13d had decreased from 44.2% in 2003 to 13.5% in 2008 and was replaced by MLST239-t037-dt13g and other new emerging of MRSA clones that accounted for 68.5% of MRSA strains in 2008. ST239-t037-dt13g could easily be transmitted in this hospital as they have spread from two wards (in 2003) to six different wards (in 2008). This dt13g which was cultured from wound and blood samples (in 2003) and infected tissue, wound, pus and bone as well as colonized respiratory tract (nasal swabs, sputum, nasopharyngeal secretion) and catheter tips in year 2008. This further suggests the changes of MRSA clones in UMMC over the study periods.

Both ST239-t037-dt13d and ST239-t421-dt13d might have a stronger survival advantage when compared to ST239-t037-dt13l, ST239-t037-dt13m, ST239-t037-dt14h, ST239-t037-dt13i, ST20-t1544-dt14c, ST239-t037-dt15l and ST239-t037-dt15m as they were present throughout the study periods.

5.14 Limitation of the study

The limitations of this study were the number of MRSA strains in the different study periods (2003, 2004, 2007 and 2008) varied and there were no MRSA strains

from year 2005 and 2006 included in the analysis. This was mainly due to lack of proper storage maintenance of the bacterial stock cultures in the hospital as MRSA strains from the earlier years (2003 to 2007) were kept in stab cultures, which could not be kept for a longer period of time. Unlike the earlier years, MRSA strains from year 2008 onwards were kept in glycerol stock at - 85°C and they could be revived.

There are no positive controls available for the detection of *ermB, tetL, tetO, tetS, cfrA, fusB, fusC, fusD, vanA* and *vanB* resistance genes; Tn*916* and Tn*4001* transposon-associated genes; *cna, fnbB, seb, sed, see, seh, sej, eta* and *etb* virulence genes and *agr* type IV. Therefore, negative results should be treated with caution.

5.15 Consideration for future study

One fusidic acid-resistant MRSA strain (MRSA0812-33) which had multiple mutations, including three novel substitution (317Met/Trp, 596Cys/Trp and 602Glu/Lys) exhibited high levels of fusidic acid resistance (MIC 256 µg/ml). The role of this three newly found mutational change in *fusA* is unknown and needs further investigation as mutational change 461Leu/Lys alone is capable of conferring high level of fusidic acid resistance.

On the other hand, this study also addressed several items which required more precise research in the future. Clinical information of persons with community-acquired MRSA should be collected in order to compare the association of CA-MRSA and HA-MRSA in Malaysia. In addition, comparison between coagulase-negative *Staphylococcus* and MRSA in Malaysia should be studied in more detail.

CHAPTER 6

CONCLUSIONS

The objectives of this study were to investigate the antimicrobial susceptibility patterns of 14 commonly used antibiotics in this tertiary hospital, the presence of selected plasmid-mediated erythromycin, tetracycline, mupirocin and β-lacam resistance genes and the transmissibility of the erythromycin and tetracycline phenotypes as well as the chromosomal mutations involved in rifampicin and fusidic acid resistance; the prevalence of virulence gene and *agr* grouping of MRSA strains; genomic relatedness of MRSA strains by SCC*mec* typing, PCR-RFLP of *coa* gene, pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), *mec*-associated *dru* typing and *spa* typing as well as the evolution of MRSA strains in 2003, 2004, 2007 and 2008.

An increase of the tetracycline and netilmicin resistance rates and occurrence of multidrug-resistant (MDR) strains from year 2008 when compared to year 2003 were observed. The antibiotic resistance rates and the occurrence of MDR strains were also higher when compared to a previous UMMC report (Thong *et al.*, 2009). Moreover, the erythromycin, clindamycin, gentamicin and ciprofloxacin resistance rates remain high over a six-year period. Tetracycline-resistant strains often show co-resistance towards ciprofloxacin and erythromycin. Vancomycin remains the most active agent 'in-vitro' against *S. aureus* infection followed by linezolid and teicoplanin.

Erythromycin, tetracycline, gentamicin and mupirocin resistance is always attributed to the presence of resistance genes. The *ermA* and *tetM* were the predominant genes detected in erythromycin- and tetracycline-resistant strains, respectively whereas *ileS2* gene was the most frequent gene detected in mupirocin-resistant strains. The presence of *ermA*, *ermC* and *msrA* genes were associated with erythromycin resistance

whereas presence of *tetK* and *tetM* genes were associated with tetracycline resistance among Malaysian MRSA strains. The presence of *ileS2* and *mupA* genes were responsible for mupirocin resistance while the presence of *aac(6')-aph(2")* gene was responsible for aminoglycoside (gentamicin and netilmicin) resistance among Malaysian MRSA strains.

Associations of resistance genes (*ermC, tetM* and *tetK*) with mobile genetic elements such as transposons possibly enhance the spread of resistant traits in MRSA. Transmissibility of the erythromycin phenotype in MRSA strains was demonstrated in the transformation experiments carried out. Results indicated that *ermA* gene was plasmid-encoded thereby increasing their mobility and transmissibility.

Different amino acid alterations are responsible for rifampicin and fusidic acid resistance among Malaysian MRSA strains. The presence of double mutation in *rpoB* gene often involved in high-rifampicin resistance (MIC 8 µg/ml) while amino acid alteration at 461Leu/Lys often linked with high-fusidic acid resistance (MIC 256 µg/ml).

Increase of MRSA strains with toxin genes over the six-years period signal the potential loss of the usage of antimicrobial agents in treating MRSA infections as MRSA strain with virulence factors is normally resistant to host immune systems and other antimicrobial agents. *agr* type I and SCC*mec* type III remained as the predominant *agr* and SCC*mec* type among MRSA strains in UMMC. The *pvl* gene was more common among invasive strains than colonization strains

DNA fingerprinting carried out using gel-based fingerprinting methods (PCR-RFLP of *coa* gene and PFGE) and sequence typing methods (MLST, *spa* and *mec*-associated *dru* typing) showed that MRSA strains in this tertiary hospital were genetically related. The MDR MRSA clinical isolates from UMMC were mostly

genetically related, suggesting that few predominant clones of the species are involved in infection.

MLST type ST239 and *spa* type t037 are the predominant MRSA clone in this tertiary hospital in Malaysia. Moreover, 23 different *dru* types were associated with ST239-t037 with ten *dru* types being introduced in later years suggesting that they might have evolved from dt13d and dt15n might also be possibly evolved from dt15l.

The combined analysis by MLST-*spa*-*dru* types indicated the changes of MRSA clones at UMMC in Malaysia where ST239-t037-dt13d and other MRSA clones in 2003 were replaced by ST239-t037-dt13g and other new emerging *spa* and *dru* types.

Correlation between DNA profiles and resistotypes was observed. Isolates with indistinguishable PFGE profiles often have similar antibiotic susceptibility patterns even though there are variations in certain antibiograms in distinct clones of MRSA.

On the other hand, no direct correlation between DNA profiles and virulotypes were observed as strains with identical DNA profiles frequently belonged to different virulotypes. This was not surprising as majority of the virulence determinants were found to be encoded on transmissible mobile elements and their acquisition would, at most, lead to minimal changes in the genetic profiles of the recipient strains.

The data from this current study may act as reference for monitoring mupirocin, rifampicin, fusidic acid and the prevalence of virulence among Malaysian MRSA strains over extended time periods. Furthermore, the data in this study underline the necessity of surveillance typing in order to control MRSA strains in this hospital.

BIBLIOGRAPHY

Afroz, S., Kobayashi, N., Nagashima, S., Alam, M. M., Hossain, A. B., Rahman, M. A., . . . Hossain, M. A. (2008). Genetic characterization of *Staphylococcus aureus* isolates carrying panton-valentine leukocidin genes in Bangladesh. *Japanese Journal of Infectious Disease, 61*(5), 393-396.

Ahmad, N., Nawi, S., Rajasekaran, G., Maning, N., Aziz, M. N., Husin, A., & Rahman, N. I. (2010). Increased vancomycin minimum inhibitory concentration among *Staphylococcus aureus* isolates in Malaysia. *Journal of Medical Microbiology, 59*(Pt 12), 1530-1532.

Ahmad, N., Ruzan, I. N., Abd Ghani, M. K., Hussin, A., Nawi, S., Aziz, M. N., . . . Eow, V. L. (2009). Characteristics of community- and hospital-acquired meticillin-resistant *Staphylococcus aureus* strains carrying SCC*mec* type IV isolated in Malaysia. *Journal of Medical Microbiology, 58*(Pt 9), 1213-1218.

Aires-de-Sousa, M., Correia, B., & de Lencastre, H. (2008). Changing patterns in frequency of recovery of five methicillin-resistant *Staphylococcus aureus* clones in Portuguese hospitals: surveillance over a 16-year period. *Journal of Clinical Microbiology, 46*(9), 2912-2917.

Aires de Sousa, M., Crisostomo, M. I., Sanches, I. S., Wu, J. S., Fuzhong, J., Tomasz, A., & de Lencastre, H. (2003). Frequent recovery of a single clonal type of multidrug-resistant *Staphylococcus aureus* from patients in two hospitals in Taiwan and China. *Journal of Clinical Microbiololgy, 41*(1), 159-163.

Akpaka, P. E., Kissoon, S., Swanston, W. H., & Monteil, M. (2006). Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus* isolates from Trinidad & Tobago. *Annal Clinical of Microbiology and Antimicrobial, 5*, 16.

Almer, L. S., Shortridge, V. D., Nilius, A. M., Beyer, J. M., Soni, N. B., Bui, M. H., . . . Flamm, R. K. (2002). Antimicrobial susceptibility and molecular characterization of community-acquired methicillin-resistant *Staphylococcus aureus. Diagnostic Microbiology and Infectious Disease, 43*(3), 225-232.

Alreshidi, M. A., & Mariana, N. S. (2011). Increasing rate of detection of fusidic acid resistance in methicillin-resistant *Staphylococcus aureus* isolated from clinical samples in Malaysia. *Medical Journal of Malaysia, 66*(3), 276.

Al-Talib, H., Hasan, H., Yean, C. Y., Al-Ashwal, S. M., & Ravichandran, M. (2011). Fatal necrotizing pneumonia caused by Panton-Valentine leukocidin-producing hospital-acquired *Staphylococcus aureus*: a case report. *Japanese Journal of Infectious Disease, 64*(1), 58-60.

Al-Talib, H. I., Yean, C. Y., Al-Jashamy, K., & Hasan, H. (2010). Methicillin-resistant *Staphylococcus aureus* nosocomial infection trends in Hospital Universiti Sains Malaysia during 2002-2007. *Ann Saudi Med, 30*(5), 358-363.

Amaral, M. M., Coelho, L. R., Flores, R. P., Souza, R. R., Silva-Carvalho, M. C., Teixeira, L. A., . . . Figueiredo, A. M. (2005). The predominant variant of the

Brazilian epidemic clonal complex of methicillin-resistant *Staphylococcus aureus* has an enhanced ability to produce biofilm and to adhere to and invade airway epithelial cells. *Journal of Infectious Disease, 192*(5), 801-810.

Amorim, M. L., Faria, N. A., Oliveira, D. C., Vasconcelos, C., Cabeda, J. C., Mendes, A. C., . . . de Lencastre, H. (2007). Changes in the clonal nature and antibiotic resistance profiles of methicillin-resistant *Staphylococcus aureus* isolates associated with spread of the EMRSA-15 clone in a tertiary care Portuguese hospital. *Journal of Clinical Microbiology, 45*(9), 2881-2888.

Anthony, R. M., Connor, A. M., Power, E. G., & French, G. L. (1999). Use of the polymerase chain reaction for rapid detection of high-level mupirocin resistance in staphylococci. *European Journal of Clinical Microbiology and Infectious Disease, 18*(1), 30-34.

Arciola, C. R., Campoccia, D., Gamberini, S., Baldassarri, L., & Montanaro, L. (2005). Prevalence of *cna, fnbA* and *fnbB* adhesin genes among *Staphylococcus aureus* isolates from orthopedic infections associated to different types of implant. *FEMS Microbiology Letters, 246*(1), 81-86.

Agerso, Y., Pedersen, A. G., & Aarestrup, F. M. (2006). Identification of Tn*5397*-like and Tn*916*-like transposons and diversity of the tetracycline resistance gene *tet(M)* in enterococci from humans, pigs and poultry. *Journal of Antimicrobial Chemotherapy, 57*(5), 832-839.

Argudin, M. A., Mendoza, M. C., & Rodicio, M. R. (2010). Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins (Basel), 2*(7), 1751-1773.

Arias, C. A., Vallejo, M., Reyes, J., Panesso, D., Moreno, J., Castaneda, E., . . . Quinn, J. P. (2008). Clinical and microbiological aspects of linezolid resistance mediated by the *cfr* gene encoding a 23S rRNA methyltransferase. *Journal of Clinical Microbiology, 46*(3), 892-896.

Baranovich, T., Zaraket, H., Shabana, II, Nevzorova, V., Turcutyuicov, V., & Suzuki, H. (2010). Molecular characterization and susceptibility of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates from hospitals and the community in Vladivostok, Russia. *Clinical Microbiology and Infections, 16*(6), 575-582.

Bell, J. M., & Turnidge, J. D. (2002). High prevalence of oxacillin-resistant *Staphylococcus aureus* isolates from hospitalized patients in Asia-Pacific and South Africa: results from SENTRY antimicrobial surveillance program, 1998-1999. *Antimicrobial Agents and Chemotherapy,* 46(3), 879-881.

Besier, S., Ludwig, A., Brade, V., & Wichelhaus, T. A. (2005). Compensatory adaptation to the loss of biological fitness associated with acquisition of fusidic acid resistance in *Staphylococcus aureus. Antimicrobial Agents and Chemotherapy, 49*(4), 1426-1431.

Besier, S., Ludwig, A., Brade, V., & Wichelhaus, T. A. (2003). Molecular analysis of fusidic acid resistance in *Staphylococcus aureus*. *Molecular Microbiology, 47*(2), 463-469.

Besier, S., Ludwig, A., Zander, J., Brade, V., & Wichelhaus, T. A. (2008). Linezolid resistance in *Staphylococcus aureus*: gene dosage effect, stability, fitness costs, and cross-resistances. *Antimicrobial Agents and Chemotherapy, 52*(4), 1570-1572.

Bertrand, S., Huys, G., Yde, M., D'Haene, K., Tardy, F., Vrints, M., . . . Collard, J. M. (2005). Detection and characterization of *tet(M)* in tetracycline-resistant *Listeria* strains from human and food-processing origins in Belgium and France. *Journal of Medical Microbiology, 54*(Pt 12), 1151-1156.

Borchardt, S.M. (2005). Outbreak of methicillin-resistant *Staphylococcus aureus* skin infections among high school athletes in Illinois. *Illinois Infectious Disease Report, 2(3).*

Boyce, J. M., Cookson, B., Christiansen, K., Hori, S., Vuopio-Varkila, J., Kocagoz, S., . . . Pittet, D. (2005). Meticillin-resistant *Staphylococcus aureus*. *Lancet Infectious Disease, 5*(10), 653-663.

Brown, D. F., Edwards, D. I., Hawkey, P. M., Morrison, D., Ridgway, G. L., Towner, K. J., & Wren, M. W. (2005). Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* (MRSA). *Journal of Antimicrobial Chemotherapy, 56*(6), 1000-1018.

Brown, J. W., & Grilli, A. (1998). An emerging superbug. *Staphylococcus aureus* becomes less susceptible to vancomycin. *MLO Medical Laboratory Obstretic, 30*(1), 26-32; quiz 34-25.

Campanile, F., Bongiorno, D., Borbone, S., & Stefani, S. (2009). Hospital-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) in Italy. *Annals of Clinical Microbiology and Antimicrobial, 8*, 22.

Campanile, F., Bongiorno, D., Borbone, S and Stefani, S. (2010). Methicillin-resistant *Staphylococcus aureus* evolution – the multiple facets of an old pathogen. *European Infectious Disease,* 4(1), 70-76.

Campbell, K. M., Vaughn, A. F., Russell, K. L., Smith, B., Jimenez, D. L., Barrozo, C. P., . . . Ryan, M. A. (2004). Risk factors for community-associated methicillin-resistant *Staphylococcus aureus* infections in an outbreak of disease among military trainees in San Diego, California, in 2002. *Journal of Clinical Microbiology, 42*(9), 4050-4053.

Castanheira, M., Watters, A. A., Bell, J. M., Turnidge, J. D., & Jones, R. N. (2010a). Fusidic acid resistance rates and prevalence of resistance mechanisms among *Staphylococcus* spp. isolated in North America and Australia, 2007-2008. *Antimicrobial Agents and Chemotherapy, 54*(9), 3614-3617.

Castanheira, M., Watters, A. A., Mendes, R. E., Farrell, D. J., & Jones, R. N. (2010b). Occurrence and molecular characterization of fusidic acid resistance mechanisms among *Staphylococcus* spp. from European countries (2008). *Journal of Antimicrobial Chemotherapy, 65*(7), 1353-1358.

Chambers, H. F. (1997). Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. *Clinical Microbiology Review, 10*(4), 781-791.

Chambers, H. F. (2001). The changing epidemiology of *Staphylococcus aureus*? *Emerging Infectious Disease, 7*(2), 178-182.

Chavez-Bueno, S., Bozdogan, B., Katz, K., Bowlware, K. L., Cushion, N., Cavuoti, D., . . . Appelbaum, P. C. (2005). Inducible clindamycin resistance and molecular epidemiologic trends of pediatric community-acquired methicillin-resistant *Staphylococcus aureus* in Dallas, Texas. *Antimicrobial Agents and Chemotherapy, 49*(6), 2283-2288.

Chen, H. J., Hung, W. C., Tseng, S. P., Tsai, J. C., Hsueh, P. R., & Teng, L. J. (2010). Fusidic acid resistance determinants in *Staphylococcus aureus* clinical isolates. *Antimicrobial Agents and Chemotherapy, 54*(12), 4985-4991.

Chen, C. M., Huang, M., Chen, H. F., Ke, S. C., Li, C. R., Wang, J. H., & Wu, L. T. (2011). Fusidic acid resistance among clinical isolates of methicillin-resistant *Staphylococcus aureus* in a Taiwanese hospital. *BMC Microbiology, 11*, 98.

Chongtrakool, P., Ito, T., Ma, X. X., Kondo, Y., Trakulsomboon, S., Tiensasitorn, C., . . . Hiramatsu, K. (2006). Staphylococcal cassette chromosome *mec* (SCC*mec*) typing of methicillin-resistant *Staphylococcus aureus* strains isolated in 11 Asian countries: a proposal for a new nomenclature for SCC*mec* elements. *Antimicrobial Agents and Chemotherapy, 50*(3), 1001-1012.

Chopra, I., & Roberts, M. (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology Molecular Biology Review, 65*(2), 232-260

Chow, V. C., Hawkey, P. M., Chan, E. W., Chin, M. L., Au, T. K., Fung, D. K., & Chan, R. C. (2007). High-level gentamicin resistance mediated by a Tn*4001*-like transposon in seven nonclonal hospital isolates of *Streptococcus pasteurianus*. *Antimicrobial Agents and Chemotherapy, 51*(7), 2508-2513.

Chung, M., Dickinson, G., De Lencastre, H., & Tomasz, A. (2004). International clones of methicillin-resistant *Staphylococcus aureus* in two hospitals in Miami, Florida. *Journal of Clinical Microbiology, 42*(2), 542-547.

Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, Twenty informational supplement. Approved standard MS100-S20. Wayne: CLSI; 2010.

Collery, M. M., Smyth, D. S., Twohig, J. M., Shore, A. C., Coleman, D. C., & Smyth, C. J. (2008). Molecular typing of nasal carriage isolates of *Staphylococcus aureus* from an Irish university student population based on toxin gene PCR, *agr* locus types and multiple locus, variable number tandem repeat analysis. *Journal of Medical Microbiology, 57*(Pt 3), 348-358.

Colombari, V., Mayer, M. D., Laicini, Z. M., Mamizuka, E., Franco, B. D., Destro, M. T., & Landgraf, M. (2007). Foodborne outbreak caused by *Staphylococcus*

aureus: phenotypic and genotypic characterization of strains of food and human sources. *Journal of Food Protection, 70*(2), 489-493.

Conceicao, T., Tavares, A., Miragaia, M., Hyde, K., Aires-de-Sousa, M., & de Lencastre, H. (2010). Prevalence and clonality of methicillin-resistant *Staphylococcus aureus* (MRSA) in the Atlantic Azores islands: predominance of SCC*mec* types IV, V and VI. *European Journal of Clinical Microbiology and Infectious Disease, 29*(5), 543-550.

Corrente, M., Normanno, G., Martella, V., Bellacicco, A. L., Quaglia, N. C., Dambrosio, A., . . . Buonavoglia, C. (2007). Comparison of methods for the detection of methicillin resistance in *Staphylococcus aureus* isolates from food products. *Letter of Applied Microbiology, 45*(5), 535-539.

Dale, G. E., Then, R. L., & Stuber, D. (1993). Characterization of the gene for chromosomal trimethoprim-sensitive dihydrofolate reductase of *Staphylococcus aureus* ATCC 25923. *Antimicrobial Agents and Chemotherapy, 37*(7), 1400-1405.

David, M. Z., Glikman, D., Crawford, S. E., Peng, J., King, K. J., Hostetler, M. A., . . . Daum, R. S. (2008). What is community-associated methicillin-resistant *Staphylococcus aureus*? *Journal of Infectious Disease, 197*(9), 1235-1243.

Demir, C., Aslantas, O., Duran, N., Ocak, S and Ozer, B. (2011). Investigation of toxin genes in *Staphylococcus aureus* strains isolates in Mustafa Kemal University Hospital. *Turkey Journal of Medical Science, 41*(2), 343-352.

de Sousa, M. A., & de Lencastre, H. (2003). Evolution of sporadic isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) in hospitals and their similarities to isolates of community-acquired MRSA. *Journal of Clinical Microbiology, 41*(8), 3806-3815.

de Vries, L. E., Christensen, H., Skov, R. L., Aarestrup, F. M., & Agerso, Y. (2009). Diversity of the tetracycline resistance gene *tet(M)* and identification of Tn*916*- and Tn*5801*-like (Tn*6014*) transposons in *Staphylococcus aureus* from humans and animals. *Journal of Antimicrobial Chemotherapy, 64*(3), 490-500.

Deurenberg, R.H., Kalenic, S., Friedrich, A.W., van Tiel, F.H., & Stobberingh, E.E. (2006). Molecular epidemiology of methicillin-resistant *Staphylococcus aureus*. *FORMATEX, 2007*, 766-777.

Deurenberg, R. H., & Stobberingh, E. E. (2008). The evolution of *Staphylococcus aureus*. *Infections, Genetic and Evolution, 8*(6), 747-763.

Deurenberg, R. H., Vink, C., Kalenic, S., Friedrich, A. W., Bruggeman, C. A., & Stobberingh, E. E. (2007). The molecular evolution of methicillin-resistant *Staphylococcus aureus*. *Clinical Microbiology and Infections, 13*(3), 222-235.

Dorsch, M.R. (2007). Rapid detection of bacterial antibiotic resistance: preliminary evaluation of PCR assays targeting tetracycline resistance genes. Human protection and performance division, Commonwealth of Australia. *DSTO-TR-2059*.

do Carmo, L.S., Dias, R.S., Linardi, V.R., de Sena, M.J., & dos Santos, D.A. (2003). An outbreak of staphylococcal food poisoining in the municipality of Passos, MG, Brazil. *Brazilian Archieve Biology and Technology, 46*(4), 581-586.

Doucet-Populaire, F., Trieu-Cuot, P., Dosbaa, I., Andremont, A., & Courvalin, P. (1991). Inducible transfer of conjugative transposon Tn*1545* from *Enterococcus faecalis* to *Listeria monocytogenes* in the digestive tracts of gnotobiotic mice. *Antimicrobial Agents and Chemotherapy, 35*(1), 185-187.

D'Souza, N., Rodrigues, C., & Mehta, A. (2010). Molecular characterization of methicillin-resistant *Staphylococcus aureus* with emergence of epidemic clones of sequence type (ST) 22 and ST772 in Mumbai, India. *Journal of Clinical Microbiology, 48*(5), 1806-1811.

El-Din, S.S.S., El-Rehewy, M.S., Ghazaly, M.M, & Abd-Elhamid, M.H. (2011). Biofilm formation by blood stream staphylococcal isolates from febrile pediatric cancer patients at South Egypt cancer institute. *Journal of American Science, 7*, 674-686.

El Helali, N., Carbonne, A., Naas, T., Kerneis, S., Fresco, O., Giovangrandi, Y., . . . Astagneau, P. (2005). Nosocomial outbreak of staphylococcal scalded skin syndrome in neonates: epidemiological investigation and control. *Journal of Hospital Infections, 61*(2), 130-138.

El-Mahdy, T.S., Aballa, S., El-Domany, R., & Snelling, A.M. (2010). Investigation of MLST and tetracycline resistance in coagulase-negative staphylococci isolated from the skin of Egyptian acne patients and controls. *Journal of American Science*, *6*, 880-888.

Enright, M. C., Day, N. P., Davies, C. E., Peacock, S. J., & Spratt, B. G. (2000). Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *Journal of Clinical Microbiology, 38*(3), 1008-1015.

Enright, M. C., Robinson, D. A., Randle, G., Feil, E. J., Grundmann, H., & Spratt, B. G. (2002). The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proceedings of the National Acedemy of Sciences of the United States of America, 99*(11), 7687-7692.

Faria, N. A., Oliveira, D. C., Westh, H., Monnet, D. L., Larsen, A. R., Skov, R., & de Lencastre, H. (2005). Epidemiology of emerging methicillin-resistant *Staphylococcus aureus* (MRSA) in Denmark: a nationwide study in a country with low prevalence of MRSA infection. *Journal of Clinical Microbiology, 43*(4), 1836-1842.

Feil, E. J., Nickerson, E. K., Chantratita, N., Wuthiekanun, V., Srisomang, P., Cousins, R., . . . Peacock, S. J. (2008). Rapid detection of the pandemic methicillin-resistant *Staphylococcus aureus* clone ST 239, a dominant strain in Asian hospitals. *Journal of Clinical Microbiology, 46*(4), 1520-1522.

Feizabadi, M. M., Shokrzadeh, L., Sayady, S., & Asadi, S. (2008). Transposon Tn5281 is the main distributor of the aminoglycoside modifying enzyme gene among isolates of *Enterococcus faecalis* in Tehran hospitals. *Canadian Journal of Microbiology, 54*(10), 887-890.

Feng, Y., Chen, C. J., Su, L. H., Hu, S., Yu, J., & Chiu, C. H. (2008). Evolution and pathogenesis of *Staphylococcus aureus*: lessons learned from genotyping and comparative genomics. *FEMS Microbiology Review, 32*(1), 23-37.

Ferry, T., Perpoint, T., Vandenesch, F., & Etienne, J. (2005). Virulence determinants in *Staphylococcus aureus* and their involvement in clinical syndromes. *Current Infectious Disease Report, 7*(6), 420-428.

Fong, R. K., Low, J., Koh, T. H., & Kurup, A. (2009). Clinical features and treatment outcomes of vancomycin-intermediate *Staphylococcus aureus* (VISA) and heteroresistant vancomycin-intermediate *Staphylococcus aureus* (hVISA) in a tertiary care institution in Singapore. *European Journal of Clinical Microbiology and Infectious Disease, 28*(8), 983-987.

Fontana, J., Stout, A., Bolstorff, B., & Timperi, R. (2003). Automated ribotyping and pulsed-field gel electrophoresis for rapid identification of multidrug-resistant *Salmonell*a serotype newport. *Emerging Infectious Disease, 9*(4), 496-499.

Freeman, D. J., Falkiner, F. R., & Keane, C. T. (1989). New method for detecting slime production by coagulase negative staphylococci. *Journal of Clinical Pathology, 42*(8), 872-874.

Gadepalli, R., Dhawan, B., Kapil, A., Sreenivas, V., Jais, M., Gaind, R., . . . Udo, E. E. (2009). Clinical and molecular characteristics of nosocomial meticillin-resistant *Staphylococcus aureus* skin and soft tissue isolates from three Indian hospitals. *Journal of Hospital Infections, 73*(3), 253-263.

Gardella, N., Picasso, R., Predari, S. C., Lasala, M., Foccoli, M., Benchetrit, G., . . . Gutkind, G. (2005). Methicillin-resistant *Staphylococcus aureus* strains in Buenos Aires teaching hospitals: replacement of the multidrug resistant South American clone by another susceptible to rifampin, minocycline and trimethoprim-sulfamethoxazole. *Revista Argentina de Microbiologia, 37*(3), 156-160.

Ghasemzadeh-Moghaddam, H., Ghaznavi-Rad, E., Sekawi, Z., Yun-Khoon, L., Aziz, M. N., Hamat, R. A., . . . Neela, V. (2011). Methicillin-susceptible *Staphylococcus aureus* from clinical and community sources are genetically diverse. *International Journal of Medical Microbiology, 301*(4), 347-353.

Ghaznavi-Rad, E., Goering, R. V., Nor Shamsudin, M., Weng, P. L., Sekawi, Z., Tavakol, M., . . . Neela, V. (2011). *mec*-associated *dru* typing in the epidemiological analysis of ST239 MRSA in Malaysia. *European Journal of Clinical Microbiology and Infectious Disease, 30*(11), 1365-1369.

Ghaznavi-Rad, E., Nor Shamsudin, M., Sekawi, Z., Khoon, L. Y., Aziz, M. N., Hamat, R. A., . . . Neela, V. (2010). Predominance and emergence of clones of hospital-

acquired methicillin-resistant *Staphylococcus aureus* in Malaysia. *Journal of Clinical Microbiology, 48*(3), 867-872.

Gilbert, M., MacDonald, J., Gregson, D., Siushansian, J., Zhang, K., Elsayed, S., . . . Conly, J. (2006). Outbreak in Alberta of community-acquired (USA300) methicillin-resistant *Staphylococcus aureus* in people with a history of drug use, homelessness or incarceration. *CMAJ, 175*(2), 149-154.

Goering, R. V., Morrison, D., Al-Doori, Z., Edwards, G. F. S., & Gemmell, C. G. (2008a). Usefulness of *mec*-associated direct repeat unit (*dru*) typing in the epidemiological analysis of highly clonal methicillin-resistant *Staphylococcus aureus* in Scotland. *Clinical Microbiology and Infection, 14*(10), 964-969.

Goh, S. H., Byrne, S. K., Zhang, J. L., & Chow, A. W. (1992). Molecular typing of *Staphylococcus aureus* on the basis of coagulase gene polymorphisms. *Journal of Clinical Microbiology, 30*(7), 1642-1645.

Hallin, M., Denis, O., Deplano, A., De Mendonca, R., De Ryck, R., Rottiers, S., & Struelens, M. J. (2007). Genetic relatedness between methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*: results of a national survey. *Journal of Antimicrobial Chemotherapy, 59*(3), 465-472.

Harmsen, D., Claus, H., Witte, W., Rothganger, J., Turnwald, D., & Vogel, U. (2003). Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. *Journal of Clinical Microbiology, 41*(12), 5442-5448.

Harris, L. G., Foster, S. J., & Richards, R. G. (2002). An introduction to *Staphylococcus aureus*, and techniques for identifying and quantifying *S. aureus* adhesins in relation to adhesion to biomaterials: review. *European Cells & Materials, 4*, 39-60.

Hauschild, T., Sacha, P., Wieczorek, P., Zalewska, M., Kaczynska, K., & Tryniszewska, E. (2008). Aminoglycosides resistance in clinical isolates of *Staphylococcus aureus* from a University Hospital in Bialystok, Poland. *Folia Histochemica et Cytobiologica, 46*(2), 225-228.

Hayman, S., Wilson, A. P., Singer, M., & Bellingan, G. (2007). Effect of linezolid and teicoplanin on skin staphylococci. *Journal of Antimicrobial Chemotherapy, 59*(6), 1281-1282.

Heym, B., Le Moal, M., Armand-Lefevre, L., & Nicolas-Chanoine, M. H. (2002). Multilocus sequence typing (MLST) shows that the 'Iberian' clone of methicillin-resistant *Staphylococcus aureus* has spread to France and acquired reduced susceptibility to teicoplanin. *Journal of Antimicrobial Chemotherapy, 50*(3), 323-329.

Himabindu, M., Muthamilselvan, D.S., Bishi, D.K and Verma, R.S. (2009). Molecular analysis of coagulase gene polymorphism in clinical isolates of methicillin resistant *Staphylococcus aureus* by restriction fragment length polymorphism based genotyping. *American Journal of Infectious Disease*, *5*(2), 170-176.

Hiramatsu, K., Hanaki, H., Ino, T., Yabuta, K., Oguri, T., & Tenover, F. C. (1997). Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *Journal of Antimicrobial Chemotherapy, 40*(1), 135-136.

Hiramatsu, K. (2001). Vancomycin-resistant *Staphylococcus aureus*: a new model of antibiotic resistance. *Lancet Infectious Disease, 1*(3), 147-155.

Hisata, K., Kuwahara-Arai, K., Yamanoto, M., Ito, T., Nakatomi, Y., Cui, L., . . . Hiramatsu, K. (2005). Dissemination of methicillin-resistant staphylococci among healthy Japanese children. *Journal of Clinical Microbiology, 43*(7), 3364-3372.

Hodel-Christian, S. L., & Murray, B. E. (1991). Characterization of the gentamicin resistance transposon Tn*5281* from *Enterococcus faecalis* and comparison to staphylococcal transposons Tn*4001* and Tn*4031*. *Antimicrobial Agents and Chemotherapy, 35*(6), 1147-1152.

Holmes, A., Ganner, M., McGuane, S., Pitt, T. L., Cookson, B. D., & Kearns, A. M. (2005). *Staphylococcus aureus* isolates carrying Panton-Valentine leucocidin genes in England and Wales: frequency, characterization, and association with clinical disease. *Journal of Clinical Microbiology, 43*(5), 2384-2390.

Hookey, J. V., Richardson, J. F., & Cookson, B. D. (1998). Molecular typing of *Staphylococcus aureus* based on PCR restriction fragment length polymorphism and DNA sequence analysis of the coagulase gene. *Journal of Clinical Microbiology, 36*(4), 1083-1089.

Hortiwakul, R., Chayakul, P and Ingviya, N. (2004). In vitro activities of linezolid, vancomycin, fosfomycin and fusidic acid against methicillin-resistant *Staphylococcus aureus* (MRSA). *Journal of Infectious Disease and Antimicrobial Agents, 21,* 7-10.

Howden, B. P., & Grayson, M. L. (2006). Dumb and dumber--the potential waste of a useful antistaphylococcal agent: emerging fusidic acid resistance in *Staphylococcus aureus. Clinical Infectious Disease, 42*(3), 394-400.

Howden, B. P., Johnson, P. D., Charles, P. G., & Grayson, M. L. (2004). Failure of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* infections. *Clinical Infectious Disease, 39*(10), 1544; author reply 1544-1545.

Howden, B. P., McEvoy, C. R., Allen, D. L., Chua, K., Gao, W., Harrison, P. F., . . . Stinear, T. P. (2011). Evolution of multidrug resistance during *Staphylococcus aureus* infection involves mutation of the essential two component regulator WalKR. *Plos Pathogens, 7*(11), e1002359.

Howden, B. P., Stinear, T. P., Allen, D. L., Johnson, P. D., Ward, P. B., & Davies, J. K. (2008). Genomic analysis reveals a point mutation in the two-component sensor gene *graS* that leads to intermediate vancomycin resistance in clinical *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy, 52*(10), 3755-3762.

Hsueh, P. R., Lee, S. Y., Perng, C. L., Chang, T. Y., & Lu, J. J. (2010). Clonal dissemination of meticillin-resistant and vancomycin-intermediate *Staphylococcus aureus* in a Taiwanese hospital. *International Journal of Antimicrobial Agents, 36*(4), 307-312.

Hu, D. L., Omoe, K., Inoue, F., Kasai, T., Yasujima, M., Shinagawa, K., & Nakane, A. (2008). Comparative prevalence of superantigenic toxin genes in meticillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolates. *Journal of Medical Microbiology, 57*(Pt 9), 1106-1112.

Huletsky, A., Giroux, R., Rossbach, V., Gagnon, M., Vaillancourt, M., Bernier, M., . . . Bergeron, M. G. (2004). New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. *Journal of Clinical Microbiology, 42*(5), 1875-1884.

Hussain, A., Robinson, G., Malkin, J., Duthie, M., Kearns, A., & Perera, N. (2007). Purpura fulminans in a child secondary to Panton-Valentine leukocidin-producing *Staphylococcus aureus. Journal of Medical Microbiology, 56*(Pt 10), 1407-1409.

Ikeda, T., Tamate, N., Yamaguchi, K., & Makino, S. (2005). Mass outbreak of food poisoning disease caused by small amounts of staphylococcal enterotoxins A and H. *Applied Environmental Microbiology, 71*(5), 2793-2795.

Ishino, K., Tsuchizaki, N., Ishikawa, J., & Hotta, K. (2007). Usefulness of PCR-restriction fragment length polymorphism typing of the coagulase gene to discriminate arbekacin-resistant methicillin-resistant *Staphylococcus aureus* strains. *Journal of Clinical Microbiology, 45*(2), 607-609.

Jarraud, S., Mougel, C., Thioulouse, J., Lina, G., Meugnier, H., Forey, F., . . . Vandenesch, F. (2002). Relationships between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles), and human disease. *Infections and Immunity, 70*(2), 631-641.

Jones, T. F., Creech, C. B., Erwin, P., Baird, S. G., Woron, A. M., & Schaffner, W. (2006). Family outbreaks of invasive community-associated methicillin-resistant *Staphylococcus aureus* infection. *Clinical Infectious Disease, 42*(9), e76-78.

Jones, T. F., Kellum, M. E., Porter, S. S., Bell, M., & Schaffner, W. (2002). An outbreak of community-acquired foodborne illness caused by methicillin-resistant *Staphylococcus aureus*. *Emerging Infectious Disease, 8*(1), 82-84.

Jones, C. H., Tuckman, M., Howe, A. Y., Orlowski, M., Mullen, S., Chan, K., & Bradford, P. A. (2006). Diagnostic PCR analysis of the occurrence of methicillin and tetracycline resistance genes among *Staphylococcus aureus* isolates from phase 3 clinical trials of tigecycline for complicated skin and skin structure infections. *Antimicrobial Agents and Chemotherapy, 50*(2), 505-510.

Kadlec, K., & Schwarz, S. (2010). Identification of the novel *dfrK*-carrying transposon Tn*559* in a porcine methicillin-susceptible *Staphylococcus aureus* ST398 strain. *Antimicrobial Agents and Chemotherapy, 54*(8), 3475-3477.

Kahl, B. C., Mellmann, A., Deiwick, S., Peters, G., & Harmsen, D. (2005). Variation of the polymorphic region X of the protein A gene during persistent airway infection of cystic fibrosis patients reflects two independent mechanisms of genetic change in *Staphylococcus aureus*. *Journal of Clinical Microbiology, 43*(1), 502-505.

Karami, S., Rahbar, M., & Yousefi, J.V. (2007). Evaluation of five phenotypic methods for detection of methicillin resistant *Staphylococcus aureus* (MRSA). *Iranian Journal of Pathology, 6*(1), 27-31.

Kassis, C., Hachem, R., Raad, II, Perego, C. A., Dvorak, T., Hulten, K. G., . . . Chemaly, R. F. (2011). Outbreak of community-acquired methicillin-resistant *Staphylococcus aureus* skin infections among health care workers in a cancer center. *American Journal of Infections Control, 39*(2), 112-117.

Kaya, E.G., Karakoc, E., Yagci, S., & Yucel, M. (2009). Evaluation of phenotypic and genotypic methods for detection of methicillin resistnace in *Staphylococcus aureus*. *African Journal of Microbiology Research, 3*(12), 925-929.

Kehrenberg, C., & Schwarz, S. (2006). Distribution of florfenicol resistance genes *fexA* and *cfr* among chloramphenicol-resistant *Staphylococcus* isolates. *Antimicrobial Agents and Chemotherapy, 50*(4), 1156-1163.

Kerttula, A. M., Lyytikainen, O., Vuopio-Varkila, J., Ibrahem, S., Agthe, N., Broas, M., . . . Virolainen, A. (2005). Molecular epidemiology of an outbreak caused by methicillin-resistant *Staphylococcus aureus* in a health care ward and associated nursing home. *Journal of Clinical Microbiology, 43*(12), 6161-6163.

Khan, F., Shukla, I., Rizvi, M., Mansoor, T., & Sharma, S.C. (2011). Detection of biofilm formation in *Staphylococcus aureus*. Does it have a role in treatment of MRSA infections. *Trends in Medical Research, 6*(2), 116-123.

Kim, M. N., Pai, C. H., Woo, J. H., Ryu, J. S., & Hiramatsu, K. (2000). Vancomycin-intermediate *Staphylococcus aureus* in Korea. *Journal of Clinical Microbiology, 38*(10), 3879-3881.

Klein, E., Smith, D. L., & Laxminarayan, R. (2007). Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus*, United States, 1999-2005. *Emerging Infectious Disease, 13*(12), 1840-1846.

Klingenberg, C., Sundsfjord, A., Ronnestad, A., Mikalsen, J., Gaustad, P., & Flaegstad, T. (2004). Phenotypic and genotypic aminoglycoside resistance in blood culture isolates of coagulase-negative staphylococci from a single neonatal intensive care unit, 1989-2000. *Journal of Antimicrobial Chemotherapy, 54*(5), 889-896.

Kluytmans, J., van Leeuwen, W., Goessens, W., Hollis, R., Messer, S., Herwaldt, L., . . . van Leuwen, N. (1995). Food-initiated outbreak of methicillin-resistant *Staphylococcus aureu*s analyzed by pheno- and genotyping. *Journal of Clinical Microbiology, 33*(5), 1121-1128.

Ko, K. S., Lee, J. Y., Suh, J. Y., Oh, W. S., Peck, K. R., Lee, N. Y., & Song, J. H. (2005). Distribution of major genotypes among methicillin-resistant *Staphylococcus aureus* clones in Asian countries. *Journal of Clinical Microbiology, 43*(1), 421-426.

Kobayashi, H. (2006). National hospital infection surveillance on methicillin-resistant *Staphylococcus aureus. Dermatology, 212 Suppl 1*, 1-3.

Kondo, Y., Ito, T., Ma, X. X., Watanabe, S., Kreiswirth, B. N., Etienne, J., & Hiramatsu, K. (2007). Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. *Antimicrobial Agents and Chemotherapy, 51*(1), 264-274.

Kozitskaya, S., Cho, S. H., Dietrich, K., Marre, R., Naber, K., & Ziebuhr, W. (2004). The bacterial insertion sequence element *IS256* occurs preferentially in nosocomial *Staphylococcus epidermidis* isolates: association with biofilm formation and resistance to aminoglycosides. *Infections and Immunity, 72*(2), 1210-1215.

Krishnan, P. U., Miles, K., & Shetty, N. (2002). Detection of methicillin and mupirocin resistance in *Staphylococcus aureus* isolates using conventional and molecular methods: a descriptive study from a burns unit with high prevalence of MRSA. *Journal of Clinical Pathology, 55*(10), 745-748.

Kumar, J. D., Negi, Y. K., Gaur, A., & Khanna, D. (2009). Detection of virulence genes in *Staphylococcus aureus* isolated from paper currency. *Intenationl Journal of Infectious Disease, 13*(6), e450-455.

Lannergard, J., Norstrom, T., & Hughes, D. (2009). Genetic determinants of resistance to fusidic acid among clinical bacteremia isolates of *Staphylococcus aureus. Antimicrobial Agents and Chemotherapy, 53*(5), 2059-2065.

Larsen, A. R., Bocher, S., Stegger, M., Goering, R., Pallesen, L. V., & Skov, R. (2008). Epidemiology of European community-associated methicillin-resistant *Staphylococcus aureus* clonal complex 80 type IV strains isolated in Denmark from 1993 to 2004. *Journal of Clinical Microbiology, 46*(1), 62-68.

Lawrence, C., Cosseron, M., Mimoz, O., Brun-Buisson, C., Costa, Y., Samii, K., . . . Leclercq, R. (1996). Use of the coagulase gene typing method for detection of carriers of methicillin-resistant *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy, 37*(4), 687-696.

Le Loir, Y., Baron, F., & Gautier, M. (2003). *Staphylococcus aureus* and food poisoning. *Genetics and Molecular Research, 2*(1), 63-76.

Lee, K., Chang, C. L., Lee, N. Y., Kim, H. S., Hong, K. S., & Cho, H. C. (2000). Korean Nationwide Surveillance of Antimicrobial Resistance of Bacteria in 1998. *Yonsei Medical Journal, 41*(4), 497-506.

Leski, T. A., Gniadkowski, M., Skoczynska, A., Stefaniuk, E., Trzcinski, K., & Hryniewicz, W. (1999). Outbreak of mupirocin-resistant staphylococci in a

hospital in Warsaw, Poland, due to plasmid transmission and clonal spread of several strains. *Journal of Clinical Microbiology, 37*(9), 2781-2788.

Levy, S.B & Marshall, B. (2004). Antibacterial resistance worldwide: causes challenges and responses. *Nature Medicine, 10*(12), S122-S29.

Li, S., Skov, R. L., Han, X., Larsen, A. R., Larsen, J., Sorum, M., . . . Ito, T. (2011). Novel types of staphylococcal cassette chromosome *mec* elements identified in clonal complex 398 methicillin-resistant *Staphylococcus aureus* strains. *Antimicrobial Agents and Chemotherapy, 55*(6), 3046-3050.

Lim, H. S., Lee, H., Roh, K. H., Yum, J. H., Yong, D., Lee, K., & Chong, Y. (2006). Prevalence of inducible clindamycin resistance in staphylococcal isolates at a Korean tertiary care hospital. *Yonsei Medical Journal, 47*(4), 480-484.

Lim, K. T., Hanifah, Y. A., Mohd Yusof, M. Y., & Thong, K. L. (2010). Prevalence of mupirocin resistance in methicillin-resistant *Staphylococcus aureus* strains isolated from a Malaysian hospital. *Japanese Journal of Infectious Disease, 63*(4), 286-289.

Lim, V. K., & Zulkifli, H. I. (1987). Methicillin resistant *Staphylococcus aureus* in a Malaysian neonatal unit. *Singapore Medical Journal, 28*(2), 176-179.

Lina, G., Boutite, F., Tristan, A., Bes, M., Etienne, J., & Vandenesch, F. (2003). Bacterial competition for human nasal cavity colonization: role of Staphylococcal *agr* alleles. *Applied and Environmental Microbiology, 69*(1), 18-23.

Lina, G., Piemont, Y., Godail-Gamot, F., Bes, M., Peter, M. O., Gauduchon, V., . . . Etienne, J. (1999). Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clinical Infectious Disease, 29*(5), 1128-1132.

Lindstedt, B. A. (2005). Multiple-locus variable number tandem repeats analysis for genetic fingerprinting of pathogenic bacteria. *Electrophoresis, 26*(13), 2567-2582.

Lindsay, J. A., & Holden, M. T. (2006). Understanding the rise of the superbug: investigation of the evolution and genomic variation of *Staphylococcus aureus*. *Functional and Integrative Genomics, 6*(3), 186-201.

Liu, G. Y. (2009). Molecular pathogenesis of *Staphylococcus aureu*s infection. *Pediatric Research, 65*(5 Pt 2), 71R-77R.

Livermore, D. M., Winstanley, T. G., & Shannon, K. P. (2001). Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes. *Journal of Antimicrobial Chemotherapy, 48*(Suppl 1), 87-102.

Longmore, M., Wilkinson, I.B., Davidson, E.H., Foulkes, A., & Mafi, A.R. (2008). Oxford handbook of clinical medicine. 7th Ed, Oxford University Press, UK.

Lowy, F. D. (2003). Antimicrobial resistance: the example of *Staphylococcus aureus*. *The Journal of Clinical Investigation, 111*(9), 1265-1273.

Lulitanond, A., Engchanil, C., Chaimanee, P., Vorachit, M., Ito, T., & Hiramatsu, K. (2009). The first vancomycin-intermediate *Staphylococcus aureus* strains isolated from patients in Thailand. *Journal of Clinical Microbiology, 47*(7), 2311-2316.

Ma, X. X., Ito, T., Chongtrakool, P., & Hiramatsu, K. (2006). Predominance of clones carrying panton-Valentine leukocidin genes among methicillin-resistant *Staphylococcus aureus* strains isolated in Japanese hospitals from 1979 to 1985. *Journal of Clinical Microbiology, 44*(12), 4515-4527.

Malachowa, N., Sabat, A., Gniadkowski, M., Krzyszton-Russjan, J., Empel, J., Miedzobrodzki, J., . . . Hryniewicz, W. (2005). Comparison of multiple-locus variable-number tandem-repeat analysis with pulsed-field gel electrophoresis, *spa* typing, and multilocus sequence typing for clonal characterization of *Staphylococcus aureus* isolates. *Journal of Clinical Microbiology, 43*(7), 3095-3100.

Mallick, S.K., Basak, S and Bose, S. (2009). Inducible clindamycin resistance in *Staphylococcus aureus*-A therapeutic challenge. *Journal of Clinical Diagnostic Research, 3,* 1513-1518.

Martin-Lopez, J. V., Perez-Roth, E., Claverie-Martin, F., Diez Gil, O., Batista, N., Morales, M., & Mendez-Alvarez, S. (2002). Detection of *Staphylococcus aureus* clinical isolates harboring the *ica* gene cluster needed for biofilm establishment. *Journal of Clinical Microbiology, 40*(4), 1569-1570.

Martineau, F., Picard, F. J., Lansac, N., Menard, C., Roy, P. H., Ouellette, M., & Bergeron, M. G. (2000). Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrobial Agents and Chemotherapy, 44*(2), 231-238.

Masters, P. A., O'Bryan, T. A., Zurlo, J., Miller, D. Q., & Joshi, N. (2003). Trimethoprim-sulfamethoxazole revisited. *Archieve of Internal Medicine, 163*(4), 402-410.

Matthews, P. C., Taylor, A., Byren, I., & Atkins, B. L. (2007). Teicoplanin levels in bone and joint infections: are standard doses subtherapeutic? *Journal of Infections, 55*(5), 408-413.

McDougal, L. K., Steward, C. D., Killgore, G. E., Chaitram, J. M., McAllister, S. K., & Tenover, F. C. (2003). Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. *Journal of Clinical Microbiology, 41*(11), 5113-5120.

McLaws, F., Chopra, I., & O'Neill, A. J. (2008). High prevalence of resistance to fusidic acid in clinical isolates of *Staphylococcus epidermidis*. *Journal of Antimicrobial Chemotherapy, 61*(5), 1040-1043.

McNamara, P. J., Milligan-Monroe, K. C., Khalili, S., & Proctor, R. A. (2000). Identification, cloning, and initial characterization of rot, a locus encoding a regulator of virulence factor expression in *Staphylococcus aureus*. *Journal of Bacteriology, 182*(11), 3197-3203.

Mertz, P. M., Cardenas, T. C., Snyder, R. V., Kinney, M. A., Davis, S. C., & Plano, L. R. (2007). *Staphylococcus aureus* virulence factors associated with infected skin lesions: influence on the local immune response. *Archieve of Dermatology, 143*(10), 1259-1263.

Mick, V., Dominguez, M. A., Tubau, F., Linares, J., Pujol, M., & Martin, R. (2010). Molecular characterization of resistance to Rifampicin in an emerging hospital-associated Methicillin-resistant *Staphylococcus aureus* clone ST228, Spain. *BMC Microbiology, 10*, 68.

Milheirico, C., Oliveira, D. C., & de Lencastre, H. (2007). Update to the multiplex PCR strategy for assignment of *mec* element types in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy, 51*(9), 3374-3377.

Miller, G. H., Sabatelli, F. J., Hare, R. S., Glupczynski, Y., Mackey, P., Shlaes, D., . . . Shaw, K. J. (1997). The most frequent aminoglycoside resistance mechanisms--changes with time and geographic area: a reflection of aminoglycoside usage patterns? Aminoglycoside Resistance Study Groups. *Clinical Infectious Disease, 24 Suppl 1*, S46-62.

Mims, C., Dockrell, H.M., Goering, R.V., Roitt, I., Wakelin, D and Zuckerman, M. (2004). Medical microbiology. Spain: Elsevier Limited.

Ministry of Health (2008). National surveillance of antibiotic resistance report. Malaysia: Ministry of Health. http://www.imr.gov.my/report/nsar.htm

Mitani, N., Koizumi, A., Sano, R., Masutani, T., Murakawa, K., Mikasa, K., & Okamoto, Y. (2005). Molecular typing of methicillin-resistant *Staphylococcus aureus* by PCR-RFLP and its usefulness in an epidemiological study of an outbreak. *Japanese Journal of Infectious Disease, 58*(4), 250-252.

Monecke, S., Ehricht, R., Slickers, P., Wiese, N., & Jonas, D. (2009). Intra-strain variability of methicillin-resistant *Staphylococcus aureus* strains ST228-MRSA-I and ST5-MRSA-II. *European Journal of Clinical Microbiology Infectious Disease, 28*(11), 1383-1390.

Moore, P. C., & Lindsay, J. A. (2001). Genetic variation among hospital isolates of methicillin-sensitive *Staphylococcus aureu*s: evidence for horizontal transfer of virulence genes. *Journal of Clinical Microbiology, 39*(8), 2760-2767.

Murchan, S., Kaufmann, M. E., Deplano, A., de Ryck, R., Struelens, M., Zinn, C. E., . . . Cookson, B. (2003). Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. *Journal of Clinical Microbiology, 41*(4), 1574-1585.

National Antibiotic Guideline 2008, Ministry of Health, Malaysia. Retrieved from: http://www.pharmacy.gov.my/aeimages//File/National_Antibiotic_Guideline_2008_edit(2). pdf, accessed June 2011.

Neela, V., Ehsanollah, G. R., Zamberi, S., Van Belkum, A., & Mariana, N. S. (2009a). Prevalence of panton-valentine leukocidin genes among carriage and invasive *Staphylococcus aureus* isolates in Malaysia. *International Journal of Infectious Disease, 13*(3), e131-132.

Neela, V., Mohd Zafrul, A., Mariana, N. S., van Belkum, A., Liew, Y. K., & Rad, E. G. (2009b). Prevalence of ST9 methicillin-resistant *Staphylococcus aureus* among pigs and pig handlers in Malaysia. *Journal of Clinical Microbiology, 47*(12), 4138-4140.

Neela, V., Sasikumar, M., Ghaznavi, G. R., Zamberi, S., & Mariana, S. (2008). In vitro activities of 28 antimicrobial agents against methicillin-resistant *Staphylococcus aureus* (MRSA) from a clinical setting in Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health, 39*(5), 885-892.

Ng, L. K., Martin, I., Alfa, M., & Mulvey, M. (2001). Multiplex PCR for the detection of tetracycline resistant genes. *Molecular Cellular Probes, 15*(4), 209-215.

Norazah, A., Koh, Y. T., Ghani Kamel, A., Alias, R., & Lim, V. K. (2001). Mupirocin resistance among Malaysian isolates of methicillin-resistant *Staphylococcus aureu*s. *International Journal of Antimicrobial Agents, 17*(5), 411-414.

Norazah, A., Lim, V. K., Koh, Y. T., Rohani, M. Y., Zuridah, H., Spencer, K., . . . Kamel, A. G. (2002). Molecular fingerprinting of fusidic acid- and rifampicin-resistant strains of methicillin-resistant *Staphylococcus aureus* (MRSA) from Malaysian hospitals. *Journal of Medical Microbiology, 51*(12), 1113-1116.

Norazah, A., Lim, V. K., Rohani, M. Y., Alfizah, H., Koh, Y. T., & Kamel, A. G. (2003). A major methicillin-resistant *Staphylococcus aureus* clone predominates in Malaysian hospitals. *Epidemiology and Infections, 130*(3), 407-411.

Norazah, A., Salbiah, N., Nurizzat, M., & Santhana, R. (2009). Vancomycin treatment failure in a vancomycin susceptible methicillin-resistant *Staphylococcus aureus* (MRSA) infected patient. *Medical Journal Malaysia, 64*(2), 166-167.

Nor Shamsudin, M., Sekawi, Z., van Belkum, A., & Neela, V. (2008). First community-acquired meticillin-resistant *Staphylococcus aureus* in Malaysia. *Journal of Medical Microbiology, 57*(Pt 9), 1180-1181.

Norstrom, T., Lannergard, J., & Hughes, D. (2007). Genetic and phenotypic identification of fusidic acid-resistant mutants with the small-colony-variant phenotype in *Staphylococcus aureus. Antimicrobial Agents and Chemotherapy, 51*(12), 4438-4446.

O'Brien, F. G., Coombs, G. W., Pearman, J. W., Gracey, M., Moss, F., Christiansen, K. J., & Grubb, W. B. (2009). Population dynamics of methicillin-susceptible and -resistant *Staphylococcus aureus* in remote communities. *Journal of Antimicrobial Chemotherapy, 64*(4), 684-693.

Occelli, P., Blanie, M., Sanchez, R., Vigier, D., Dauwalder, O., Darwiche, A., . . . Venier, A. G. (2007). Outbreak of staphylococcal bullous impetigo in a maternity ward linked to an asymptomatic healthcare worker. *Journal of Hospital Infections, 67*(3), 264-270.

Okuma, K., Iwakawa, K., Turnidge, J. D., Grubb, W. B., Bell, J. M., O'Brien, F. G., . . . Hiramatsu, K. (2002). Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. *Journal of Clinical Microbiology, 40*(11), 4289-4294.

Oliveira, D. C., & de Lencastre, H. (2002). Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy, 46*(7), 2155-2161.

Olorunfemi, O.B., Onasanya, A.A and Adetuyi, F.C. (2005). Genetic variation and relationship in *Staphylococcus aureus* isolates from human and food samples using random amplified polymorphic DNAs. *African Journal of Bacteriology, 4*(7), 611-614.

Olsen, J. E., Christensen, H., & Aarestrup, F. M. (2006). Diversity and evolution of *blaZ* from *Staphylococcus aureus* and coagulase-negative staphylococci. *Journal of Antimicrobial Chemotherapy, 57*(3), 450-460.

O'Neill, A. J., McLaws, F., Kahlmeter, G., Henriksen, A. S., & Chopra, I. (2007). Genetic basis of resistance to fusidic acid in staphylococci. *Antimicrobial Agents and Chemotherapy, 51*(5), 1737-1740.

O'Neill, A. J., Cove, J. H., & Chopra, I. (2001). Mutation frequencies for resistance to fusidic acid and rifampicin in *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy, 47*(5), 647-650.

O'Neill, A. J., & Chopra, I. (2006). Molecular basis of fusB-mediated resistance to fusidic acid in *Staphylococcus aureus*. *Molecular Microbiology, 59*(2), 664-676.

Ortega, E., Abriouel, H., Lucas, R., & Galvez, A. (2010). Multiple Roles of *Staphylococcus aureus* Enterotoxins: Pathogenicity, Superantigenic Activity, and Correlation to Antibiotic Resistance. *Toxins (Basel), 2*(8), 2117-2131.

Ostyn, A., De Buyser, M.L., Guillier, F., Groult, J., Felix, B., Salah, S., Delmas, G and Hennekinne, J.A. (2010). First evidence of a food poisoning outbreak due to staphylococcal enterotoxin type E, France, 2009. *Euro Surveill*. *15*(13). pil=19528. Available online: http://www.eurosurveillance.org/VierArticle.

Park, H.K., Woo, S.Y., Jung, Y.J., Lee, E.O., Cha, J.E., Cha, J.E., Park, H.S and Lee, S.J. (2008). Detection of virulence genes of *Staphylococcus aureus* and *Staphylococcus epidermidis* isolated from suprapubic urine from infants with fever. *Journal of Bacteriology and Virology, 38*(4), 189-196.

Pathirage, H. (2008). Panton valentine leukocidin (PVL) positive staphylococcal infection: an emerging infection across the world. *Sri Lanka Journal of Child and Health, 37*, 109-111.

Pawun, V., Jiraphongsa, C., Puttamasute, S., Putta, R., Wongnai, A., Jaima, T., Tithsayatikom, P and Wattanasri, S. (2008). An outbreak of hospital-acquired *Staphylococcus aureus* skin infection among newborns, Nan Province, Thailand. *Euro Surveill, 14* (43). Retrieve from http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19372.

Peacock, S. J., Moore, C. E., Justice, A., Kantzanou, M., Story, L., Mackie, K., . . . Day, N. P. (2002). Virulent combinations of adhesin and toxin genes in natural populations of *Staphylococcus aureus*. *Infections and Immunity, 70*(9), 4987-4996.

Peerayeh, S.N., Azimian, A., Nejad, Q.B and Kashi, M. (2009). Prevalence of *agr* specificity groups among *Staphylococcus aureus* isolates from University Hospitals in Tehran. *Labmedicine, 40*(1), 27-29.

Perez-Roth, E., Lopez-Aguilar, C., Alcoba-Florez, J., & Mendez-Alvarez, S. (2006). High-level mupirocin resistance within methicillin-resistant *Staphylococcus aureus* pandemic lineages. *Antimicrobial Agents and Chemotherapy, 50*(9), 3207-3211.

Perez-Vazquez, M., Vindel, A., Marcos, C., Oteo, J., Cuevas, O., Trincado, P., . . . Campos, J. (2009). Spread of invasive Spanish *Staphylococcus aureus spa*-type t067 associated with a high prevalence of the aminoglycoside-modifying enzyme gene ant(4')-Ia and the efflux pump genes *msrA/msrB*. *Journal of Antimicrobial Chemotherapy, 63*(1), 21-31.

Persson, L., Johansson, C., & Ryden, C. (2009). Antibodies to *Staphylococcus aureus* bone sialoprotein-binding protein indicate infectious osteomyelitis. *Clinical Vaccine and Immunology, 16*(6), 949-952.

Plata, K., Rosato, A. E., & Wegrzyn, G. (2009). *Staphylococcus aureus* as an infectious agent: overview of biochemistry and molecular genetics of its pathogenicity. *Acta Biochimica Polonica, 56*(4), 597-612.

Podbielska, A., Gaεkowska, H and Olszewski, W.L. (2011). Staphylococcal and enterococcal virulence- a review. *Central European Journal of Immunology, 36*(1), 56-64.

Pourmand, M.R., Memariani, M., Hoseini, M., & Yazdchi, S.B. (2009). High prevalence of SEA gene among clinical isolates of *Staphylococcus aureus* in Tehran. *Acta Medical Iranica, 47*, 357-361.

Prevost, G., Couppie, P., Prevost, P., Gayet, S., Petiau, P., Cribier, B., . . . Piemont, Y. (1995). Epidemiological data on *Staphylococcus aureus* strains producing synergohymenotropic toxins. *Journal of Medical Microbiology, 42*(4), 237-245.

Qi, W., Ender, M., O'Brien, F., Imhof, A., Ruef, C., McCallum, N., & Berger-Bachi, B. (2005). Molecular epidemiology of methicillin-resistant *Staphylococcus aureus*

in Zurich, Switzerland (2003): prevalence of type IV SCC*mec* and a new SCC*mec* element associated with isolates from intravenous drug users. *Journal of Clinical Microbiology, 43*(10), 5164-5170.

Quintiliani, R., Jr., Evers, S., & Courvalin, P. (1993). The *vanB* gene confers various levels of self-transferable resistance to vancomycin in enterococci. *Journal of Infectious Disease, 167*(5), 1220-1223.

Rampal, L. (1983). A food poisoning outbreak due to *Staphylococcus aureus*, Kapar, Malaysia, 1983. *Medical Journal of Malaysia, 38*(4), 294-298.

Ramsey, M. A., Bradley, S. F., Kauffman, C. A., & Morton, T. M. (1996). Identification of chromosomal location of *mupA* gene, encoding low-level mupirocin resistance in staphylococcal isolates. *Antimicrobial Agents and Chemotherapy, 40*(12), 2820-2823.

Ray, B and Bhunia, A. (2008). Fundamental food microbiology. 4th Ed, CRC Press, United States of America: Taylor and Francis Group.

Rijnders, M. I., Deurenberg, R. H., Boumans, M. L., Hoogkamp-Korstanje, J. A., Beisser, P. S., & Stobberingh, E. E. (2009). Population structure of *Staphylococcus aureus* strains isolated from intensive care unit patients in the netherlands over an 11-year period (1996 to 2006). *Journal of Clinical Microbiology, 47*(12), 4090-4095.

Robinson, D. A., Monk, A. B., Cooper, J. E., Feil, E. J., & Enright, M. C. (2005). Evolutionary genetics of the accessory gene regulator (*agr*) locus in *Staphylococcus aureus*. *Journal of Bacteriology, 187*(24), 8312-8321.

Rohani, M. Y., Raudzah, A., Lau, M. G., Zaidatul, A. A., Salbiah, M. N., Keah, K. C., . . . Zainuldin, T. (2000). Susceptibility pattern of *Staphylococcus aureus* isolated in Malaysian hospitals. *International Journal of Antimicrobial Agents, 13*(3), 209-213.

Rohani, M.Y., Zainuldin, M.T., Koay, A.S and Lau, M.G. (1999). Antibiotic resistance patterns of bacteria isolated in Malaysian hospital. *International Medical Journal, 6*(1), 47-51.

Rossney, A. S., Shore, A. C., Morgan, P. M., Fitzgibbon, M. M., O'Connell, B., & Coleman, D. C. (2007). The emergence and importation of diverse genotypes of methicillin-resistant *Staphylococcus aureus* (MRSA) harboring the Panton-Valentine leukocidin gene (*pvl*) reveal that *pvl* is a poor marker for community-acquired MRSA strains in Ireland. *Journal of Clinical Microbiology, 45*(8), 2554-2563.

Rotger, M., Trampuz, A., Piper, K. E., Steckelberg, J. M., & Patel, R. (2005). Phenotypic and genotypic mupirocin resistance among Staphylococci causing prosthetic joint infection. *Journal of Clinical Microbiology, 43*(8), 4266-4268.

Rouch, D. A., Byrne, M. E., Kong, Y. C., & Skurray, R. A. (1987). The *aacA-aphD* gentamicin and kanamycin resistance determinant of Tn*4001* from

Staphylococcus aureus: expression and nucleotide sequence analysis. *Journal of General Microbiology, 133*(11), 3039-3052.

Rouch, D. A., Messerotti, L. J., Loo, L. S., Jackson, C. A., & Skurray, R. A. (1989). Trimethoprim resistance transposon Tn*4003* from *Staphylococcus aureus* encodes genes for a dihydrofolate reductase and thymidylate synthetase flanked by three copies of IS*257*. *Molecular Microbiology, 3*(2), 161-175.

Ruppitsch, W., Indra, A., Stoger, A., Mayer, B., Stadlbauer, S., Wewalka, G., & Allerberger, F. (2006). Classifying *spa* types in complexes improves interpretation of typing results for methicillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology*, *44*(7), 2442-2448.

Sabat, A., Krzyszton-Russjan, J., Strzalka, W., Filipek, R., Kosowska, K., Hryniewicz, W., . . . Potempa, J. (2003). New method for typing *Staphylococcus aureus* strains: multiple-locus variable-number tandem repeat analysis of polymorphism and genetic relationships of clinical isolates. *Journal of Clinical Microbiology, 41*(4), 1801-1804.

Sabat, A., Melles, D. C., Martirosian, G., Grundmann, H., van Belkum, A., & Hryniewicz, W. (2006). Distribution of the serine-aspartate repeat protein-encoding *sdr* genes among nasal-carriage and invasive *Staphylococcus aureus* strains. *Journal of Clinical Microbiology*, *44*(3), 1135-1138.

Saha, B., Singh, A. K., Ghosh, A., & Bal, M. (2008). Identification and characterization of a vancomycin-resistant *Staphylococcus aureus* isolated from Kolkata (South Asia). *Journal of Medical Microbiology, 57*(Pt 1), 72-79.

Sam, I. C., Kahar-Bador, M., Chan, Y. F., Loong, S. K., & Mohd Nor Ghazali, F. (2008). Multisensitive community-acquired methicillin-resistant *Staphylococcus aureus* infections in Malaysia. *Diagnostic Microbiology and Infectious Disease, 62*(4), 437-439.

Sambrook, J, and Russel, D. W. (2001). Molecular Cloning: A laboratory Manual. 3rd edition. New York : Cold Spring Laboratory Press.

Sanakal, R.D and Kaliwal, B.B. (2011). Vancomycin resistance genes in various organisms- an insilico study. *International Journal of Biometric and Bioinformatics*, *5*(2), 111-129.

Sachse, F., von Eiff, C., Becker, K., & Rudack, C. (2008). Anti-inflammatory effects of ciprofloxacin in *S. aureus* Newman induced nasal inflammation in vitro. *Journal of Inflamatory (Lond), 5*, 11.

Saderi, H., Owlia, P., & Shahrbanooie, R. (2005). Vancomycin resistance among clinical isolates of *Staphylococcus aureus. Archieves of Iranian Medicine, 8*(2), 100-103.

Sanchez Garcia, M., De la Torre, M. A., Morales, G., Pelaez, B., Tolon, M. J., Domingo, S., . . . Picazo, J. (2010). Clinical outbreak of linezolid-resistant *Staphylococcus aureus* in an intensive care unit. *JAMA, 303*(22), 2260-2264.

Sanjiv, K., Kataria, A.K., Sharma, R., & Singh, G. (2008). Epidemiological typing of *Staphylococcus aureus* by DNA restriction fragment length polymorphism of *coa* gene. *Veterinarski Archiv, 78*(1), 31-38.

Saunders, A., Panaro, L., McGeer, A., Rosenthal, A., White, D., Willey, B. M., . . . Katz, K. (2007). A nosocomial outbreak of community-associated methicillin-resistant *Staphylococcus aureus* among healthy newborns and postpartum mothers. *Canadian Journal of Infectious Disease and Medical Microbiology, 18*(2), 128-132.

Sauer, P., Sila, J., Stosova, T., Vecerova, R., Hejnar, P., Vagnerova, I., . . . Koukalova, D. (2008). Prevalence of genes encoding extracellular virulence factors among meticillin-resistant *Staphylococcus aureus* isolates from the University Hospital, Olomouc, Czech Republic. *Journal of Medical Microbiology, 57*(Pt 4), 403-410.

Schenk, S., & Laddaga, R. A. (1992). Improved method for electroporation of *Staphylococcus aureus*. *FEMS Microbiology Letters, 73*(1-2), 133-138.

Schlebusch, S., Price, G. R., Hinds, S., Nourse, C., Schooneveldt, J. M., Tilse, M. H., . . . Nimmo, G. R. (2010). First outbreak of PVL-positive non multiresistant MRSA in a neonatal ICU in Australia: comparison of MALDI-TOF and SNP-plus-binary gene typing. *European Journal of Clinical Microbiology and Infectious Disease, 29*(10), 1311-1314.

Schmitz, F. J., Fluit, A. C., Gondolf, M., Beyrau, R., Lindenlauf, E., Verhoef, J., . . . Jones, M. E. (1999). The prevalence of aminoglycoside resistance and corresponding resistance genes in clinical isolates of staphylococci from 19 European hospitals. *Journal of Antimicrobial Chemotherapy, 43*(2), 253-259.

Schmitz, F. J., Krey, A., Sadurski, R., Verhoef, J., Milatovic, D., & Fluit, A. C. (2001). Resistance to tetracycline and distribution of tetracycline resistance genes in European *Staphylococcus aureus* isolates. *Journal of Antimicrobial Chemotherapy, 47*(2), 239-240.

Schmitz, F. J., Petridou, J., Fluit, A. C., Hadding, U., Peters, G., & von Eiff, C. (2000). Distribution of macrolide-resistance genes in *Staphylococcus aureus* blood-culture isolates from fifteen German university hospitals. M.A.R.S. Study Group. Multicentre Study on Antibiotic Resistance in Staphylococci. *European Journal of Clinical Microbiology and Infectious Disease, 19*(5), 385-387.

Scholar, E.M and Pratt, W.B. (2000). The antimicrobial drugs. 2nd Ed, New York: Oxford.

Seguin, J. C., Walker, R. D., Caron, J. P., Kloos, W. E., George, C. G., Hollis, R. J., . . . Pfaller, M. A. (1999). Methicillin-resistant *Staphylococcus aureus* outbreak in a veterinary teaching hospital: potential human-to-animal transmission. *Journal of Clinical Microbiology, 37*(5), 1459-1463.

Sekiguchi, J., Fujino, T., Saruta, K., Konosaki, H., Nishimura, H., Kawana, A., . . . Kirikae, T. (2004). Prevalence of erythromycin-, tetracycline-, and aminoglycoside- resistance genes in methicillin-resistant *Staphylococcus aureus*

in hospitals in Tokyo and Kumamoto. *Japanese Journal of Infectious Disease, 57*(2), 74-77.

Shimeld, L.A and Rodger, A.T. (1998). Essential of diagnostic microbiology New York: Delmar Publisher.

Shittu, A. O., & Lin, J. (2006). Antimicrobial susceptibility patterns and characterization of clinical isolates of *Staphylococcus aureus* in KwaZulu-Natal province, South Africa. *BMC Infectious Disease, 6*, 125.

Shopsin, B., Gomez, M., Montgomery, S. O., Smith, D. H., Waddington, M., Dodge, D. E., . . . Kreiswirth, B. N. (1999). Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *Journal of Clinical Microbiology, 37*(11), 3556-3563.

Shore, A. C., Deasy, E. C., Slickers, P., Brennan, G., O'Connell, B., Monecke, S., . . . Coleman, D. C. (2011). Detection of staphylococcal cassette chromosome *mec* type XI carrying highly divergent *mecA, mecI, mecR1, blaZ,* and *ccr* genes in human clinical isolates of clonal complex 130 methicillin-resistant *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy, 55*(8), 3765-3773.

Shore, A. C., Rossney, A. S., Kinnevey, P. M., Brennan, O. M., Creamer, E., Sherlock, O., . . . Coleman, D. C. (2010). Enhanced discrimination of highly clonal ST22-methicillin-resistant *Staphylococcus aureus* IV isolates achieved by combining spa, dru, and pulsed-field gel electrophoresis typing data. *Journal of Clinical Microbiology, 48*(5), 1839-1852.

Shukla, S. K., Bernard, K. A., Harney, M., Frank, D. N., & Reed, K. D. (2003). *Corynebacterium nigricans* sp. nov.: proposed name for a black-pigmented *Corynebacterium* species recovered from the human female urogenital tract. *Journal of Clinical Microbiology, 41*(9), 4353-4358.

Singh, A., Goering, R. V., Simjee, S., Foley, S. L., & Zervos, M. J. (2006). Application of molecular techniques to the study of hospital infection. *Clinical Microbiology Review, 19*(3), 512-530.

Skov, R., Smyth, R., Larsen, A. R., Bolmstrom, A., Karlsson, A., Mills, K., . . . Kahlmeter, G. (2006). Phenotypic detection of methicillin resistance in *Staphylococcus aureus* by disk diffusion testing and Etest on Mueller-Hinton agar. *Journal of Clinical Microbiology, 44*(12), 4395-4399.

Song, J. H., Hiramatsu, K., Suh, J. Y., Ko, K. S., Ito, T., Kapi, M., . . . Lee, N. Y. (2004). Emergence in Asian countries of *Staphylococcus aureus* with reduced susceptibility to vancomycin. *Antimicrobial Agents and Chemotherapy, 48*(12), 4926-4928.

Song, J. H., Hsueh, P. R., Chung, D. R., Ko, K. S., Kang, C. I., Peck, K. R., . . . Van, P. H. (2011). Spread of methicillin-resistant *Staphylococcus aureus* between the community and the hospitals in Asian countries: an ANSORP study. *Journal of Antimicrobial Chemotherapy, 66*(5), 1061-1069.

Spiliopoulou, I., Petinaki, E., Papandreou, P., & Dimitracopoulos, G. (2004). *erm*(C) is the predominant genetic determinant for the expression of resistance to macrolides among methicillin-resistant *Staphylococcus aureus* clinical isolates in Greece. *Journal of Antimicrobial Chemotherapy, 53*(5), 814-817.

Stranden, A., Frei, R., & Widmer, A. F. (2003). Molecular typing of methicillin-resistant *Staphylococcus aureus*: can PCR replace pulsed-field gel electrophoresis? *Journal of Clinical Microbiology, 41*(7), 3181-3186.

Svetitsky, S., Leibovici, L., & Paul, M. (2009). Comparative efficacy and safety of vancomycin versus teicoplanin: systematic review and meta-analysis. *Antimicrobial Agents and Chemotherapy, 53*(10), 4069-4079.

Taiwo, S.S., Bamigboye, T.B., Odaro, O., Aefioye, O.A.A., & Fadiora, O. (2011). Vancomycin intermediate and high level vancomycin resistant *Staphylococcus aureus* clinical isolates in Osogbo, Nigeria. *Microbiology Research, 2*(1).

Tenover, F. C., Arbeit, R. D., Goering, R. V., Mickelsen, P. A., Murray, B. E., Persing, D. H., & Swaminathan, B. (1995). Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *Journal of Clinical Microbiology, 33*(9), 2233-2239.

Tenover, F. C., & Moellering, R. C., Jr. (2007). The rationale for revising the Clinical and Laboratory Standards Institute vancomycin minimal inhibitory concentration interpretive criteria for *Staphylococcus aureus*. *Clinical Infectious Disease, 44*(9), 1208-1215.

The SunLab Homepage. Information retrieved online from URL http://sunlab.ustc.edu.cn/protocol/gene_deletion_in_SA.htm, accessed on 8th March 2012.

Thong, K. L., Junnie, J., Liew, F. Y., Yusof, M. Y., & Hanifah, Y. A. (2009). Antibiograms and molecular subtypes of methicillin-resistant *Staphylococcus aureus* in local teaching hospital, Malaysia. *Journal of Microbiology and Biotechnology, 19*(10), 1265-1270.

Thong, K.L., Lai, K.S., Puthucheary, S.D., Koh, Y.T., Ahmad, N and Yassin, R.M. (2007). Subtyping of *S. enterica* serovar Muenchen by pulse-field gel electrophoresis, plasmid profiling and antimicrobial susceptibility testing. *Malaysia Journal of Sciences, 26*(2), 1-13.

Thuong, C. T., Nguyen, D. T., Ngo, T. H., Nguyen, T. M., Le, V. T., To, S. D., . . . Schultsz, C. (2007). An outbreak of severe infections with community-acquired MRSA carrying the Panton-Valentine leukocidin following vaccination. *PLoS One, 2*(9), e822.

Tiwari, H. K., Sapkota, D., Gaur, A., Mathuria, J. P., Singh, A., & Sen, M. R. (2008). Molecular typing of clinical *Staphylococcus aureus* isolates from northern India using coagulase gene PCR-RFLP. *The Southeast Asian Journal of Tropical Medicine and Public Health, 39*(3), 467-473.

Tiwari, H. K., & Sen, M. R. (2006). Emergence of vancomycin resistant *Staphylococcus aureus* (VRSA) from a tertiary care hospital from northern part of India. *BMC Infectious Disease, 6*, 156.

Towner, K, J and Cockayne, A. (1993). Molecular Methods for microbial identification and typing. Nottingham: United Kingdom: Chapman and Hall.

Traber, K. E., Lee, E., Benson, S., Corrigan, R., Cantera, M., Shopsin, B., & Novick, R. P. (2008). *agr* function in clinical *Staphylococcus aureus* isolates. *Microbiology, 154*(Pt 8), 2265-2274.

Trakulsomboon, S., Danchaivijitr, S., Rongrungruang, Y., Dhiraputra, C., Susaemgrat, W., Ito, T., & Hiramatsu, K. (2001). First report of methicillin-resistant *Staphylococcus aureus* with reduced susceptibility to vancomycin in Thailand. *Journal of Clinical Microbiology, 39*(2), 591-595.

Tristan, A., Bes, M., Meugnier, H., Lina, G., Bozdogan, B., Courvalin, P., . . . Etienne, J. (2007a). Global distribution of Panton-Valentine leukocidin--positive methicillin-resistant *Staphylococcus aureus*, 2006. *Emerging Infectious Disease, 13*(4), 594-600.

Tristan, A., Ferry, T., Durand, G., Dauwalder, O., Bes, M., Lina, G., . . . Etienne, J. (2007b). Virulence determinants in community and hospital meticillin-resistant *Staphylococcus aureus. Journal of Hospital Infections, 65 Suppl 2*, 105-109.

Udo, E. E., Al-Sweih, N., Mokaddas, E., Johny, M., Dhar, R., Gomaa, H. H., . . . Rotimi, V. O. (2006). Antibacterial resistance and their genetic location in MRSA isolated in Kuwait hospitals, 1994-2004. *BMC Infectios Disease, 6*, 168.

Udo, E. E., O'Brien, F. G., Al-Sweih, N., Noronha, B., Matthew, B., & Grubb, W. B. (2008). Genetic lineages of community-associated methicillin-resistant *Staphylococcus aureu*s in Kuwait hospitals. *Journal of Clinical Microbiology, 46*(10), 3514-3516.

Udo, E. E., Pearman, J. W., & Grubb, W. B. (1993). Genetic analysis of community isolates of methicillin-resistant *Staphylococcus aureus* in Western Australia. *Journal of Hospital Infections, 25*(2), 97-108.

Vakulenko, S. B., & Mobashery, S. (2003). Versatility of aminoglycosides and prospects for their future. *Clinical Microbiology Review, 16*(3), 430-450

Vali, L., Davies, S. E., Lai, L. L., Dave, J., & Amyes, S. G. (2008). Frequency of biocide resistance genes, antibiotic resistance and the effect of chlorhexidine exposure on clinical methicillin-resistant *Staphylococcus aureus* isolates. *Journal of Antimicrobial Chemotherapy, 61*(3), 524-532. doi: 10.1093/jac/dkm520

van Belkum, A., Tassios, P. T., Dijkshoorn, L., Haeggman, S., Cookson, B., Fry, N. K., . . . Struelens, M. (2007). Guidelines for the validation and application of typing methods for use in bacterial epidemiology. *Clinical Microbiology and Infections, 13 Suppl 3*, 1-46.

Vandenesch, F., Naimi, T., Enright, M. C., Lina, G., Nimmo, G. R., Heffernan, H., . . . Etienne, J. (2003). Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. *Emerging Infectious Disease, 9*(8), 978-984.

Varaldo, P. E., Montanari, M. P., & Giovanetti, E. (2009). Genetic elements responsible for erythromycin resistance in streptococci. *Antimicrob Agents and Chemotherapy, 53*(2), 343-353.

Varela, A. S., Luttrell, M. P., Howerth, E. W., Moore, V. A., Davidson, W. R., Stallknecht, D. E., & Little, S. E. (2004). First culture isolation of *Borrelia lonestari*, putative agent of southern tick-associated rash illness. *Journal of Clinical Microbiology, 42*(3), 1163-1169.

Velasco, D., del Mar Tomas, M., Cartelle, M., Beceiro, A., Perez, A., Molina, F., . . . Bou, G. (2005). Evaluation of different methods for detecting methicillin (oxacillin) resistance in *Staphylococcus aureus. Journal of Antimicrobial Chemotherapy, 55*(3), 379-382.

Villedieu, A., Diaz-Torres, M. L., Hunt, N., McNab, R., Spratt, D. A., Wilson, M., & Mullany, P. (2003). Prevalence of tetracycline resistance genes in oral bacteria. *Antimicrobial Agents and Chemotherapy, 47*(3), 878-882.

Voss, A., & Doebbeling, B. N. (1995). The worldwide prevalence of methicillin-resistant *Staphylococcus aureus. International Journal of Antimicrobial Agents, 5*(2), 101-106.

Wang, J. T., Fang, C. T., Chen, Y. C., Wu, C. L., Chen, M. L., & Chang, S. C. (2007). Staphylococcal cassette chromosome mec in MRSA, Taiwan. *Emerging Infectious Disease, 13*(3), 494-497.

Wang, Y., Huang, T.P., Chang, Y.C., & Shih, D.Y.C. (2003). Subtyping of enterotoxin C strains isolated from food poisoning outbreaks in Taiwan. *Journal of Food Drug Analysis*, 11(3), 239-245.

Wang, Y., Wu, C. M., Lu, L. M., Ren, G. W., Cao, X. Y., & Shen, J. Z. (2008). Macrolide-lincosamide-resistant phenotypes and genotypes of *Staphylococcus aureus* isolated from bovine clinical mastitis. *Veterinary Microbiology, 130*(1-2), 118-125.

Wang, L., Yu, F., Yang, L., Li, Q., Zhang, X., Zeng, Y., & Xu, Y. (2010). Prevalence of virulence genes and biofilm formation among *Staphylococcus aureus* clinical isolates associated with lower respiratory infection. *African Journal of Micorbiology Research, 4*(23), 2566-2569.

Wannet, W. J., Spalburg, E., Heck, M. E., Pluister, G. N., Tiemersma, E., Willems, R. J., . . . Etienne, J. (2005). Emergence of virulent methicillin-resistant *Staphylococcus aureus* strains carrying Panton-Valentine leucocidin genes in The Netherlands. *Journal of Clinical Microbiology, 43*(7), 3341-3345.

Weigelt, J.A. (2007). MRSA. New York: Informa Healthcare USA, Inc.

Wertheim, H. F., Melles, D. C., Vos, M. C., van Leeuwen, W., van Belkum, A., Verbrugh, H. A., & Nouwen, J. L. (2005). The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infectious Disease, 5*(12), 751-762.

Westh, H., Hougaard, D. M., Vuust, J., & Rosdahl, V. T. (1995). Prevalence of erm gene classes in erythromycin-resistant *Staphylococcus aureus* strains isolated between 1959 and 1988. *Antimicrobial Agents and Chemotherapy, 39*(2), 369-373.

Whitehead, K. A., Colligon, J., & Verran, J. (2005). Retention of microbial cells in substratum surface features of micrometer and sub-micrometer dimensions. *Colloids and Surfaces B: Biointerfaces, 41*(2-3), 129-138.

Wichelhaus, T. A., Boddinghaus, B., Besier, S., Schafer, V., Brade, V., & Ludwig, A. (2002). Biological cost of rifampin resistance from the perspective of *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy, 46*(11), 3381-3385.

Wisplinghoff, H., Ewertz, B., Wisplinghoff, S., Stefanik, D., Plum, G., Perdreau-Remington, F., & Seifert, H. (2005). Molecular evolution of methicillin-resistant *Staphylococcus aureus* in the metropolitan area of Cologne, Germany, from 1984 to 1998. *Journal of Clinical Microbiology, 43*(11), 5445-5451.

Wolk, D. M., Blyn, L. B., Hall, T. A., Sampath, R., Ranken, R., Ivy, C., . . . Carroll, K. C. (2009). Pathogen profiling: rapid molecular characterization of *Staphylococcus aureus* by PCR/electrospray ionization-mass spectrometry and correlation with phenotype. *Journal of Clinical Microbiology, 47*(10), 3129-3137.

Wyllie, D. H., Crook, D. W., & Peto, T. E. (2006). Mortality after *Staphylococcus aureus* bacteraemia in two hospitals in Oxfordshire, 1997-2003: cohort study. *BMJ, 333*(7562), 281.

Xu, Z., Li, L., Chu, J., Peters, B.M., Harris, M.L., Li, B., Shi, L., & Shirtliff, E. (2011). Development and application of loop-mediated isothermal amplification assays on rapid detection of various types of staphylococci strains. *Food Research International*. doi:10.1016/j.foodres.2011.04.042.

Xu, B. L., Zhang, G., Ye, H. F., Feil, E. J., Chen, G. R., Zhou, X. M., . . . Pan, W. B. (2009). Predominance of the Hungarian clone (ST 239-III) among hospital-acquired meticillin-resistant *Staphylococcus aureus* isolates recovered throughout mainland China. *Journal of Hospital Infections, 71*(3), 245-255.

Yamaguchi, T., Nishifuji, K., Sasaki, M., Fudaba, Y., Aepfelbacher, M., Takata, T., . . . Sugai, M. (2002). Identification of the *Staphylococcus aureus etd* pathogenicity island which encodes a novel exfoliative toxin, ETD, and EDIN-B. *Infections and Immunity, 70*(10), 5835-5845.

Yamamoto, T., Nishiyama, A., Takano, T., Yabe, S., Higuchi, W., Razvina, O., & Shi, D. (2010). Community-acquired methicillin-resistant *Staphylococcus aureus*: community transmission, pathogenesis, and drug resistance. *Journal of Infections and Chemotherapy, 16*(4), 225-254.

Yanagihara, K., Kaneko, Y., Sawai, T., Miyazaki, Y., Tsukamoto, K., Hirakata, Y., . . . Kohno, S. (2002). Efficacy of linezolid against methicillin-resistant or vancomycin-insensitive *Staphylococcus aureus* in a model of hematogenous pulmonary infection. *Antimicrobial Agents and Chemotherapy, 46*(10), 3288-3291.

Yu, J., Wu, J., Francis, K. P., Purchio, T. F., & Kadurugamuwa, J. L. (2005). Monitoring in vivo fitness of rifampicin-resistant *Staphylococcus aureus* mutants in a mouse biofilm infection model. *Journal of Antimicrobial Chemotherapy, 55*(4), 528-534.

Yun, H. J., Lee, S. W., Yoon, G. M., Kim, S. Y., Choi, S., Lee, Y. S., . . . Kim, S. (2003). Prevalence and mechanisms of low- and high-level mupirocin resistance in staphylococci isolated from a Korean hospital. *Journal of Antimicrobial Chemotherapy, 51*(3), 619-623.

Zetola, N., Francis, J. S., Nuermberger, E. L., & Bishai, W. R. (2005). Community-acquired meticillin-resistant *Staphylococcus aureus*: an emerging threat. *Lancet Infectious Disease, 5*(5), 275-286.

Zhang, K., McClure, J. A., Elsayed, S., Louie, T., & Conly, J. M. (2005). Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome *mec* types I to V in methicillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology, 43*(10), 5026-5033.

Zhu, W., Clark, N. C., McDougal, L. K., Hageman, J., McDonald, L. C., & Patel, J. B. (2008). Vancomycin-resistant *Staphylococcus aureus* isolates associated with *Inc18*-like *vanA* plasmids in Michigan. *Antimicrobial Agents and Chemotherapy, 52*(2), 452-457.

Zuccarelli, A. J., Roy, I., Harding, G. P., & Couperus, J. J. (1990). Diversity and stability of restriction enzyme profiles of plasmid DNA from methicillin-resistant *Staphylococcus aureus*. *Journal of Clinical Microbiology, 28*(1), 97-102.

APPENDIX 1

Bacterial Strains Information

Strains	Specimen	Ward Isolated	Year
MRSA2	Nasal swab	Orthopaedic	2003
MRSA3	Nasal swab	Orthopaedic	2003
MRSA4	Nasal swab	Orthopaedic	2003
MRSA8	Nasal swab	Orthopaedic	2003
MRSA0301-1	Wound swab	Orthopaedic	2003
MRSA0301-28	Tissue	Orthopaedic	2003
MRSA0302-4	Tissue	Orthopaedic	2003
MRSA0304-16	Nasal swab	ICU	2003
MRSA0305-10	Urine	Orthopaedic	2003
MRSA0305-18	Nasal swab	Orthopaedic	2003
MRSA0305-23	Nasal swab	Orthopaedic	2003
MRSA0306-10	Nasophargeal secretion	Paediatric	2003
MRSA0306-14	Bone	Orthopaedic	2003
MRSA0306-15	Pus	Medical	2003
MRSA0306-18	Bone	Orthopaedic	2003
MRSA0306-26	Wound swab	Orthopaedic	2003
MRSA0306-7	Wound swab	Orthopaedic	2003
MRSA0307-1	Wound swab	Orthopaedic	2003
MRSA0307-10	Nasophargeal secretion	Obstetric and gynaecology	2003
MRSA0307-14	Wound swab	ICU	2003
MRSA0307-20	Wound swab	Orthopaedic	2003
MRSA0307-23	Wound swab	Orthopaedic	2003
MRSA0307-25	Body fluid	Orthopaedic	2003
MRSA0307-5	Wound swab	Orthopaedic	2003
MRSA0307-9	Nasal swab	CICU	2003
MRSA0308-1	Tracheal secretion	Surgical	2003
MRSA0308-10	Tissue	Orthopaedic	2003
MRSA0308-22	Blood	ICU	2003
MRSA0308-23	Blood	ICU	2003
MRSA0308-24	Blood	ICU	2003
MRSA0308-28	Nasal swab	Orthopaedic	2003
MRSA0309-10	Tissue	Orthopaedic	2003
MRSA0309-11	Nasal swab	ICU	2003
MRSA0309-9	Nasal swab	Paediatric	2003
MRSA0310-19	Wound swab	Orthopaedic	2003
MRSA0310-23	Wound swab	Orthopaedic	2003
MRSA0310-26	Tracheal secretion	ICU	2003
MRSA0310-9	Wound swab	Orthopaedic	2003
MRSA0311-1	Tracheal secretion	ICU	2003
MRSA0311-21	Sputum	Orthopaedic	2003
MRSA0311-23	Tracheal secretion	ICU	2003
MRSA0311-4	Pus	Surgical	2003
MRSA0311-7	Tissue	Orthopaedic	2003
MRSA0311-8	Wound swab	Orthopaedic	2003
MRSA0311-9	Wound swab	Orthopaedic	2003
MRSA0312-13	Pus	Medical	2003

(continue)

Strain No	Specimen	Ward isolated	Year
MRSA0312-15	Blood	Medical	2003
MRSA0312-17	Tracheal secretion	Surgical	2003
MRSA0312-2	Slough	Orthopaedic	2003
MRSA0312-3	Pus	Other	2003
MRSA0312-30	Tissue	Orthopaedic	2003
MRSA0312-35	Chest tube	Surgical	2003
MRSA0401-13	Wound swab	Orthopaedic	2004
MRSA0402-21	Nasophargeal secretion	Paediatric	2004
MRSA0402-8	Tracheal secretion	ICU	2004
MRSA0403-20	Sputum	Dialysis	2004
MRSA0405-20	Blood	Medical	2004
MRSA0406-8	Blood	Other	2004
MRSA0408-33	Blood	Dialysis	2004
MRSA0408-34	Wound swab	Surgical	2004
MRSA0409-17	Shoulder swab	Surgical	2004
MRSA0701-15	Sputum	Medical	2007
MRSA0701-26	Wound swab	Surgical	2007
MRSA0703-8	Wound swab	Surgical	2007
MRSA0704-15	Nasal swab	Orthopaedic	2007
MRSA0704-18	Wound swab	Orthopaedic	2007
MRSA0704-20	Blood	CICU	2007
MRSA0704-3	Wound swab	ICU	2007
MRSA0705-13	Blood	Medical	2007
MRSA0705-17	Tracheal secretion	Medical	2007
MRSA0705-7	Tracheal secretion	Medical	2007
MRSA0705-8	Blood	Dialysis	2007
MRSA0707-17	Nasophargeal secretion	Paediatric	2007
MRSA0707-26	Nasal swab	Medical	2007
MRSA0708-1	Nasal swab	Surgical	2007
MRSA0708-10	Tracheal secretion	Medical	2007
MRSA0709-22	Tracheal secretion	Medical	2007
MRSA0801-1	Wound swab	Medical	2008
MRSA0801-13	Blood	Medical	2008
MRSA0801-16	Sputum	Mecial	2008
MRSA0801-2	Wound swab	Surgical	2008
MRSA0801-21	Pus	Surgical	2008
MRSA0801-26	Tracheal secretion	Orthopaedic	2008
MRSA0801-27	Tissue	ICU	2008
MRSA0801-30	Bone	Orthopaedic	2008
MRSA0801-4	Tracheal secretion	Medical	2008
MRSA0801-9	Sputum	Medical	2008
MRSA0802-14	Nasal swab	Orthopaedic	2008
MRSA0802-19	Nasopharygeal secretion	Paediatric	2008
MRSA0802-2	Nasal swab	Orthopaedic	2008
MRSA0802-3	Wound swab	Surgical	2008
MRSA0803-28	Nasal swab	ICU	2008
MRSA0803-29	Sputum	Surgical	2008
MRSA0803-30	Tissue	Orthopaedic	2008
MRSA003-35	Tracheal secretion	Orthopaedic	2008

(continue)

Strain No	Specimen	Ward isolated	
MRSA0804-1	Nasal swab	Cardiac care unit	2008
MRSA0804-14	Nasal swab	Orthopaedic	2008
MRSA0804-20	Nasal swab	Medical	2008
MRSA0804-24	Tissue	Medical	2008
MRSA0805-1	Nasopharygeal secretion	Paediatric	2008
MRSA0805-10	Wound swab	Orthopaedic	2008
MRSA0805-11	Nasal swab	Cardiac care unit	2008
MRSA0805-15	Tissue	Orthopaedic	2008
MRSA0805-17	Sputum	Medical	2008
MRSA0805-19	Pus	Orthopaedic	2008
MRSA0805-20	Urine	Orthopaedic	2008
MRSA0805-21	Tip	Medical	2008
MRSA0805-22	Tracheal secretion	Medical	2008
MRSA0805-23	Tracheal secretion	Cardiac care unit	2008
MRSA0805-24	Wound swab	Medical	2008
MRSA0805-3	Nasal swab	Surgical	2008
MRSA0805-4	Sputum	Dialysis	2008
MRSA0805-5	Urine	Surgical	2008
MRSA0805-6	Nasal swab	Orthopaedic	2008
MRSA0805-9	Pus	Psychiatry	2008
MRSA0806-1	Blood	Dialysis	2008
MRSA0806-11	Wound swab	Medical	2008
MRSA0806-13	Nasal swab	Dialysis	2008
MRSA0806-14	Nasal swab	Orthopaedic	2008
MRSA0806-18	Nasal swab	Orthopaedic	2008
MRSA0806-21	Blood	Surgical	2008
MRSA0806-22	Nasal swab	ICU	2008
MRSA0806-26	Nasal swab	Surgical	2008
MRSA0806-33	Nasopharygeal secretion	Paediatric	2008
MRSA0807-1	Nasal swab	Orthopaedic	2008
MRSA0807-13	Nasal Swab	Surgical	2008
MRSA0807-14	Urine	Surgical	2008
MRSA0807-19	Tracheal secretion	Other	2008
MRSA0807-7	Nasal swab	Surgical	2008
MRSA0807-8	Nasal swab	Surgical	2008
MRSA0808-17	Sputum	Medical	2008
MRSA0808-19	Wound swab	Orthopaedic	2008
MRSA0808-21	Bone	Orthopaedic	2008
MRSA0808-24	Wound swab	Obstetric and gynaecology	2008
MRSA0808-25	Tip	Neurosurgical	2008
MRSA0808-26	Nasal swab	CICU	2008
MRSA0808-35	Wound swab	Surgical	2008
MRSA0809-1	Graft	Surgical	2008
MRSA0809-10	Sputum	Medical	2008
MRSA0809-14	Tracheal secretion	Medical	2008
MRSA0809-15	Tracheal secretion	Medical	2008
MRSA0809-24	Tracheal secretion	Medical	2008
MRSA0809-25	Sputum	Dialysis	2008
MRSA0809-27	Nasal swab	Medical	2008

(continue)

Strain No	Resistance profiles		
MRSA0809-30	Sputum	Medical	2008
MRSA0809-32	Pus	Dialysis	2008
MRSA0809-33	Tissue	Medical	2008
MRSA0809-36	Urine	Medical	2008
MRSA0809-38	Tracheal secretion	Surgical	2008
MRSA0810-10	Tracheal secretion	Medical	2008
MRSA0810-13	Wound swab	Dialysis	2008
MRSA0810-15	Sputum	Surgical	2008
MRSA0810-16	Tracheal secretion	Medical	2008
MRSA0810-17	Sputum	Medical	2008
MRSA0810-18	Nasal swab	Orthopaedic	2008
MRSA0810-2	Nasal swab	Medical	2008
MRSA0810-22	Nasal swab	Medical	2008
MRSA0810-23	Tracheal secretion	Medical	2008
MRSA0810-6	Sputum	Medical	2008
MRSA0810-7	Pus	Medical	2008
MRSA0810-9	Pus	Orthopaedic	2008
MRSA0811-10	Urine	Surgical	2008
MRSA0811-11	Sputum	Orthopaedic	2008
MRSA0811-13	Nasopharygeal secretion	Medical	2008
MRSA0811-16	Nasal swab	Medical	2008
MRSA0811-2	Sputum	Surgical	2008
MRSA0811-22	Nasal swab	Cardiac care unit	2008
MRSA0811-24	Nasal swab	Medical	2008
MRSA0811-25	Tip	Surgical	2008
MRSA0811-26	Tracheal secretion	Surgical	2008
MRSA0811-28	Nasal swab	Surgical	2008
MRSA0811-30	Wound swab	Orthopaedic	2008
MRSA0811-5	Tracheal secretion	ICU	2008
MRSA0811-8	Pus	Surgical	2008
MRSA0812-1	Nasal swab	Orthopaedic	2008
MRSA0812-11	Tracheal secretion	Surgical	2008
MRSA0812-15	Sputum	Medical	2008
MRSA0812-17	Nasopharygeal secretion	Paediatric	2008
MRSA0812-2	Nasal swab	Orthopaedic	2008
MRSA0812-22	Sputum	Surgical	2008
MRSA0812-23	Sputum	Medical	2008
MRSA0812-27	Wound swab	Orthopaedic	2008
MRSA0812-30	Tracheal secretion	Medical	2008
MRSA0812-31	Sputum	Medical	2008
MRSA0812-33	Tracheal secretion	ICU	2008
MRSA0812-35	Pus	Medical	2008
MRSA0812-36	Tissue	ICU	2008
MRSA0812-37	Tissue	Orthopaedic	2008

APPENDIX 2
Media, buffers and solution

All media and solutions were prepared with distilled or deionised water. Most of the media were sterilized by autoclaving at 15 psi at 121°C for 20 minutes, unless otherwise stated.

1. Tryptone soy agar (Oxoid Ltd, UK)

Tryptone soy agar	16.0 g
Distilled water up to 400 ml.	

2. Tryptone Soy broth (Oxoid Ltd, UK)

Tryptone soy broth	12.0 g
Distilled water up to 100 ml	

3. Luria-bertani (LB) agar

Tryptone (Oxoid Ltd, UK)	4.0 g
Yeast extracts (Oxoid Ltd, UK)	2.0 g
Sodium chloride (Merk, USA)	2.0 g
Bacteriological agar (Oxoid Ltd, UK)	6.0 g
Distilled water up to 400 ml	

4. Luria-bertani (LB) broth

Sodium chloride (Merck, USA)	1.0 g
Yeast extract (Oxoid Ltd, UK)	1.0 g
Tryptone (Oxoid Ltd, UK)	2.0 g
Distilled water up to 200 ml	

5. Mannitol salt agar (Oxoid Ltd, UK)

Mannitol salt agar	44.0 g
Distilled water up to 400 ml	

6. Meuller-hinton agar (BD-BBL, USA)

Meuller-hinton II agar	15.2 g
Distilled water up to 400 ml	

7. Brain-heart Infusion agar (Oxoid Ltd, UK)

Brain-heart infusion agar	20.8 g
Distilled water up to 400 ml	

8. 50% glycerol

Ultrapure glycerol (Invitrogen, USA)	25 ml
Distilled water up to 50 ml	

9. 0.85% Sodium chloride

   Sodium chloride (Merck, USA) 3.2 g

   Distilled water up to 400 ml

10. Lysozyme (10 mg/ml)

    Lysozyme (Sigma, USA) 0.01 g

    Sterile deonised water up to 1 ml and filter sterilized

11. Lysostaphin (1 mg/ml)

    Lysostaphin (Sigma, USA) 0.001 g

    Sterile deonised water up to 1 ml

12. 1M Tris, pH 8.0 (molecular weight = 121.44 g)

    Trizma base (Sigma, USA) 48.45 g

    Deonised water up to 360 ml

    The pH of the solution was adjusted to 8.0 by adding concentrated HCl and top up with deonised water up to 400 ml.

13. 0.5M Ethylenediaminetetraacetic acid, pH 8.0 (molecular weight = 372.44)

    Ethylenediaminetetraacetic acid (EDTA) (Invitrogen, USA) 74.4 g

    Deonised water up to 360 ml

    The pH of the solution was adjusted to 8.0 by adding NaOH and top up with deonised water up to 400 ml.

14. Solution I (TES) (10mM Tris, 1 mM EDTA and 0.1mM NaCl, pH8.0)

    1M Tris, pH 8.0 4 ml

    0.5M EDTA pH 8.0 0.8 ml

    Sodium chloride (molecular weight = 58.44 g) 23.38 g

    Sterile deonised water up to 400 ml

15. 10% Sodium dodecyle supphate (SDS)

    Sodium dodecyle sulphate (Invitrogen, USA) 10 g

    Sterile deonised water up to 100 ml. Do not autoclave.

16. 10N NaOH

    NaOH (Sigma, USA) (molecular weight = 40 g) 40 g

    Sterile deonised water up to 100 ml and do not autoclave.

17. Solution II (1% SDS, 0.2N NaOH)

    10% SDS 1 ml

    10N NaOH 0.2 ml

    Sterile deonised water up to 10 ml and used immediately.

18. 70% ethanol

Ultrapure ethanol (Sigma, USA)	70 ml
Sterile deonised water	30 ml

19. 0.5M sucrose

Sucrose (molecular weight = 342.30 g) (BDH, USA)	17.12 g
Sterile deonised water up to 100 ml and filter sterilized.	

20. 10% Glycerol

Ultrapure glycerol (Invitrogen, USA)	10 ml
Sterile deonised water up to 100 ml	

21. GYT Medium

Glycerol (Invitrogen, USA)	10 ml
Yeast extract (Oxoid Ltd, UK)	0.125 g
Tryptone (Oxoid Ltd, UK)	0.125 g
Deonised water up to 100 ml	

22. 0.2 M sodium hydrogen maleate

Maleic acid (Sigma, USA)	13.7 g
NaOH (Sigma, USA)	4.0 g
Deonised water up to 500 ml	

23. Pennasay broth

Antibiotic Medium 3 (BD, USA)	17.5 g
Deonised water up to 250 ml	

24. 2X SMM broth

0.2 M sodium hydrogen maleate	25 ml
0.1 N NaOH	40 ml
pH adjusted to 6.5 before addition of	
1M $MgCl_2$ (Promega Ltd, USA)	5 ml
Sucrose (Sigma, USA)	42.7g
Sterile deonsed water to 125 ml and filter sterilzed	

25. SMMP broth (pH 7.0)

2 X SMM	55 ml
4 X Pennasay broth	40 ml
10% (w/v) bovine albumin (Sigma, USA)	5 ml
pH adjusted to 7.0 and filter sterilized	

26. Congo red agar

Congo red (WR Prolabo, France)	0.2 g
Sucrose (Sigma, USA)	10 g

27. Tris-EDTA (TE) buffer (10mM Tris : 1mM EDTA, pH8.0)

1 M Tris, pH 8.0	10 ml
0.5 M EDTA, pH 8.0	2 ml

Deionised water to 1000 ml.

28. Proteinase K (10.0mg/ml)

Proteinase K (Promega, USA)	100 mg

Sterile deionised water to 10.0ml

29. 1.0% Seakem gold agarose

Seakem gold agarose (Cambrex Bio-Science, Rockland USA)	0.1g
TE buffer	10 ml

Swirl gently to disperse agarose and microwave for 30 seconds. Mix gently and repeat for 10-second interval until the agarose is completely dissolved.

30. Cell suspension buffer (100.0mM Tris, 100mM EDTA, pH8.0)

1 M Tris, pH 8.0	10 ml
0.5 M EDTA, pH 8.0	20 ml

Deionised water to 100 ml

31. 10% Sarcosyl (N-Lauryl-Sarcosine [Molecular weight = 293.39g]

Sodium N-lauroyl-sarcosinate Solution (Fluka, USA)	10 ml

Deionised water to 100 ml

32. Cell lysis buffer (50mM Tris, 50mM EDTA, pH8.0 + 1.0% Sarcosine)

1.0M Tris, pH 8.0	25 ml
0.5M EDTA, pH 8.0	50 ml
10% Sarcosyl	50 ml

Deionised water to 500 ml

33. Pre-Restriction buffer (per plug slice)

10 X RE Buffer (Promega, USA)	10 □ l
BSA (Promega, USA)	1 □ l
Deionised water	89 ml

34. Restriction buffer mixture (per plug slice)

10 X RE buffer (Promega, USA)	10 µl
Enzyme (Promega, USA)	2 µl
BSA (Promega, USA)	1 µl
Deionised water	87 µl

35. 10X Tris-Borate EDTA Buffer (TBE), pH8.3

Trizma-base (Sigma, USA)	121.1 g

Othoboric Acid (Merck, USA)	61.8 g
EDTA (Ultra Pure Grade) (Invitrogen, USA)	0.745 g

Deionised water to 1000 ml

The pH of the stock solution was adjusted pH to 8.3 and autoclaved. It was then diluted to 0.5X for routine use.

APPENDIX 3

Antimicrobial resistance profiles of the 188 MRSA strains

Strains	Resistance profiles
MRSA2	ERY, GEN, CIP, TET, OXA, SXT
MRSA3	ERY, GEN, CIP, OXA, SXT
MRSA4	ERY, GEN, CIP, OXA, SXT
MRSA8	ERY, GEN, CIP, TET, OXA, SXT
MRSA0301-1	ERY, CIP, OXA, SXT
MRSA0301-28	ERY, GEN,CIP. TET, OXA, SXT
MRSA0302-4	ERY, GEN, RD, CIP, NET, FD, OXA, SXT
MRSA0304-16	ERY, GEN, LZD, CIP, OXA
MRSA0305-10	ERY, GEN, NET, OXA
MRSA0305-18	ERY, GEN, OXA, SXT
MRSA0305-23	ERY, GEN, DA, CIP, TET, OXA, SXT
MRSA0306-10	ERY, GEN, CIP, TET, OXA, SXT
MRSA0306-14	ERY, GEN, CIP, NET, OXA, SXT
MRSA0306-15	ERY, GEN, CIP, OXA
MRSA0306-18	OXA
MRSA0306-26	ERY, GEN, CIP, NET, OXA
MRSA0306-7	ERY, GEN, CIP, OXA, SXT
MRSA0307-1	ERY, GEN, CIP, NET, OXA
MRSA0307-10	ERY, GEN, CIP, NET, OXA
MRSA0307-14	ERY, GEN, CIP, OXA
MRSA0307-20	ERY, GEN, CIP, NET, OXA, SXT
MRSA0307-23	ERY, GEN, CIP, FD, OXA
MRSA0307-25	ERY, GEN, CIP, OXA, SXT
MRSA0307-5	ERY, GEN, CIP, NET, OXA
MRSA0307-9	ERY, CIP, OXA
MRSA0308-1	ERY, GEN, CIP, OXA
MRSA0308-10	ERY GEN, RD, CIP, TET, OXA, SXT
MRSA0308-22	ERY, GEN, CIP, OXA
MRSA0308-23	ERY, GEN, RF, CIP, TE, FD, OXA, SXT
MRSA0308-24	ERY, GEN, CIP, OXA
MRSA0308-28	ERY, GEN, CIP, OXA, SXT
MRSA0309-10	ERY, GEN, CIP, OXA, SXT
MRSA0309-11	ERY, GEN, CIP, OXA
MRSA0309-9	ERY, GEN, CIP, NET, OXA
MRSA0310-19	ERY, GEN, CIP, OXA
MRSA0310-23	ERY, GEN, DA, CIP, NET, TET, OXA, SXT
MRSA0310-26	ERY, GEN, RD, CIP, NET, FD, OXA
MRSA0310-9	ERY, GEN, CIP, OXA
MRSA0311-1	ERY, GEN, CIP, OXA
MRSA0311-21	ERY, CIP, TET, OXA, SXT
MRSA0311-23	ERY, GEN, TEC, CIP, OXA, SXT
MRSA0311-4	ERY, GEN, CIP, NET, OXA
MRSA0311-7	ERY, GEN, CIP, OXA
MRSA0311-8	ERY, GEN, CIP, OXA
MRSA0311-9	ERY, GEN, CIP, OXA
MRSA0312-13	ERY, GEN, OXA

(continue)

Strain No	Resistance profiles
MRSA0312-15	ERY, GEN, CIP, OXA
MRSA0312-17	ERY, GEN, CIP, NET, OXA
MRSA0312-2	ERY, GEN, CIP, OXA
MRSA0312-3	ERY, GEN
MRSA0312-30	ERY, GEN, CIP, OXA
MRSA0312-35	MUP, TEC, TET, FD, OXA
MRSA0401-13	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0402-21	ERY, LZD, MUP, DA, CIP, TET, OXA, SXT
MRSA0402-8	ERY, GEN, MUP, TET, OXA, SXT
MRSA0403-20	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0405-20	ERY, GEN, CIP, OXA
MRSA0406-8	ERY, GEN, MUP, CIP, NET, TET, OXA
MRSA0408-33	ERY, GEN, MUP, CIP, OXA
MRSA0408-34	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0409-17	ERY, CIP, NET, TET, OXA, SXT
MRSA0701-15	ERY, GEN, CIP, OXA
MRSA0701-26	OXA
MRSA0703-8	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0704-15	ERY, GEN, CIP, TET, OXA, SXT
MRSA0704-18	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0704-20	ERY, GEN, CIP, NET, OXA
MRSA0704-3	ERY, GEN, MUP, CIP, TET, OXA, SXT
MRSA0705-13	ERY, GEN, RD, CIP, TET, FD, OXA, SXT
MRSA0705-17	ERY, GEN, CIP, OXA
MRSA0705-7	GEN, CIP. OXA
MRSA0705-8	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0707-17	OXA
MRSA0707-26	ERY, GEN, CIP, TET, OXA
MRSA0708-1	ERY, CIP, OXA
MRSA0708-10	ERY, CIP, OXA
MRSA0709-22	ERY, CIP, OXA
MRSA0801-1	ERY, GEN, CIP, NET, TET, OXA
MRSA0801-13	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0801-16	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0801-2	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0801-21	ERY, GEN, LZD, CIP, NET, OXA, SXT
MRSA0801-26	ERY, GEN, CIP, TET, OXA, SXT
MRSA0801-27	ERY, GEN, MUP, CIP, NET, TET, OXA
MRSA0801-30	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0801-4	ERY, GEN, CIP, TET, OXA
MRSA0801-9	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0802-14	ERY, GEN, CIP, NET, OXA, SXT
MRSA0802-19	OXA
MRSA0802-2	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0802-3	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0803-28	ERY, CIP, OXA, SXT
MRSA0803-29	ERY, GEN, CIP, TET, OXA, SXT
MRSA0803-30	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA003-35	ERY, GEN, CIP, NET, TET, OXA, SXT

(continue)

Strain No	Resistance profiles
MRSA0804-1	ERY, CIP, OXA, SXT
MRSA0804-14	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0804-20	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0804-24	ERY, GEN, CIP, OXA
MRSA0805-1	ERY, FD, OXA, SXT
MRSA0805-10	GEN, CIP, OXA, SXT
MRSA0805-11	ERY, GEN, CIP, TET, OXA, SXT
MRSA0805-15	ERY, GEN, MUP, DA, CIP, TE, FD, OXA, SXT
MRSA0805-17	ERY, GEN, CIP, NET, TET, FD, OXA, SXT
MRSA0805-19	ERY, GEN, RD, CIP, OXA
MRSA0805-20	ERY, GEN, MUP, CIP, NET, TET, OXA, SXT
MRSA0805-21	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0805-22	ERY, GEN, CIP, TET, OXA, SXT
MRSA0805-23	ERY, GEN, CIP, OXA, SXT
MRSA0805-24	ERY, GEN, CIP, TET, OXA, SXT
MRSA0805-3	ERY, GEN, NET, TET, OXA
MRSA0805-4	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0805-5	ERY, GEN, TET, OXA, SXT
MRSA0805-6	ERY, GEN, CIP, TET, OXA, SXT
MRSA0805-9	ERY, CIP, OXA, SXT
MRSA0806-1	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0806-11	OXA
MRSA0806-13	OXA
MRSA0806-14	ERY, GEN, OXA, SXT
MRSA0806-18	ERY, GEN, CIP, NET, OXA
MRSA0806-21	ERY, CIP, OXA, SXT
MRSA0806-22	ERY, GEN, CIP, NET, OXA, SXT
MRSA0806-26	ERY, GEN, NET, TET, OXA
MRSA0806-33	ERY, GEN, CIP, OXA
MRSA0807-1	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0807-13	ERY, GEN, CIP, TET, OXA, SXT
MRSA0807-14	ERY, GEN, CIP, NET, OXA
MRSA0807-19	ERY, GEN, CIP, OXA
MRSA0807-7	ERY, GEN, CIP, TET, OXA, SXT
MRSA0807-8	ERY, GEN, CIP, NET, TET, OXA
MRSA0808-17	ERY, GEN, MUP, CIP, NET, TET, OXA, SXT
MRSA0808-19	ERY, GEN, CIP, OXA
MRSA0808-21	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0808-24	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0808-25	ERY, GEN, CIP, OXA
MRSA0808-26	ERY, GEN, CIP, NET, OXA
MRSA0808-35	ERY, GEN, TET, OXA, SXT
MRSA0809-1	ERY, GEN, DA, CIP, TET, OXA, SXT
MRSA0809-10	ERY, CIP, TET, OXA, SXT
MRSA0809-14	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0809-15	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0809-24	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0809-25	ERY, GEN, CIP, NET, OXA, SXT
MRSA0809-27	ERY, CIP, OXA, SXT

(continue)

Strain No	Resistance profiles
MRSA0809-30	ERY. GEN, CIP, NET, TET, OXA, SXT
MRSA0809-32	ERY, GEN, RD, DA, CIP, TET, FD, OXA, SXT
MRSA0809-33	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0809-36	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0809-38	ERY, GEN, CIP, NET, TET, OXA
MRSA0810-10	ERY, CIP, FD, OXA
MRSA0810-13	ERY, GEN. CIP, TET, OXA, SXT
MRSA0810-15	ERY, GEN, CIP, NET, OXA
MRSA0810-16	ERY, GEN, CIP, OXA
MRSA0810-17	ERY, CIP, FD, OXA
MRSA0810-18	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0810-2	ERY, CIP, TET, OXA, SXT
MRSA0810-22	ERY, CIP, TET, OXA, SXT
MRSA0810-23	ERY, GEN, CIP, TET, OXA
MRSA0810-6	ERY, CIP, FD, OXA
MRSA0810-7	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0810-9	ERY, GEN, CIP, NET, OXA, SXT
MRSA0811-10	ERY, GEN, CIP, OXA
MRSA0811-11	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0811-13	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0811-16	ERY, GEN, NET, TET, OXA, SXT
MRSA0811-2	ERY, CIP, OXA, SXT
MRSA0811-22	ERY, CIP, OXA, SXT
MRSA0811-24	ERY, GEN, OXA, SXT
MRSA0811-25	ERY, GEN, RD, CIP, NET, TET, FD, OXA
MRSA0811-26	ERY, GEN, CIP, OXA, SXT
MRSA0811-28	ERY, GEN, CIP, TET, OXA, SXT
MRSA0811-30	ERY, CIP, TET, OXA, SXT
MRSA0811-5	ERY, GEN, CIP, TET, OXA
MRSA0811-8	ERY, GEN, CIP, TET, OXA, SXT
MRSA0812-1	ERY, CIP, OXA, SXT
MRSA0812-11	OXA
MRSA0812-15	ERY, GEN, CIP, NET, OXA, SXT
MRSA0812-17	ERY, GEN, CIP, TET, OXA, SXT
MRSA0812-2	ERY, GEN, DA, CIP, TET, OXA, SXT
MRSA0812-22	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0812-23	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0812-27	ERY, GEN, NET, TET, OXA, SXT
MRSA0812-30	ERY, GEN, CIP, TET, FD, OXA, SXT
MRSA0812-31	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0812-33	ERY, GEN, RD, CIP, TET, FD, OXA, SXT
MRSA0812-35	ERY, GEN, CIP, NET, TET, OXA, SXT
MRSA0812-36	ERY, GEN, CIP, OXA, SXT
MRSA0812-37	ERY, GEN, CIP, NET, TET, OXA, SXT

Abbreviations: CIP, ciprofloxacin; DA, clindamycin; ERY, erythromycin; FD, fusidic acid; GEN, gentamicin; LZD, linezolid; NET, netilmicin; RD, rifampicin; TEC, teicoplanin; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole; MUP, mupirocin; OXA, oxacillin

APPENDIX 4

Standard Nucleotide-nucleotide BLAST search results for DNA sequence of the amplified beta-lactam resistant gene (*blaZ*) from MRSA0810-6

> gb|DQ016068.1| Staphylococcus aureus strain E19778 beta-lactamase (blaZ) gene, partial cds
Length=519

Score = 470 bits (254), Expect = 3e-129
Identities = 264/269 (98%), Gaps = 0/269 (0%)
Strand=Plus/Minus

```
Query  23   TATTATAAGGTACTTGTTCTAACAAAATAGCACTATTTATCGCTTTTGAAGTTGAAGCAG  82
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  288  TATTATAAGGTACTTGTTCTAACAAAATAGCACTATTTATCGCTTTTGAAGTTGAAGCAT  229

Query  83   AGGCAAATCTCTTATCGGCATTAAATTTTACTTCCTTACCACTTTTAGTATCTAATGCAT  142
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  228  AGGCAAATCTCTTATCGGCATTAAATTTTACTTCCTTACCACTTTTAGTATCTAATGCAT  169

Query  143  AGACACCAATATTAGCATTATATTTCTTTTCTAAATTATTTAACTCTTTAGCATGTGAAC  202
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  168  AGACACCAATATTAGCATTATATTTCTTTTCTAAATTATTTAACTCTTTAGCATGTGAAC  109

Query  203  TGGGTGAATTACATGCACTTAGAACTAAAGCAATTGCAATTAAAAGTATTAACTTTTTCA  262
            ||| ||||||||||||| |||||||||||||||||||||||||||||||||||||||||||
Sbjct  108  TGGTTGAATTACATGCACTTAAAACTAAAGCAATTGCAATTAAAAGTATTAACTTTTTCA  49

Query  263  AAAGATACCCTCCGATATTACAGTTGTAA  291
            ||| |||||||| ||||||||||||||
Sbjct  48   AAATATACCCTCCAATATTACAGTTGTAA  20
```

APPENDIX 5

Standard Open Reading Frame BLAST search results for DNA sequence of the amplified tetracycline resistance protein class M (*tetM*) from MRSA0809-10

```
235 atggatttcttagcagaagtatatcgttcattatcagttttagat
    M  D  F  L  A  E  V  Y  R  S  L  S  V  L  D
190 ggggcaattctactgatttctgcaaaagatggcgtacaagcacaa
    G  A  I  L  L  I  S  A  K  D  G  V  Q  A  Q
145 actcgtatattatttcatgcacttaggaaaatgggattcccaca
    T  R  I  L  F  H  A  L  R  K  M  G  I  P  T
100 atctttttatcaataagattgaccaaaatggaattgatttatca
    I  F  F  I  N  K  I  D  Q  N  G  I  D  L  S
 55 acggtttatcaggatattaaagagaaacttctgccgaaattgtaa 11
    T  V  Y  Q  D  I  K  E  K  L  L  P  K  L  *
```

> gb|AEA35199.1| tetracycline resistance protein class M [uncultured bacterium]
Length=136

Score = 142 bits (359), Expect = 6e-42, Method: Compositional matrix adjust.
Identities = 70/70 (100%), Positives = 70/70 (100%), Gaps = 0/70 (0%)

```
Query  1    MDFLAEVYRSLSVLDGAILLISAKDGVQAQTRILFHALRKMGIPTIFFINKIDQNGIDLS  60
            MDFLAEVYRSLSVLDGAILLISAKDGVQAQTRILFHALRKMGIPTIFFINKIDQNGIDLS
Sbjct  44   MDFLAEVYRSLSVLDGAILLISAKDGVQAQTRILFHALRKMGIPTIFFINKIDQNGIDLS  103

Query  61   TVYQDIKEKL  70
            TVYQDIKEKL
Sbjct  104  TVYQDIKEKL  113
```

APPENDIX 6

Standard Open Reading Frame BLAST search results for DNA sequence of the amplified tetracycline resistance protein class K (*tetK)* from MRSA0704-15

```
116 ataggaacagcagtatatggaaaattatctgattatataaatata
    I  G  T  A  V  Y  G  K  L  S  D  Y  I  N  I
 71 aaaaaattgttaattattggtattagtttgagctgtcttggttca
    K  K  L  L  I  I  G  I  S  L  S  C  L  G  S
 26 ttgattgcttttattggtcanatcac 1
    L  I  A  F  I  G  X  I
```

> gb|AEA35190.1| tetracycline resistance protein class K [uncultured bacterium]
gb|AEA35191.1| tetracycline resistance protein class K [uncultured bacterium]
gb|AEA35192.1| tetracycline resistance protein class K [uncultured bacterium]
Length=54

Score = 67.8 bits (164), Expect = 3e-14, Method: Compositional matrix adjust.
Identities = 36/36 (100%), Positives = 36/36 (100%), Gaps = 0/36 (0%)

```
Query  1   IGTAVYGKLSDYINIKKLLIIGISLSCLGSLIAFIG  36
           IGTAVYGKLSDYINIKKLLIIGISLSCLGSLIAFIG
Sbjct  1   IGTAVYGKLSDYINIKKLLIIGISLSCLGSLIAFIG  36
```

APPENDIX 7

Standard Nucleotide-nucleotide BLAST search results for DNA sequence of the amplified *ermA* from MRSA0812-37

> gb|CP002643.1| Staphylococcus aureus subsp. aureus T0131, complete genome
Length=29139
Sort alignments for this subject sequence by: E value Score Percent identity Query start position Subject start position
Features in this part of subject sequence:rRNA adenine N-6-methyltransferase

Score = 174 bits (94), Expect = 7e-41
Identities = 98/100 (98%), Gaps = 1/100 (1%)
Strand=Plus/Plus

```
Query  1        ATCTGC-ACGAGCTTTGGGTTTACTATTAATGGTGGAGANGGATATAAAAATGCTCAAAA  59
                ||||||  |||||||||||||||||||||||| ||||||||||||||||
Sbjct  1733787  ATCTGCAACGAGCTTTGGGTTTACTATTAATGGTGGAGATGGATATAAAAATGCTCAAAA  1733846

Query  60       AAGTACCACCACTATATTTTCATCCTAAGCCAAGTGTAGA  99
                ||||||||||||||||||||||||||||||||||||||||
Sbjct  1733847  AAGTACCACCACTATATTTTCATCCTAAGCCAAGTGTAGA  1733886
```

APPENDIX 8

Standard Open Reading Frame BLAST search results for DNA sequence of the amplified *ermC* from MRSA0811-16

```
  107 atattgcagtttaaatttcctaaaaaccaatcctataaaatattt
        I  L  Q  F  K  F  P  K  N  Q  S  Y  K  I  F
   62 ggtaatataccttataacataagtacggatataatacgcaaaatt
        G  N  I  P  Y  N  I  S  T  D  I  I  R  K  I
   17 gtttttgatagtatagc 1
        V  F  D  S  I
```

> gb|AAL75490.1| ErmC [Staphylococcus aureus]
gb|AAL75491.1| ErmC [Staphylococcus aureus]
gb|AAL75492.1| ErmC [Staphylococcus aureus]
Length=127

Score = 72.8 bits (177), Expect = 2e-15, Method: Compositional matrix adjust.
Identities = 35/35 (100%), Positives = 35/35 (100%), Gaps = 0/35 (0%)

```
Query  1   ILQFKFPKNQSYKIFGNIPYNISTDIIRKIVFDSI  35
           ILQFKFPKNQSYKIFGNIPYNISTDIIRKIVFDSI
Sbjct  56  ILQFKFPKNQSYKIFGNIPYNISTDIIRKIVFDSI  90
```

APPENDIX 9

Standard Open Reading Frame BLAST search results for DNA sequence of the amplified *aac(6')-aph(2")* from MRSA0707-26

```
   1 attggntatggacaaatatataaaatgtatgatgagttatatact
     I G Y G Q I Y K M Y D E L Y T
  46 gattatcattatccaaaaactgatgagatagtctatggtatggat
     D Y H Y P K T D E I V Y G M D
  91 caatttataggagagccaaattattggagtaaagga 126
     Q F I G E P N Y W S K G
```

> ref|NP_115315.1| N-acetyltransferase [Staphylococcus aureus subsp. aureus Mu50]
ref|NP_816984.1| 6'-aminoglycoside N-acetyltransferase [Enterococcus faecalis V583]
ref|NP_863643.1| bifunctional aminoglycoside modifying enzyme AacA-AphD [Staphylococcus aureus] 112 more sequence titles
Length=479
GENE ID: 1119947 aacA | N-acetyltransferase [Staphylococcus aureus subsp. aureus Mu50] (10 or fewer PubMed links)

Score = 91.7 bits (226), Expect = 6e-21, Method: Compositional matrix adjust.
Identities = 42/42 (100%), Positives = 42/42 (100%), Gaps = 0/42 (0%)

```
Query  1    IGYGQIYKMYDELYTDYHYPKTDEIVYGMDQFIGEPNYWSKG  42
            IGYGQIYKMYDELYTDYHYPKTDEIVYGMDQFIGEPNYWSKG
Sbjct  70   IGYGQIYKMYDELYTDYHYPKTDEIVYGMDQFIGEPNYWSKG  111
```

APPENDIX 10

Standard Open Reading Frame BLAST search results for DNA sequence of the amplified *ileS2* from MRSA0406-8

```
  11 attattaacaataataataatatagagtggtttccttctcatatt
     I I N N N N N I E W F P S H I
  56 aaggaagggagaatggaaatttcttagaaaaatatggttgattgg
     K E G R M G N F L E N M V D W
 101 aacattggtagaaatagatattggggaacaccattaaatgtatgg
     N I G R N R Y W G T P L N V W
 146 atttgcaatgattgtaatcacgaatacgcaccaagtagtattaag
     I C N D C N H E Y A P S S I K
 191 gatttacaaaataattccatcaataaaattgatgaagatattgag
     D L Q N N S I N K I D E D I E
 236 ttgcatagaccttatgttgataatatcactcttagttgccctaag
     L H R P Y V D N I T L S C P K
 281 tgtaatgggaaaatgtctcgagtagaagaagtaatcgatgtttgg
     C N G K M S R V E E V I D V W
 326 tttgatagcggctctatgccgtttgctcagcatcattatcctttt
     F D S G S M P F A Q H H Y P F
 371 gataaccagaaaatttttaatcaacactttcnnnnnaattttatt 415
     D N Q K I F N Q H F X X N F I
```

> ref|YP_492689.1| isoleucyl-tRNA synthetase [Staphylococcus aureus subsp. aureus USA300_FPR3757]
ref|ZP_04796222.1| isoleucyl-tRNA synthetase [Staphylococcus epidermidis W23144]
ref|ZP_04824236.1| isoleucyl-tRNA synthetase [Staphylococcus epidermidis BCM-HMP0060]
18 more sequence titles Length=1024

GENE ID: 3912750 ileS | isoleucyl-tRNA synthetase [Staphylococcus aureus subsp. aureus USA300_FPR3757] (10 or fewer PubMed links)

Score = 271 bits (692), Expect = 1e-82, Method: Compositional matrix adjust.
Identities = 123/126 (98%), Positives = 124/126 (98%), Gaps = 0/126 (0%)

```
Query  10   WFPSHIKEGRMGNFLENMVDWNIGRNRYWGTPLNVWICNDCNHEYAPSSIKDLQNNSINK  69
            WFPSHIKEGRMGNFLENMVDWNIGRNRYWGTPLNVWICNDCNHEYAPSSIKDLQNNSINK
Sbjct  417  WFPSHIKEGRMGNFLENMVDWNIGRNRYWGTPLNVWICNDCNHEYAPSSIKDLQNNSINK  476

Query  70   IDEDIELHRPYVDNITLSCPKCNGKMSRVEEVIDVWFDSGSMPFAQHHYPFDNQKIFNQH  129
            IDEDIELHRPYVDNITLSCPKCNGKMSRVEEVIDVWFDSGSMPFAQHHYPFDNQKIFNQH
Sbjct  477  IDEDIELHRPYVDNITLSCPKCNGKMSRVEEVIDVWFDSGSMPFAQHHYPFDNQKIFNQH  536

Query  130  FXXNFI  135
            F +FI
Sbjct  537  FPADFI  542
```

APPENDIX 11

Standard Open Reading Frame BLAST search results for DNA sequence of the amplified *mupA* from MRSA0406-8

> gb|EF433950.1| Staphylococcus aureus strain MB1348 nonfunctional polymorphic mupirocin-resistance protein (mupA) gene, complete sequence
Length=3233

Score = 1910 bits (1034), Expect = 0.0
Identities = 1118/1158 (97%), Gaps = 26/1158 (2%)
Strand=Plus/Minus

```
Query  5     TCTTAAA-AATCGGGCTCCGTACTTATTAATTAATTCAGTTGGATTAATAACGTTTCCTT  63
             ||||||| |||| ||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1906  TCTTAAAGAATC-GGCTCCGTACTTATTAATTAATTCAGTTGGATTAATAACGTTTCCTT  1848

Query  64    TACTTTTAGACAttttttACCATTACTGTCTAGAATATGTCCTAAAGATAAAGCACGTT  123
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1847  TACTTTTAGACATTTTTTTACCATTACTGTCTAGAATATGTCCTAAAGATAAAGCACGTT  1788

Query  124   TATAAGAAGATTTTCCTTTTAGAATAGTAGAAATTACTAGTAAACTGTAAAACCAGCCTC  183
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1787  TATAAGAAGATTTTCCTTTTAGAATAGTAGAAATTACTAGTAAACTGTAAAACCAGCCTC  1728

Query  184   TCGTTTGATCAACTCCTTCTGCAATAAAATCAGCTGGAAAGTGTTGATTAAAAATTTTCT  243
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1727  TCGTTTGATCAACTCCTTCTGCAATAAAATCAGCTGGAAAGTGTTGATTAAAAATTTTCT  1668

Query  244   GGTTATCAAAAGGATAATGATGCTGAGCAAACGGCATAGAGCCGCTATCAAACCAAACAT  303
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1667  GGTTATCAAAAGGATAATGATGCTGAGCAAACGGCATAGAGCCGCTATCAAACCAAACAT  1608

Query  304   CGATTACTTCTTCTACTCGAGACATTTTCCCATTACACTTAGGGCAACTAAGAGTGATAT  363
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1607  CGATTACTTCTTCTACTCGAGACATTTTCCCATTACACTTAGGGCAACTAAGAGTGATAT  1548

Query  364   TATCAACATAAGGTCTATGCAACTCAATATCTTCATCAATTTTATTGATGGAATTATTTT  423
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1547  TATCAACATAAGGTCTATGCAACTCAATATCTTCATCAATTTTATTGATGGAATTATTTT  1488

Query  424   GTAAATCCTTAATACTACTTGGTGCGTATTCGTGATTACAATCATTGCAAATCCATACAT  483
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1487  GTAAATCCTTAATACTACTTGGTGCGTATTCGTGATTACAATCATTGCAAATCCATACAT  1428

Query  484   TTAATGGTGTTCCCCAATATCTATTTCTACCAATGTTCCAATCAACCATATTTTCTAAGA  543
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1427  TTAATGGTGTTCCCCAATATCTATTTCTACCAATGTTCCAATCAACCATATTTTCTAAGA  1368

Query  544   AATTTCCCATTCTCCCTTCCTTAATATGAGAAGGAAACCACTCTATATTATTATTATTGT  603
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1367  AATTTCCCATTCTCCCTTCCTTAATATGAGAAGGAAACCACTCTATATTATTATTATTGT  1308

Query  604   TAATAATTTCATTCTTAAAATTAGTTGTTTTAATAAACCAACCTTCCATCGCATAATATA  663
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1307  TAATAATTTCATTCTTAAAATTAGTTGTTTTAATAAACCAACCTTCCATCGCATAATATA  1248

Query  664   TCAAAGGATTACCACATCTCCAACAATGAGGATAATTATGCTCatatttttgtttttat  723
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1247  TCAAAGGATTACCACATCTCCAACAATGAGGATAATTATGCTCATATTTTTGTTTTTTAT  1188

Query  724   ataaaagttgtttttggataataattttatgatttctatatcactattttAGCTTTAT  783
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1187  ATAAAAGTTGTTTTTTGGATAATAATTTTATGATTTCTATATCACTATTTTTAGCTTTAT  1128

Query  784   TACCAACTAATTCAGGGAACCTATCATTATATACTCCTTCTCTTGTTATAACATTTAAGA  843
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1127  TACCAACTAATTCAGGGAACCTATCATTATATACTCCTTCTCTTGTTATAACATTTAAGA  1068

Query  844   AATCCAAATCACGCTCTAAAACCAATTGGTAGTCATCTTCCC-ATGAGCTGGTGCTATAT  902
             |||||||||||||||||||||||||||||||| ||||||||||||||
Sbjct  1067  AATCCAAATCACGCTCTAAAACCAATTGGTAGTCATCTTCCCCATGAGCTGGTGCTATAT  1008

Query  903   GAACAATACCAGTTCCTTCTGAGTTAGTAACAA-TTCTCCATCAACAACGTAATATGCAT  961
             |||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  1007  GAACAATACCAGTTCCTTCTGAGTTAGTAACAAATTCTCCATCAACAACGTAATATGCAT  948

Query  962   TAACTAAAACCGTCGCTT-CAAA-GGNNGGA-TGTATTTTNAATTTATTAANTTACTTNC  1018
             ||||||| |||||||| |||| ||  ||| |||||||| |||| ||||| ||||| |
Sbjct  947   TAACTAAA-CCGTCGCTTTCAAAAGGA-GGAATGTATTTTAAATTAATTAAATTACTTCC  890

Query  1019  TGAAAGGGTATCA-TA-TTTCGTATTTTTTCAGTTAATNATAGANT-AAT-AGGATCTTG  1074
             ||||| ||||||| || |||||||||| |||||| | ||||| | ||| || |||| |
Sbjct  889   TGAAAAGGTATCAATAATTTCGTATTTTT-CAGTTATT-ATAGAATTAATTAG-ATCT-G  834

Query  1075  TAGCTA-GATAATA-TACTCAATTTCTACCCCNANTTTTTGA-TANN-A-GATCTT-ANT  1128
             |||||| |||| || |||||| |||||||||  | |||||||  ||   | |||||| | |
Sbjct  833   TAGCTAAGATA-TAATACTCATTTTCTACCCGAA-TTTTTGAATAATTAAGATCTTTATT  776

Query  1129  TATAGCTA-TGCTACATT  1145
             ||||||| |||||||| |
Sbjct  775   TATAGCTAATGCTACATT  758
```

APPENDIX 12

Standard Nucleotide-nucleotide BLAST search results for DNA sequence of the amplified macrolide efflux (*msrA*) from MRSA0812-36

> gb|EF092840.1| Staphylococcus epidermidis macrolide efflux resistance (msrA) gene, partial cds
Length=974

Score = 187 bits (101), Expect = 1e-44
Identities = 105/107 (98%), Gaps = 0/107 (0%)
Strand=Plus/Minus

```
Query  1    CTACACCATTTGCACCTACGAGCGCTATATTTTTGCCATATGGTATTTGGAATCGTACTT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  877  CTACACCATTTGCACCTACGAGCGCTATATTTTTGCCATATGGTATTTGGAATCGTACTT  818

Query  61   GTGTTAGCAGTTTTTGACTCCCTTTAACCAATGTTAAATTTTGTGCA  107
            |||||||||||||||| |||||||||||||| |||||||||
Sbjct  817  GTGTTAGCAGTTTTTGACTTCCTTTAACCAATGTTAGATTTTGTGCA  771
```

APPENDIX 13

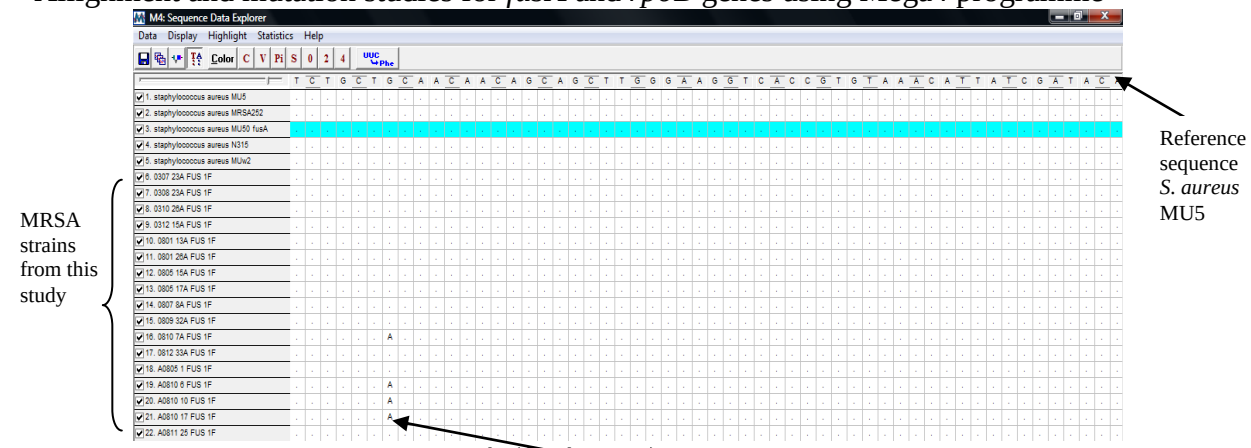
Standard Nucleotide-nucleotide BLAST search results for DNA sequence of the amplified transposon Tn*5801*-like tetracycline resistance protein (*tetM*) from MRSA0805-15**.**

> gb|EU918655.2| Staphylococcus aureus strain 1680 transposon Tn5801-like tetracycline resistance protein (tetM) gene, complete cds
Length=5520
Score = 1243 bits (673), Expect = 0.0
Identities = 676/677 (99%), Gaps = 1/677 (0%)
Strand=Plus/Minus

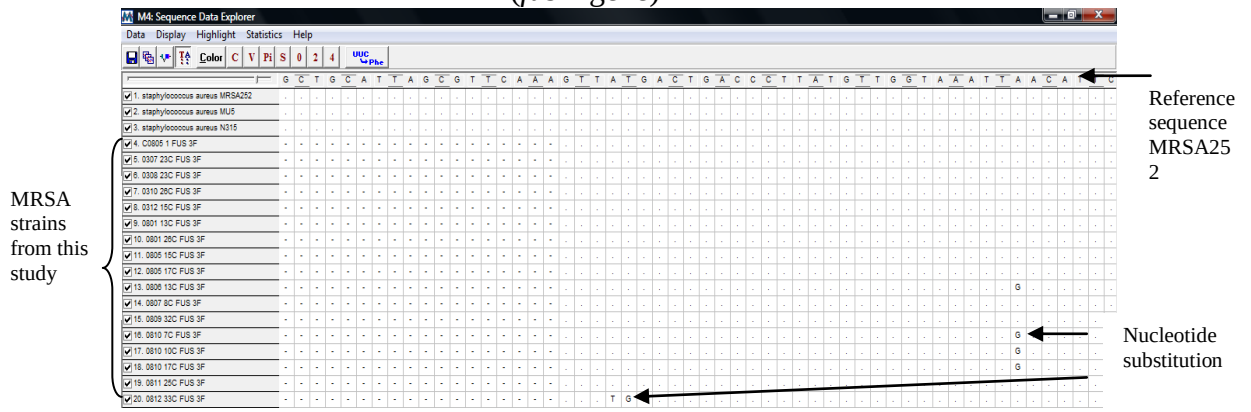
```
Query  1    TTAATGATAAGTGGATTTTCTCCCATACTGATTAACAAGGAAGCATGAGAATGCCGTAAC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5516 TTAATGATAAGTGGATTTTCTCCCATACTGATTAACAAGGAAGCATGAGAATGCCGTAAC  5457

Query  61   CCATGGATTCTGATTCGATGGATACCAGCCAGCTTAGCATAGCGTTCAATAGCATATGAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5456 CCATGGATTCTGATTCGATGGATACCAGCCAGCTTAGCATAGCGTTCAATAGCATATGAT  5397

Query  121  AGTGTATGTTTTTGGGTAGGAATACCGTTATAGCTCATTACAAAGTCTGTCTGAATTAGA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5396 AGTGTATGTTTTTGGGTAGGAATACCGTTATAGCTCATTACAAAGTCTGTCTGAATTAGA  5337

Query  181  TTTTGTTGTACTTCCTTCCATTCGGATAAATAAGTTAATGTGCATTTATCTAATACAATA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5336 TTTTGTTGTACTTCCTTCCATTCGGATAAATAAGTTAATGTGCATTTATCTAATACAATA  5277

Query  241  TGACGAACGCTCGCCTTTGTTTTTGGTTCAACAAATCTATAATTCGATTGATTCTTGTAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5276 TGACGAACGCTCGCCTTTGTTTTTGGTTCAACAAATCTATAATTCGATTGATTCTTGTAA  5217

Query  301  TAGAGTGTTTTGTTGATGGTTAGTACTCCTGAGTCAAAATCAATATCTTCCCATTGAATG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5216 TAGAGTGTTTTGTTGATGGTTAGTACTCCTGAGTCAAAATCAATATCTTCCCATTGAATG  5157

Query  361  GCAGTAGCTTCACCAATTCGCATTCCAGTCATAAATAAAAACCACAGAGAGATAAATAAA  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5156 GCAGTAGCTTCACCAATTCGCATTCCAGTCATAAATAAAAACCACAGAGAGATAAATAAA  5097

Query  421  AAGTGTTGATAGTAATCTTCTTTGTAAATTAGAGAGATTActttttcaaattcttctttt  480
            ||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5096 AAGTGTTGATAGTAATCTTCTTTGTAAATTAGAGAGATTACTTTTTCAAATTCTTCTTTT  5037

Query  481  gtccaaaattcaattttagacttttgctttttcACATTGCCAATAATCTTAGATGGATTT  540
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  5036 GTCCAAAATTCAATTTTAGACTTTTGCTTTTTCACATTGCCAATAATCTTAGATGGATTT  4977

Query  541  GTGGTGGTTATCCCAAGGACAATTGCTCGATCCATAGCAACTGAAAATAGTCCTTGAACA  600
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4976 GTGGTGGTTATCCCAAGGACAATTGCTCGATCCATAGCAACTGAAAATAGTCCTTGAACA  4917

Query  601  GCTCTTACATAAGAAGACTTACACTTCTTTGATAATTTTAGTTGCCAATTTTGCACATCA  660
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  4916 GCTCTTACATAAGAAGACTTACACTTCTTTGATAATTTTAGTTGCCAATTTTGCACATCA  4857

Query  661  ATAGGCTCTAATATCGG  677
            ||||||| |||||||
Sbjct  4856 ATAGGCTC-AATATCGG  4841
```

APPENDIX 14

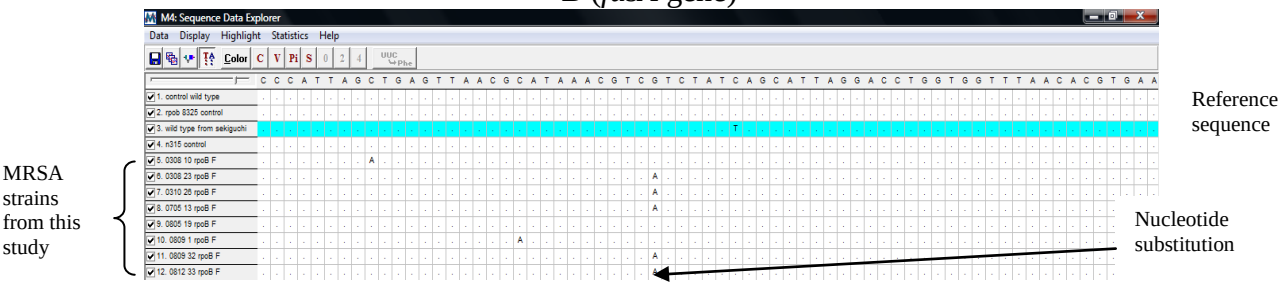
Allignment and mutation studies for *fus*A and *rpoB* genes using Mega4 programme



A (*fus*A gene)



B (*fusA* gene)



C (*rpoB* gene)

APPENDIX 15

GenBank flat file for mutational changes in *rpoB* gene for MRSA0308-10

LOCUS HQ914953 375 bp DNA linear BCT 27-JUL-2011
DEFINITION *Staphylococcus aureus* strain MRSA0308-10 RNA polymerase subunit beta (*rpoB*) gene, partial cds.
ACCESSION HQ914953
VERSION HQ914953
KEYWORDS
SOURCE *Staphylococcus aureus*
ORGANISM *Staphylococcus aureus*
Bacteria; Firmicutes; Bacillales; *Staphylococcus.*
REFERENCE 1 (bases 1 to 375)
AUTHORS Lim,K.T., Abu Hanifah,Y., Mohd Yusof,M.Y. and Thong,K.L.
TITLE Prevalence and molecular characterizaiton of rifampicin resistance in *Staphylococcus aureus* isolated in Malaysia
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 375)
AUTHORS Lim,K.T., Abu Hanifah,Y., Mohd Yusof,M.Y. and Thong,K.L.
TITLE Direct Submission
JOURNAL Submitted (21-JAN-2011) Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia
FEATURES Location/Qualifiers
source 1..375
/organism="Staphylococcus aureus"
/mol_type="genomic DNA"
/strain="MRSA0308-10"
/host="Homo sapiens"
/db_xref="taxon:1280"
/country="Malaysia"
gene <1..>375
/gene="rpoB"
CDS <1..>375
/gene="rpoB"
/codon_start=1
/transl_table=11
/product="RNA polymerase subunit beta"
/protein_id="AEK94022"
/translation="MERVVRERMSIQDTESITPQQLINIRPVIASIKEFFGSSQLSQF
MDQANPLDELTHKRRLSALGPGGLTRERAQMEVRDVHYSHYGRMCPIETPEGPNIGLI
NSLSSYARVNEFGFIETPYRKVE"
ORIGIN
1 atggaaagag ttgtacgtga aagaatgtca attcaagata ctgagtctat cacacctcaa
61 caattaatta atattcgacc tgttattgca tctattaaag aattctttgg tagctctcaa
121 ttatcacaat tcatggacca agcaaaccca ttagatgagt taacgcataa acgtcgtcta
181 tcagcattag gacctggtgg tttaacacgt gaacgtgctc aaatggaagt acgtgacgtt
241 cactactctc actatggccg tatgtgtcca attgaaacac ctgagggacc aaacattgga
301 ttgattaact cattatcaag ttatgcacgt gtaaatgaat tcggctttat tgaaacacca
361 tatcgtaaag ttgaa

APPENDIX 16

GenBank flat file for mutational changes in *rpoB* gene for MRSA0308-23

LOCUS HQ914957 374 bp DNA linear BCT 27-JUL-2011
DEFINITION *Staphylococcus aureus* strain MRSA0308-23 RNA polymerase subunit beta (*rpoB*) gene, partial cds.
ACCESSION HQ914957
VERSION HQ914957
KEYWORDS .
SOURCE *Staphylococcus aureus*
ORGANISM *Staphylococcus aureus*
Bacteria; Firmicutes; Bacillales; *Staphylococcus.*
REFERENCE 1 (bases 1 to 374)
AUTHORS Lim,K.T., Abu Hanifah,Y., Mohd Yusof,M.Y. and Thong,K.L.
TITLE Prevalence and molecular characterizaiton of rifampicin resistance in *Staphylococcus aureus* isolated in Malaysia
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 374)
AUTHORS Lim,K.T., Abu Hanifah,Y., Mohd Yusof,M.Y. and Thong,K.L.
TITLE Direct Submission
JOURNAL Submitted (21-JAN-2011) Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia
FEATURES Location/Qualifiers
source 1..374
/organism="Staphylococcus aureus"
/mol_type="genomic DNA"
/strain="MRSA0308-23"
/host="Homo sapiens"
/db_xref="taxon:1280"
/country="Malaysia"
gene <1..>374
/gene="rpoB"
CDS <1..>374
/gene="rpoB"
/codon_start=1
/transl_table=11
/product="RNA polymerase subunit beta"
/protein_id="AEK94026"
/translation="MERVVRERMSIQDTESITPQQLINIRPVIASIKEFFGSSQLSQF
MDQANPLAELTHKRHLSALGPGGLTRERAQMEVRDVHYSHYGRMCPIQTPEGPNIGLI
NSLSSYARVNEFGFIETPYRKV"
ORIGIN
1 atggaaagag ttgtacgtga aagaatgtca attcaagata ctgagtctat cacacctcaa
61 caattaatta atattcgacc tgttattgca tctattaaag aattctttgg aagctctcaa
121 ttatcacaat tcatggacca agcaaaccca ttagctgagt taacgcataa acgtcatcta
181 tcagcattag gacctggtgg tttaacacgt gaacgtgctc aaatggaagt acgtgacgtt
241 cactactctc actatggccg tatgtgtcca attcaaacac ctgagggacc aaacattgga
301 ttgattaact cattatcaag ttatgcacgt gtaaatgaat tcggctttat tgaaacacca
361 tatcgtaaag ttga

APPENDIX 17

GenBank flat file for mutational changes in *rpoB* gene for MRSA0310-26

LOCUS HQ914958 384 bp DNA linear BCT 27-JUL-2011
DEFINITION *Staphylococcus aureus* strain MRSA0310-26 RNA polymerase subunit beta (*rpoB*) gene, partial cds.
ACCESSION HQ914958
VERSION HQ914958
KEYWORDS .
SOURCE *Staphylococcus aureus*
ORGANISM *Staphylococcus aureus*
Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE 1 (bases 1 to 384)
AUTHORS Lim,K.T., Abu Hanifah,Y., Mohd Yusof,M.Y. and Thong,K.L.
TITLE Prevalence and molecular characterizaiton of rifampicin resistance in *Staphylococcus aureus* isolated in Malaysia
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 384)
AUTHORS Lim,K.T., Abu Hanifah,Y., Mohd Yusof,M.Y. and Thong,K.L.
TITLE Direct Submission
JOURNAL Submitted (21-JAN-2011) Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia
FEATURES Location/Qualifiers
source 1..384
/organism="Staphylococcus aureus"
/mol_type="genomic DNA"
/strain="MRSA0310-26"
/host="Homo sapiens"
/db_xref="taxon:1280"
/country="Malaysia"
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/gene="rpoB"
CDS <1..>384
/gene="rpoB"
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/transl_table=11
/product="RNA polymerase subunit beta"
/protein_id="AEK94027"
/translation="GVVENXVVRERMSIQDTESITPQQLINIRPVIASIKEFFGSSQL
SQFMDQANPLAELTHKRHLSALGPGGLTRERAQMEVRDVHYSHYGRMCPIQTPEGPNI
GLINSLSSYARVNEFGFIETPYRKVE"
ORIGIN
1 ggtgtagttg agaacngagt tgtacgtgaa agaatgtcaa ttcaagatac tgagtctatc
61 acacctcaac aattaattaa tattcgacct gttattgcat ctattaaaga attctttggt
121 agctctcaat tatcacaatt catggaccaa gcaaacccat tagctgagtt aacgcataaa
181 cgtcatctat cagcattagg acctggtggt ttaacacgtg aacgtgctca aatggaagta
241 cgtgacgttc actactctca ctatggccgt atgtgtccaa ttcaaacacc tgagggacca
301 aacattggat tgattaactc attatcaagt tatgcacgtg taaatgaatt cggctttatt
361 gaaacaccat atcgtaaagt tgaa

APPENDIX 18

GenBank flat file for mutational changes in *rpoB* gene for MRSA0705-13

LOCUS HQ914959 378 bp DNA linear BCT 27-JUL-2011
DEFINITION *Staphylococcus aureus* strain MRSA0705-13 RNA polymerase subunit beta (*rpoB)* gene, partial cds.
ACCESSION HQ914959
VERSION HQ914959
KEYWORDS .
SOURCE *Staphylococcus aureus*
ORGANISM *Staphylococcus aureus*
Bacteria; Firmicutes; Bacillales; *Staphylococcus.*
REFERENCE 1 (bases 1 to 378)
AUTHORS Lim,K.T., Abu Hanifah,Y., Mohd Yusof,M.Y. and Thong,K.L.
TITLE Prevalence and molecular characterizaiton of rifampicin resistance in *Staphylococcus aureus* isolated in Malaysia
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 378)
AUTHORS Lim,K.T., Abu Hanifah,Y., Mohd Yusof,M.Y. and Thong,K.L.
TITLE Direct Submission
JOURNAL Submitted (21-JAN-2011) Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia
FEATURES Location/Qualifiers
source 1..378
/organism="Staphylococcus aureus"
/mol_type="genomic DNA"
/strain="MRSA0705-13"
/host="Homo sapiens"
/db_xref="taxon:1280"
/country="Malaysia"
gene <1..>378
/gene="rpoB"
CDS <1..>378
/gene="rpoB"
/codon_start=1
/transl_table=11
/product="RNA polymerase subunit beta"
/protein_id="AEK94028"
/translation="RMERVVRERMSIQDTESITPQQLINIRPVIASIKEFFGSSQLSQ
FMDQANPLAELTHKRHLSALGPGGLTRERAQMEVRDVHYSHYGRMCPIQTPEGPNIGL
INSLSSYARVNEFGFIETPYRKVE"
ORIGIN
1 agaatggaaa gagttgtacg tgaaagaatg tcaattcaag atactgagtc tatcacacct
61 caacaattaa ttaatattcg acctgttatt gcatctatta aagaattctt tggtagctct
121 caattatcac aattcatgga ccaagcaaac ccattagctg agttaacgca taaacgtcat
181 ctatcagcat taggacctgg tggtttaaca cgtgaacgtg ctcaaatgga agtacgtgac
241 gttcactact ctcactatgg ccgtatgtgt ccaattcaaa cacctgaggg accaaacatt
301 ggattgatta actcattatc aagttatgca cgtgtaaatg aattcggctt tattgaaaca
361 ccatatcgta aagttgaa

APPENDIX 19

GenBank flat file for mutational changes in *rpoB* gene for MRSA0809-1

```
//
LOCUS       HQ914960               360 bp    DNA     linear  BCT 27-JUL-2011
DEFINITION  Staphylococcus aureus strain MRSA0809-1 RNA polymerase subunit beta (rpoB) gene,
            partial cds.
ACCESSION   HQ914960
VERSION     HQ914960
KEYWORDS    .
SOURCE      Staphylococcus aureus
ORGANISM  Staphylococcus aureus
          Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE  1  (bases 1 to 360)
AUTHORS  Lim,K.T., Abu Hanifah,Y., Mohd Yusof,M.Y. and Thong,K.L.
TITLE     Prevalence and molecular characterizaiton of rifampicin resistance in Staphylococcus aureus isolated in Malaysia
JOURNAL  Unpublished
REFERENCE  2  (bases 1 to 360)
AUTHORS  Lim,K.T., Abu Hanifah,Y., Mohd Yusof,M.Y. and Thong,K.L.
TITLE     Direct Submission
JOURNAL  Submitted (21-JAN-2011) Institute of Biological Sciences, Faculty of Science, University of
             Malaya, Kuala Lumpur 50603, Malaysia
FEATURES             Location/Qualifiers
     source          1..360
                     /organism="Staphylococcus aureus"
                     /mol_type="genomic DNA"
                     /strain="MRSA0809-1"
                     /host="Homo sapiens"
                     /db_xref="taxon:1280"
                     /country="Malaysia"
     gene            <1..>360
                     /gene="rpoB"
     CDS             <1..>360
                     /gene="rpoB"
                     /codon_start=1
                     /transl_table=11
                     /product="RNA polymerase subunit beta"
                     /protein_id="AEK94029"
                     /translation="RERMSIQDTESITPQQLINIRPVIASIKEFFGSSQLSQFMDQAN
                     PLAELTNKRRLSALGPGGLTRERAQMEVRDVHYSHYGRMCPIETPEGPNIGLINSLSS
                     YARVNEFGFIETPYRKVE"
ORIGIN
        1 cgtgaaagaa tgtcaattca agatactgag tctatcacac ctcaacaatt aattaatatt
       61 cgacctgtta ttgcatctat taaagaattc tttggtagct ctcaattatc acaattcatg
      121 gaccaagcaa acccattagc tgagttaacg aataaacgtc gtctatcagc attaggacct
      181 ggtggtttaa cacgtgaacg tgctcaaatg gaagtacgtg acgttcacta ctctcactat
      241 ggccgtatgt gtccaattga aacacctgag ggaccaaaca ttggattgat taactcatta
      301 tcaagttatg cacgtgtaaa tgaattcggc tttattgaaa caccatatcg taaagttgaa
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APPENDIX 20

GenBank flat file for mutational changes in *rpoB* gene for MRSA0809-32

```
//
LOCUS       HQ914961               366 bp    DNA     linear  BCT 27-JUL-2011
DEFINITION  Staphylococcus aureus strain MRSA0809-32 RNA polymerase subunit beta (rpoB) gene,
            partial cds.
ACCESSION   HQ914961
VERSION     HQ914961
KEYWORDS    .
SOURCE      Staphylococcus aureus
ORGANISM  Staphylococcus aureus
          Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE  1  (bases 1 to 366)
AUTHORS  Lim,K.T., Abu Hanifah,Y., Mohd Yusof,M.Y. and Thong,K.L.
TITLE     Prevalence and molecular characterizaiton of rifampicin resistance in Staphylococcus aureus
          isolated in Malaysia
JOURNAL  Unpublished
REFERENCE  2  (bases 1 to 366)
AUTHORS  Lim,K.T., Abu Hanifah,Y., Mohd Yusof,M.Y. and Thong,K.L.
TITLE     Direct Submission
JOURNAL  Submitted (21-JAN-2011) Institute of Biological Sciences, Faculty of Science, University of
           Malaya, Kuala Lumpur 50603, Malaysia
FEATURES             Location/Qualifiers
   source            1..366
                     /organism="Staphylococcus aureus"
                     /mol_type="genomic DNA"
                     /strain="MRSA0809-32"
                     /host="Homo sapiens"
                     /db_xref="taxon:1280"
                     /country="Malaysia"
   gene              <1..>366
                     /gene="rpoB"
   CDS               <1..>366
                     /gene="rpoB"
                     /codon_start=1
                     /transl_table=11
                     /product="RNA polymerase subunit beta"
                     /protein_id="AEK94030"
                     /translation="VVRERMSIQDTESITPQQLINIRPVIASIKEFFGSSQLSQFMDQ
                     ANPLAELTHKRHLSALGPGGLTRERAQMEVRDVHYSHYGRMCPIQTPEGPNIGLINSL
                     SSYARVNEFGFIETPYRKVE"
ORIGIN
        1 gttgtacgtg aaagaatgtc aattcaagat actgagtcta tcacacctca acaattaatt
       61 aatattcgac ctgttattgc atctattaaa gaattctttg gtagctctca attatcacaa
      121 ttcatggacc aagcaaaccc attagctgag ttaacgcata aacgtcatct atcagcatta
      181 ggacctggtg gtttaacacg tgaacgtgct caaatggaag tacgtgacgt tcactactct
      241 cactatggcc gtatgtgtcc aattcaaaca cctgagggac caaacattgg attgattaac
      301 tcattatcaa gttatgcacg tgtaaatgaa ttcggcttta ttgaaacacc atatcgtaaa
      361 gttgaa
```

APPENDIX 21

GenBank flat file for mutational changes in *rpoB* gene for MRSA0308-10

LOCUS HQ914962 359 bp DNA linear BCT 27-JUL-2011
DEFINITION *Staphylococcus aureus* strain MRSA0811-25 RNA polymerase subunit beta (*rpoB*) gene, partial cds.
ACCESSION HQ914962
VERSION HQ914962
KEYWORDS .
SOURCE *Staphylococcus aureus*
ORGANISM *Staphylococcus aureus*
Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE 1 (bases 1 to 359)
AUTHORS Lim,K.T., Abu Hanifah,Y., Mohd Yusof,M.Y. and Thong,K.L.
TITLE Prevalence and molecular characterizaiton of rifampicin resistance in *Staphylococcus aureus* isolated in Malaysia
JOURNAL Unpublished
REFERENCE 2 (bases 1 to 359)
AUTHORS Lim,K.T., Abu Hanifah,Y., Mohd Yusof,M.Y. and Thong,K.L.
TITLE Direct Submission
JOURNAL Submitted (21-JAN-2011) Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur 50603, Malaysia
FEATURES Location/Qualifiers
source 1..359
/organism="Staphylococcus aureus"
/mol_type="genomic DNA"
/strain="MRSA0811-25"
/host="Homo sapiens"
/db_xref="taxon:1280"
/country="Malaysia"
gene <1..>359
/gene="rpoB"
CDS <1..>359
/gene="rpoB"
/codon_start=1
/transl_table=11
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/protein_id="AEK94031"
/translation="RERMSIQDTESITPQQLINIRPVIASIKEFFGSSQLSQFMDQAN
PLAELTHKRHLSALGPGGLTRERAQMEVRDVHYSHYGRMCPIQTPEGPNIGLINSLSS
YARVNEFGFIETPYRKV"
ORIGIN
1 cgtgaaagaa tgtcaattca agatactgag tctatcacac ctcaacaatt aattaatatt
61 cgacctgtta ttgcatctat taaagaattc tttggtagct ctcaattatc acaattcatg
121 gaccaagcaa acccattagc tgagttaacg cataaacgtc atctatcagc attaggacct
181 ggtggtttaa cacgtgaacg tgctcaaatg gaagtacgtg acgttcacta ctctcactat
241 ggccgtatgt gtccaattca aacacctgag ggaccaaaca ttggattgat taactcatta
301 tcaagttatg cacgtgtaaa tgaattcggc tttattgaaa caccatatcg taaagttga

APPENDIX 22

GenBank flat file for mutational changes in *rpoB* gene for MRSA0812-33

```
//
LOCUS       HQ914963               390 bp    DNA     linear  BCT 27-JUL-2011
DEFINITION  Staphylococcus aureus strain MRSA0812-33 RNA polymerase subunit beta (rpoB) gene,
            partial cds.
ACCESSION   HQ914963
VERSION     HQ914963
KEYWORDS    .
SOURCE      Staphylococcus aureus
ORGANISM  Staphylococcus aureus
          Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE  1  (bases 1 to 390)
AUTHORS  Lim,K.T., Abu Hanifah,Y., Mohd Yusof,M.Y. and Thong,K.L.
TITLE     Prevalence and molecular characterizaiton of rifampicin resistance in Staphylococcus aureus
          isolated in Malaysia
JOURNAL  Unpublished
REFERENCE  2  (bases 1 to 390)
AUTHORS  Lim,K.T., Abu Hanifah,Y., Mohd Yusof,M.Y. and Thong,K.L.
TITLE     Direct Submission
JOURNAL  Submitted (21-JAN-2011) Institute of Biological Sciences, Faculty of Science, University of
           Malaya, Kuala Lumpur 50603, Malaysia
FEATURES             Location/Qualifiers
   source          1..390
                   /organism="Staphylococcus aureus"
                   /mol_type="genomic DNA"
                   /strain="MRSA0812-33"
                   /host="Homo sapiens"
                   /db_xref="taxon:1280"
                   /country="Malaysia"
   gene            <1..>390
                   /gene="rpoB"
   CDS             <1..>390
                   /gene="rpoB"
                   /codon_start=1
                   /transl_table=11
                   /product="RNA polymerase subunit beta"
                   /protein_id="AEK94032"
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                   QLSQFMDQANPLAELTHKRHLSALGPGGLTRERAQMEVRDVHYSHYGRMCPIQTPEGP
                   NIGLINSLSSYARVNEFGFIETPYRKVE"
ORIGIN
        1 atcggtttat caagaatgga aagagttgta cgtgaaagaa tgtcaattca agatactgag
       61 tctatcacac ctcaacaatt aattaatatt cgacctgtta ttgcatctat taaagaattc
      121 tttggtagct ctcaattatc acaattcatg gaccaagcaa acccattagc tgagttaacg
      181 cataaacgtc atctatcagc attaggacct ggtggtttaa cacgtgaacg tgctcaaatg
      241 gaagtacgtg acgttcacta ctctcactat ggccgtatgt gtccaattca aacacctgag
      301 ggaccaaaca ttggattgat taactcatta tcaagttatg cacgtgtaaa tgaattcggc
      361 tttattgaaa caccatatcg naaagttgaa
//
```

APPENDIX 23

GenBank flat file for mutational changes in *fusA* gene for MRSA0307-23

```
LOCUS       JN597292               879 bp    DNA     linear   BCT 18-JAN-2012
DEFINITION  Staphylococcus aureus strain MRSA0307-23 elongation factor G (fusA)
            gene, partial cds.
ACCESSION   JN597292
VERSION     JN597292
KEYWORDS    .
SOURCE      Staphylococcus aureus
  ORGANISM  Staphylococcus aureus
            Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE   1  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Rifampicin and Fusidic Acid Resistance among Methiciilin-Resistant
            Staphylococcus aureus Strains from a Tertiary Hospital in Malaysia
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-AUG-2011) Institut of Biological Sciences, Faculty of
            Science, University of Malaya, Kuala Lumpur, Kuala Lumpur 50603,
            Malaysia
FEATURES             Location/Qualifiers
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                     /strain="MRSA0307-23"
                     /host="Homo sapiens"
                     /specimen_voucher="ST03007-23"
                     /db_xref="taxon:1280"
                     /country="Malaysia"
                     /collection_date="25-Jul-2003"
     gene            <1..>879
                     /gene="fusA"
     CDS             <1..>879
                     /gene="fusA"
                     /codon_start=1
                     /transl_table=11
                     /product="elongation factor G"
                     /protein_id="AEX93396"
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                     ADQDKMTQALVKLQEEDPTFHAHTDEETGQVIIGGMGELYLDILVDRMKKEFNVECNV
                     GAPMVSYRETFKSSAQVQGKFSRQSGGRGQYGDVHIEFTPNETGAGFEFENAIVGGVV
                     PREYIPSVEAGLKDAMENGVLAGYPLIDVKAKLYDGSYHDVDSSEMAFKIAASLALKE
                     AAKKCDPVILEPMMKVT"
ORIGIN
        1 gttatgactg acccttatgt tggtaaatta acattcttcc gtgtgtattc aggtacaatg
       61 acatctggtt catacgttaa gaactctact aaaggtaaac gtgaacgtgt aggtcgttta
      121 ttacaaatgc acgctaactc acgtcaagaa atcgatactg tatactctgg agatatcgct
      181 gctgcggtag gtcttaaaga tacaggtact ggtgatactt tatgtggtga gaaaaatgac
      241 attatcttgg aatcaatgga attcccagag ccagttattc acttatcagt agagccaaaa
      301 tctaaagctg accaagataa aatgactcaa gctttagtta aattacaaga agaagaccca
      361 acattccatg cacacactga cgaagaaact ggacaagtta tcatcggtgg tatgggtgag
      421 ctttacttag acatcttagt agaccgtatg aagaaagaat tcaacgttga atgtaacgta
      481 ggtgctccaa tggtttcata tcgtgaaaca ttcaaatcat ctgcacaagt tcaaggtaaa
      541 ttctctcgtc aatctggtgg tcgtggtcaa tacggtgatg ttcacattga attcacacca
      601 aacgaaacag gcgcaggttt cgaattcgaa aacgctatcg ttggtggtgt agttcctcgt
      661 gaatacattc catcagtaga agctggtctt aaagatgcta tggaaaatgg tgttttagca
      721 ggttatcctt taattgatgt taaagctaaa ttatatgatg gttcatacca tgatgtcgat
      781 tcatctgaaa tggccttcaa aattgctgca tcattagcac ttaaagaagc tgctaaaaaa
      841 tgtgatcctg taatcttaga accaatgatg aaagtaact
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APPENDIX 24

GenBank flat file for mutational changes in *fusA* gene for MRSA0308-23

```
//
LOCUS       JN597293               879 bp    DNA     linear   BCT 18-JAN-2012
DEFINITION  Staphylococcus aureus strain MRSA0308-23 elongation factor G (fusA)
            gene, partial cds.
ACCESSION   JN597293
VERSION     JN597293
KEYWORDS    .
SOURCE      Staphylococcus aureus
  ORGANISM  Staphylococcus aureus
            Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE   1  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Rifampicin and Fusidic Acid Resistance among Methiciilin-Resistant
            Staphylococcus aureus Strains from a Tertiary Hospital in Malaysia
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-AUG-2011) Institut of Biological Sciences, Faculty of
            Science, University of Malaya, Kuala Lumpur, Kuala Lumpur 50603,
            Malaysia
FEATURES             Location/Qualifiers
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                     /host="Homo sapiens"
                     /specimen_voucher="ST0308-23"
                     /db_xref="taxon:1280"
                     /country="Malaysia"
                     /collection_date="07-Aug-2003"
     gene            <1..>879
                     /gene="fusA"
     CDS             <1..>879
                     /gene="fusA"
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                     /transl_table=11
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                     /protein_id="AEX93397"
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                     ADQDKMTQALVKLQEEDPTFHAHTDEETGQVIIGGMGELHLDIKVDRMKKEFNVECNV
                     GAPMVSYRETFKSSAQVQGKFSRQSGGRGQYGDVHIEFTPNETGAGFEFENAIVGGVV
                     PREYIPSVEAGLKDAMENGVLAGYPLIDVKAKLYDGSYHDVDSSEMAFKIAASLALKE
                     AAKKCDPVILEPMMKVT"
ORIGIN
        1 gttatgactg acccttatgt tggtaaatta acattcttcc gtgtgtattc aggtacaatg
       61 acatctggtt catacgttaa gaactctact aaaggtaaac gtgaacgtgt aggtcgttta
      121 ttacaaatgc acgctaactc acgtcaagaa atcgatactg tatactctgg agatatcgct
      181 gctgcggtag gtcttaaaga tacaggtact ggtgatactt tatgtggtga gaaaaatgac
      241 attatcttgg aatcaatgga attcccagag ccagttattc acttatcagt agagccaaaa
      301 tctaaagctg accaagataa aatgactcaa gctttagtta aattacaaga agaagaccca
      361 acattccatg cacacactga cgaagaaact ggacaagtta tcatcggtgg tatgggtgag
      421 cttcacttag acatcaaagt agaccgtatg aagaaagaat tcaacgttga atgtaacgta
      481 ggtgctccaa tggtttcata tcgtgaaaca ttcaaatcat ctgcacaagt tcaaggtaaa
      541 ttctctcgtc aatctggtgg tcgtggtcaa tacggtgatg ttcacattga attcacacca
      601 aacgaaacag gcgcaggttt cgaattcgaa aacgctatcg ttggtggtgt agttcctcgt
      661 gaatacattc catcagtaga agctggtctt aaagatgcta tggaaaatgg tgttttagca
      721 ggttatcctt taattgatgt taaagctaaa ttatatgatg gttcatacca tgatgtcgat
      781 tcatctgaaa tggccttcaa aattgctgca tcattagcac ttaaagaagc tgctaaaaaa
      841 tgtgatcctg taatcttaga accaatgatg aaagtaact
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APPENDIX 25

GenBank flat file for mutational changes in *fusA* gene for MRSA0310-26

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LOCUS       JN597294               879 bp    DNA     linear  BCT 18-JAN-2012
DEFINITION  Staphylococcus aureus strain MRSA0310-26 elongation factor G (fusA)
            gene, partial cds.
ACCESSION   JN597294
VERSION     JN597294
KEYWORDS    .
SOURCE      Staphylococcus aureus
  ORGANISM  Staphylococcus aureus
            Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE   1  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Rifampicin and Fusidic Acid Resistance among Methiciilin-Resistant
            Staphylococcus aureus Strains from a Tertiary Hospital in Malaysia
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-AUG-2011) Institut of Biological Sciences, Faculty of
            Science, University of Malaya, Kuala Lumpur, Kuala Lumpur 50603,
            Malaysia
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /strain="MRSA0310-26"
                     /host="Homo sapiens"
                     /specimen_voucher="ST0310-26"
                     /db_xref="taxon:1280"
                     /country="Malaysia"
                     /collection_date="30-Oct-2003"
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     CDS             <1..>879
                     /gene="fusA"
                     /codon_start=1
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                     /product="elongation factor G"
                     /protein_id="AEX93398"
                     /translation="VMTDPYVGKLTFFRVYSGTMTSGSYVKNSTKGKRERVGRLLQMH
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                     ADQDKMTQALVKLQEEDPTFHAHTDEETGQVIIGGMGELHLDIKVDRMKKEFNVECNV
                     GAPMVSYRETFKSSAQVQGKFSRQSGGRGQYGDVHIEFTPNETGAGFEFENAIVGGVV
                     PREYIPSVEAGLKDAMENGVLAGYPLIDVKAKLYDGSYHDVDSSEMAFKIAASLALKE
                     AAKKCDPVILEPMMKVT"
ORIGIN
        1 gttatgactg acccttatgt tggtaaatta acattcttcc gtgtgtattc aggtacaatg
       61 acatctggtt catacgttaa gaactctact aaaggtaaac gtgaacgtgt aggtcgttta
      121 ttacaaatgc acgctaactc acgtcaagaa atcgatactg tatactctgg agatatcgct
      181 gctgcggtag gtcttaaaga tacaggtact ggtgatactt tatgtggtga gaaaaatgac
      241 attatcttgg aatcaatgga attcccagag ccagttattc acttatcagt agagccaaaa
      301 tctaaagctg accaagataa aatgactcaa gctttagtta aattacaaga agaagaccca
      361 acattccatg cacacactga cgaagaaact ggacaagtta tcatcggtgg tatgggtgag
      421 cttcacttag acatcaaagt agaccgtatg aagaaagaat tcaacgttga atgtaacgta
      481 ggtgctccaa tggtttcata tcgtgaaaca ttcaaatcat ctgcacaagt tcaaggtaaa
      541 ttctctcgtc aatctggtgg tcgtggtcaa tacggtgatg ttcacattga attcacacca
      601 aacgaaacag gcgcaggttt cgaattcgaa aacgctatcg ttggtggtgt agttcctcgt
      661 gaatacattc catcagtaga agctggtctt aaagatgcta tggaaaatgg tgttttagca
      721 ggttatcctt taattgatgt taaagctaaa ttatatgatg gttcatacca tgatgtcgat
      781 tcatctgaaa tggccttcaa aattgctgca tcattagcac ttaaagaagc tgctaaaaaa
      841 tgtgatcctg taatcttaga accaatgatg aaagtaact
```

APPENDIX 26

GenBank flat file for mutational changes in *fusA* gene for MRSA0801-26

```
//
LOCUS       JN597295               879 bp    DNA     linear   BCT 18-JAN-2012
DEFINITION  Staphylococcus aureus strain MRSA0801-26 elongation factor G (fusA)
            gene, partial cds.
ACCESSION   JN597295
VERSION     JN597295
KEYWORDS    .
SOURCE      Staphylococcus aureus
  ORGANISM  Staphylococcus aureus
            Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE   1  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Rifampicin and Fusidic Acid Resistance among Methiciilin-Resistant
            Staphylococcus aureus Strains from a Tertiary Hospital in Malaysia
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-AUG-2011) Institut of Biological Sciences, Faculty of
            Science, University of Malaya, Kuala Lumpur, Kuala Lumpur 50603,
            Malaysia
FEATURES             Location/Qualifiers
     source          1..879
                     /organism="Staphylococcus aureus"
                     /mol_type="genomic DNA"
                     /strain="MRSA0801-26"
                     /host="Homo sapiens"
                     /specimen_voucher="ST0801-2"
                     /db_xref="taxon:1280"
                     /country="Malaysia"
                     /collection_date="29-Jan-2008"
     gene            <1..>879
                     /gene="fusA"
     CDS             <1..>879
                     /gene="fusA"
                     /codon_start=1
                     /transl_table=11
                     /product="elongation factor G"
                     /protein_id="AEX93399"
                     /translation="VMTDPYVGKLTFFRVYSGTMTSGSYVKNSTKGKRERVGRLLQMH
                     ANSRQEIDTVYSGDIAAAVGLKDTGTGDTLCGEKNDIILESMEFPEPVIHLSVEPKSK
                     ADQDKMTQALVKLQEEDPTFHAHTDEETGQVIIGGMGELHLDISVDRMKKEFNVECNV
                     GAPMVSYRETFKSSAQVQGKFSRQSGGRGQYGDVHIEFTPNETGAGFEFENAIVGGVV
                     PREYIPSVEAGLKDAMENGVLAGYPLIDVKAKLYDGSYHDVDSSEMAFKIAASLALKE
                     AAKKCDPVILEPMMKVT"
ORIGIN
        1 gttatgactg acccttatgt tggtaaatta acattcttcc gtgtgtattc aggtacaatg
       61 acatctggtt catacgttaa gaactctact aaaggtaaac gtgaacgtgt aggtcgttta
      121 ttacaaatgc acgctaactc acgtcaagaa atcgatactg tatactctgg agatatcgct
      181 gctgcggtag gtcttaaaga tacaggtact ggtgatactt tatgtggtga gaaaaatgac
      241 attatcttgg aatcaatgga attcccagag ccagttattc acttatcagt agagccaaaa
      301 tctaaagctg accaagataa aatgactcaa gctttagtta aattacaaga agaagaccca
      361 acattccatg cacacactga cgaagaaact ggacaagtta tcatcggtgg tatgggtgag
      421 cttcacttag acatctcagt agaccgtatg aagaaagaat tcaacgttga atgtaacgta
      481 ggtgctccaa tggtttcata tcgtgaaaca ttcaaatcat ctgcacaagt tcaaggtaaa
      541 ttctctcgtc aatctggtgg tcgtggtcaa tacggtgatg ttcacattga attcacacca
      601 aacgaaacag gcgcaggttt cgaattcgaa aacgctatcg ttggtggtgt agttcctcgt
      661 gaatacattc catcagtaga agctggtctt aaagatgcta tggaaaatgg tgttttagca
      721 ggttatcctt taattgatgt taaagctaaa ttatatgatg gttcatacca tgatgtcgat
      781 tcatctgaaa tggccttcaa aattgctgca tcattagcac ttaaagaagc tgctaaaaaa
      841 tgtgatcctg taatcttaga accaatgatg aaagtaact
```

APPENDIX 27

GenBank flat file for mutational changes in *fusA* gene for MRSA0805-15

```
//
LOCUS       JN597296               879 bp    DNA     linear   BCT 18-JAN-2012
DEFINITION  Staphylococcus aureus strain MRSA0805-15 elongation factor G (fusA)
            gene, partial cds.
ACCESSION   JN597296
VERSION     JN597296
KEYWORDS    .
SOURCE      Staphylococcus aureus
  ORGANISM  Staphylococcus aureus
            Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE   1  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Rifampicin and Fusidic Acid Resistance among Methiciilin-Resistant
            Staphylococcus aureus Strains from a Tertiary Hospital in Malaysia
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-AUG-2011) Institut of Biological Sciences, Faculty of
            Science, University of Malaya, Kuala Lumpur, Kuala Lumpur 50603,
            Malaysia
FEATURES             Location/Qualifiers
     source          1..879
                     /organism="Staphylococcus aureus"
                     /mol_type="genomic DNA"
                     /strain="MRSA0805-15"
                     /host="Homo sapiens"
                     /specimen_voucher="ST0805-15"
                     /db_xref="taxon:1280"
                     /country="Malaysia"
                     /collection_date="12-May-2008"
     gene            <1..>879
                     /gene="fusA"
     CDS             <1..>879
                     /gene="fusA"
                     /codon_start=1
                     /transl_table=11
                     /product="elongation factor G"
                     /protein_id="AEX93400"
                     /translation="VMTDPYVGKLTFFRVYSGTMTSGSYVKNSTKGKRERVGRLLQMH
                     ANSRQEIDTVYSGDIAAAVGLKDTGTGDTLCGEKNDIILESMEFPEPVIHLSVEPKSK
                     ADQDKMTQALVKLQEEDPTFHAHTDEETGQVIIGGMGELHLDIKVDRMKKEFNVECNV
                     GAPMVSYRETFKSSAQVQGKFSRQSGGRGQYGDVHIEFTPNETGAGFEFENAIVGGVV
                     PREYIPSVEAGLKDAMENGVLAGYPLIDVKAKLYDGSYHDVDSSEMAFKIAASLALKE
                     AAKKCDPVILEPMMKVT"
ORIGIN
        1 gttatgactg acccttatgt tggtaaatta acattcttcc gtgtgtattc aggtacaatg
       61 acatctggtt catacgttaa gaactctact aaaggtaaac gtgaacgtgt aggtcgttta
      121 ttacaaatgc acgctaactc acgtcaagaa atcgatactg tatactctgg agatatcgct
      181 gctgcggtag gtcttaaaga tacaggtact ggtgatactt tatgtggtga gaaaaatgac
      241 attatcttgg aatcaatgga attcccagag ccagttattc acttatcagt agagccaaaa
      301 tctaaagctg accaagataa aatgactcaa gctttagtta aattacaaga agaagaccca
      361 acattccatg cacacactga cgaagaaact ggacaagtta tcatcggtgg tatgggtgag
      421 cttcacttag acatcaaagt agaccgtatg aagaaagaat tcaacgttga atgtaacgta
      481 ggtgctccaa tggtttcata tcgtgaaaca ttcaaatcat ctgcacaagt tcaaggtaaa
      541 ttctctcgtc aatctggtgg tcgtggtcaa tacggtgatg ttcacattga attcacacca
      601 aacgaaacag gcgcaggttt cgaattcgaa aacgctatcg ttggtggtgt agttcctcgt
      661 gaatacattc catcagtaga agctggtctt aaagatgcta tggaaaatgg tgttttagca
      721 ggttatcctt taattgatgt taaagctaaa ttatatgatg gttcatacca tgatgtcgat
      781 tcatctgaaa tggccttcaa aattgctgca tcattagcac ttaaagaagc tgctaaaaaa
      841 tgtgatcctg taatcttaga accaatgatg aaagtaact
```

APPENDIX 28

GenBank flat file for mutational changes in *fusA* gene for MRSA0805-17

```
//
LOCUS       JN597297               879 bp    DNA     linear   BCT 18-JAN-2012
DEFINITION  Staphylococcus aureus strain MRSA0805-17 elongation factor G (fusA)
            gene, partial cds.
ACCESSION   JN597297
VERSION     JN597297
KEYWORDS    .
SOURCE      Staphylococcus aureus
  ORGANISM  Staphylococcus aureus
            Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE   1  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Rifampicin and Fusidic Acid Resistance among Methiciilin-Resistant
            Staphylococcus aureus Strains from a Tertiary Hospital in Malaysia
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-AUG-2011) Institut of Biological Sciences, Faculty of
            Science, University of Malaya, Kuala Lumpur, Kuala Lumpur 50603,
            Malaysia
FEATURES             Location/Qualifiers
     source          1..879
                     /organism="Staphylococcus aureus"
                     /mol_type="genomic DNA"
                     /strain="MRSA0805-17"
                     /host="Homo sapiens"
                     /specimen_voucher="ST0805-17"
                     /db_xref="taxon:1280"
                     /country="Malaysia"
                     /collection_date="13-May-2008"
     gene            <1..>879
                     /gene="fusA"
     CDS             <1..>879
                     /gene="fusA"
                     /codon_start=1
                     /transl_table=11
                     /product="elongation factor G"
                     /protein_id="AEX93401"
                     /translation="VMTDPYVGKLTFFRVYSGTMTSGSYVKNSTKGKRERVGRLLQMH
                     ANSRQEIDTVYSGDIAAAVGLKDTGTGDTLCGEKNDIILESMEFLEPVIHLSVEPKSK
                     ADQDKMTQALVKLQEEDPTFHAHTDEETGQVIIGGMGELHLDISVDRMKKEFNVECNV
                     GAPMVSYRETFKSSAQVQGKFSRQSGGRGQYGDVHIEFTPNETGAGFEFENAIVGGVV
                     PREYIPSVEAGLKDAMENGVLAGYPLIDVKAKLYDGSYHDVDSSEMAFKIAASLALKE
                     AAKKCDPVILEPMMKVT"
ORIGIN
        1 gttatgactg acccttatgt tggtaaatta acattcttcc gtgtgtattc aggtacaatg
       61 acatctggtt catacgttaa gaactctact aaaggtaaac gtgaacgtgt aggtcgttta
      121 ttacaaatgc acgctaactc acgtcaagaa atcgatactg tatactctgg agatatcgct
      181 gctgcggtag gtcttaaaga tacaggtact ggtgatactt tatgtggtga gaaaaatgac
      241 attatcttgg aatcaatgga attcctagag ccagttattc acttatcagt agagccaaaa
      301 tctaaagctg accaagataa aatgactcaa gctttagtta aattacaaga agaagaccca
      361 acattccatg cacacactga cgaagaaact ggacaagtta tcatcggtgg tatgggtgag
      421 cttcacttag acatctcagt agaccgtatg aagaaagaat tcaacgttga atgtaacgta
      481 ggtgctccaa tggtttcata tcgtgaaaca ttcaaatcat ctgcacaagt tcaaggtaaa
      541 ttctctcgtc aatctggtgg tcgtggtcaa tacggtgatg ttcacattga attcacacca
      601 aacgaaacag gcgcaggttt cgaattcgaa aacgctatcg ttggtggtgt agttcctcgt
      661 gaatacattc catcagtaga agctggtctt aaagatgcta tggaaaatgg tgttttagca
      721 ggttatcctt taattgatgt taaagctaaa ttatatgatg gttcatacca tgatgtcgat
      781 tcatctgaaa tggccttcaa aattgctgca tcattagcac ttaaagaagc tgctaaaaaa
      841 tgtgatcctg taatcttaga accaatgatg aaagtaact
```

APPENDIX 29

GenBank flat file for mutational changes in *fusA* gene for MRSA0807-8

```
//
LOCUS       JN597298               879 bp    DNA     linear   BCT 18-JAN-2012
DEFINITION  Staphylococcus aureus strain MRSA0807-8 elongation factor G (fusA)
            gene, partial cds.
ACCESSION   JN597298
VERSION     JN597298
KEYWORDS    .
SOURCE      Staphylococcus aureus
  ORGANISM  Staphylococcus aureus
            Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE   1  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Rifampicin and Fusidic Acid Resistance among Methiciilin-Resistant
            Staphylococcus aureus Strains from a Tertiary Hospital in Malaysia
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-AUG-2011) Institut of Biological Sciences, Faculty of
            Science, University of Malaya, Kuala Lumpur, Kuala Lumpur 50603,
            Malaysia
FEATURES             Location/Qualifiers
     source          1..879
                     /organism="Staphylococcus aureus"
                     /mol_type="genomic DNA"
                     /strain="MRSA0807-8"
                     /host="Homo sapiens"
                     /specimen_voucher="ST0807-8"
                     /db_xref="taxon:1280"
                     /country="Malaysia"
                     /collection_date="18-Jul-2008"
     gene            <1..>879
                     /gene="fusA"
     CDS             <1..>879
                     /gene="fusA"
                     /codon_start=1
                     /transl_table=11
                     /product="elongation factor G"
                     /protein_id="AEX93402"
                     /translation="VMTDPYVGKLTFFRVYSGTMTSGSYVKNSTKGKRERVGRLLQMH
                     ANSRQEIDTVYSGDIAAAVGLKDTGTGDTLCGEKNDIILESMEFPEPVIHLSVEPKSK
                     ADQDKMTQALVKLQEEDPTFHAHTDEETGQVIIGGMGELYLDILVDRMKKEFNVECNV
                     GAPMVSYRETFKSSAQVQGKFSRQSGGRGQYGDVHIEFTPNETGAGFEFENAIVGGVV
                     PREYIPSVEAGLKDAMENGVLAGYPLIDVKAKLYDGSYHDVDSSEMAFKIAASLALKE
                     AAKKCDPVILEPMMKVT"
ORIGIN
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       61 acatctggtt catacgttaa gaactctact aaaggtaaac gtgaacgtgt aggtcgttta
      121 ttacaaatgc acgctaactc acgtcaagaa atcgatactg tatactctgg agatatcgct
      181 gctgcggtag gtcttaaaga tacaggtact ggtgatactt tatgtggtga gaaaaatgac
      241 attatcttgg aatcaatgga attcccagag ccagttattc acttatcagt agagccaaaa
      301 tctaaagctg accaagataa aatgactcaa gctttagtta aattacaaga agaagaccca
      361 acattccatg cacacactga cgaagaaact ggacaagtta tcatcggtgg tatgggtgag
      421 ctttacttag acatcttagt agaccgtatg aagaaagaat tcaacgttga atgtaacgta
      481 ggtgctccaa tggtttcata tcgtgaaaca ttcaaatcat ctgcacaagt tcaaggtaaa
      541 ttctctcgtc aatctggtgg tcgtggtcaa tacggtgatg ttcacattga attcacacca
      601 aacgaaacag gcgcaggttt cgaattcgaa aacgctatcg ttggtggtgt agttcctcgt
      661 gaatacattc catcagtaga agctggtctt aaagatgcta tggaaaatgg tgttttagca
      721 ggttatcctt taattgatgt taaagctaaa ttatatgatg gttcatacca tgatgtcgat
      781 tcatctgaaa tggccttcaa aattgctgca tcattagcac ttaaagaagc tgctaaaaaa
      841 tgtgatcctg taatcttaga accaatgatg aaagtaact
```

APPENDIX 30

GenBank flat file for mutational changes in *fusA* gene for MRSA0809-32

```
//
LOCUS       JN597299               879 bp    DNA     linear   BCT 18-JAN-2012
DEFINITION  Staphylococcus aureus strain MRSA0809-32 elongation factor G (fusA)
            gene, partial cds.
ACCESSION   JN597299
VERSION     JN597299
KEYWORDS    .
SOURCE      Staphylococcus aureus
  ORGANISM  Staphylococcus aureus
            Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE   1  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Rifampicin and Fusidic Acid Resistance among Methiciilin-Resistant
            Staphylococcus aureus Strains from a Tertiary Hospital in Malaysia
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-AUG-2011) Institut of Biological Sciences, Faculty of
            Science, University of Malaya, Kuala Lumpur, Kuala Lumpur 50603,
            Malaysia
FEATURES             Location/Qualifiers
     source          1..879
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                     /mol_type="genomic DNA"
                     /strain="MRSA0809-32"
                     /host="Homo sapiens"
                     /specimen_voucher="ST0809-32"
                     /db_xref="taxon:1280"
                     /country="Malaysia"
                     /collection_date="22-Sep-2008"
     gene            <1..>879
                     /gene="fusA"
     CDS             <1..>879
                     /gene="fusA"
                     /codon_start=1
                     /transl_table=11
                     /product="elongation factor G"
                     /protein_id="AEX93403"
                     /translation="VMTDPYVGKLTFFRVYSGTMTSGSYVKNSTKGKRERVGRLLQMH
                     ANSRQEIDTVYSGDIAAAVGLKDTGTGDTLCGEKNDIILESMEFPEPVIHLSVEPKSK
                     ADQDKMTQALVKLQEEDPTFHAHTDEETGQVIIGGMGELHLDIKVDRMKKEFNVECNV
                     GAPMVSYRETFKSSAQVQGKFSRQSGGRGQYGDVHIEFTPNETGAGFEFENAIVGGVV
                     PREYIPSVEAGLKDAMENGVLAGYPLIDVKAKLYDGSYHDVDSSEMAFKIAASLALKE
                     AAKKCDPVILEPMMKVT"
ORIGIN
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      121 ttacaaatgc acgctaactc acgtcaagaa atcgatactg tatactctgg agatatcgct
      181 gctgcggtag gtcttaaaga tacaggtact ggtgatactt tatgtggtga gaaaaatgac
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      301 tctaaagctg accaagataa aatgactcaa gctttagtta aattacaaga agaagaccca
      361 acattccatg cacacactga cgaagaaact ggacaagtta tcatcggtgg tatgggtgag
      421 cttcacttag acatcaaagt agaccgtatg aagaaagaat tcaacgttga atgtaacgta
      481 ggtgctccaa tggtttcata tcgtgaaaca ttcaaatcat ctgcacaagt tcaaggtaaa
      541 ttctctcgtc aatctggtgg tcgtggtcaa tacggtgatg ttcacattga attcacacca
      601 aacgaaacag gcgcaggttt cgaattcgaa aacgctatcg ttggtggtgt agttcctcgt
      661 gaatacattc catcagtaga agctggtctt aaagatgcta tggaaaatgg tgttttagca
      721 ggttatcctt taattgatgt taaagctaaa ttatatgatg gttcatacca tgatgtcgat
      781 tcatctgaaa tggccttcaa aattgctgca tcattagcac ttaaagaagc tgctaaaaaa
      841 tgtgatcctg taatcttaga accaatgatg aaagtaact
```

APPENDIX 31

GenBank flat file for mutational changes in *fusA* gene for MRSA0810-7

```
//
LOCUS       JN597300               1740 bp    DNA     linear   BCT 18-JAN-2012
DEFINITION  Staphylococcus aureus strain MRSA0810-7 elongation factor G (fusA)
            gene, partial cds.
ACCESSION   JN597300
VERSION     JN597300
KEYWORDS    .
SOURCE      Staphylococcus aureus
  ORGANISM  Staphylococcus aureus
            Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE   1  (bases 1 to 1740)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Rifampicin and Fusidic Acid Resistance among Methiciilin-Resistant
            Staphylococcus aureus Strains from a Tertiary Hospital in Malaysia
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1740)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-AUG-2011) Institut of Biological Sciences, Faculty of
            Science, University of Malaya, Kuala Lumpur, Kuala Lumpur 50603,
            Malaysia
FEATURES             Location/Qualifiers
     source          1..1740
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                     /mol_type="genomic DNA"
                     /strain="MRSA0810-7"
                     /host="Homo sapiens"
                     /specimen_voucher="ST0810-7"
                     /db_xref="taxon:1280"
                     /country="Malaysia"
                     /collection_date="07-Oct-2008"
     gene            <1..>1740
                     /gene="fusA"
     CDS             <1..>1740
                     /gene="fusA"
                     /codon_start=1
                     /transl_table=11
                     /product="elongation factor G"
                     /protein_id="AEX93404"
                     /translation="RILYYTGRIHKIGETHEGASQMDWMEQEQDRGITITSATTTAAW
                     EGHRVNIIDTPGHVDFTVEVERSLRVLDGAVTVLDAQSGVEPQTETVWRQATTYGVPR
                     IVFVNKMDKLGANFEYSVSTLHDRLQANAAPIQLPIGAEDEFEAIIDLVEMKCFKYTN
                     DLGTEIEEIEIPEDHLDRAEEARASLIEAVAETSDELMEKYLGDEEISVSELKEAIRQ
                     ATTNVEFYPVLCGTAFKNKGVQLMLDAVIDYLPSPLDVKPIIGHRASNPEEEVIAKAD
                     DSAEFAALAFKVMTDPYVGKLTFFRVYSGTMTSGSYVKNSTKGKRERVGRLLQMHANS
                     RQEIDTVYSGDIAAAVGLKDTGTGDTLCGEKNDIILESMEFPEPVIHLSVEPKSKADQ
                     DKMTQALVKLQEEDPTFHAHTDEETGQVIIGGMGELYLDILVDRMKKEFNVECNVGAP
                     MVSYRETFKSSAQVQGKFSRQSGGRGQYGDVHIEFTPNETGAGFEFENAIVGGVVPRE
                     YIPSVEAGLKDAMENGVLAGYPLIDVKAKLYDGSYHDVDSSEMAFKIAASLALKEAAK
                     KCDPVILEPMMKVT"
ORIGIN
        1 cgtattcttt attacactgg ccgtatccac aaaattggtg aaacacacga aggtgcttca
       61 caaatggact ggatggagca agaacaagac cgtggtatta ctatcacatc tgctacaaca
      121 acagcagctt gggaaggtca ccgtgtaaac attatcgata cacctggaca cgtagacttc
      181 actgtagaag ttgaacgttc attacgtgta cttgacggag cagttacagt acttgatgca
      241 caatcaggtg ttgaacctca aactgaaaca gtttggcgtc aggctacaac ttatggtgtt
      301 ccacgtatcg tatttgtaaa caaaatggac aaattaggtg ctaacttcga atactctgta
      361 agtacattac atgatcgttt acaagctaac gctgctccaa tccaattacc aattggtgcg
      421 gaagacgaat tcgaagcaat cattgactta gttgaaatga aatgtttcaa atatacaaat
      481 gatttaggta ctgaaattga agaaattgaa attcctgaag accacttaga tagagctgaa
      541 gaagctcgtg ctagcttaat cgaagcagtt gcagaaacta gcgacgaatt aatggaaaaa
      601 tatcttggtg acgaagaaat ttcagtttct gaattaaaag aagctatccg ccaagctact
      661 actaacgtag aattctaccc agtactttgt ggtacagctt tcaaaaacaa aggtgttcaa
      721 ttaatgcttg acgctgtaat tgattactta ccttcaccac tagacgttaa accaattatt
      781 ggtcaccgtg ctagcaaccc tgaagaagaa gtaatcgcga aagcagacga ttcagctgaa
      841 ttcgctgcat tagcgttcaa agttatgact gacccttatg ttggtaaatt gacattcttc
      901 cgtgtgtatt caggtacaat gacatctggt tcatacgtta agaactctac taaaggtaaa
      961 cgtgaacgtg taggtcgttt attacaaatg cacgctaact cacgtcaaga aatcgatact
     1021 gtatactctg gagatatcgc tgctgcggta ggtcttaaag atacaggtac tggtgatact
     1081 ttatgtggtg agaaaaatga cattatcttg gaatcaatgg aattcccaga gccagttatt
     1141 cacttatcag tagagccaaa atctaaagct gaccaagata aaatgactca agctttagtt
     1201 aaattacaag aagaagaccc aacattccat gcacacactg acgaagaaac tggacaagtt
     1261 atcatcggtg gtatgggtga gctttactta gacatcttag tagaccgtat gaagaaagaa
     1321 ttcaacgttg aatgtaacgt aggtgctcca atggtttcat atcgtgaaac attcaaatca
     1381 tctgcacaag ttcaaggtaa attctctcgt caatctggtg gtcgtggtca atacggtgat
     1441 gttcacattg aattcacacc aaacgaaaca ggcgcaggtt tcgaattcga aaacgctatc
     1501 gttggtggtg tagttcctcg tgaatacatt ccatcagtag aagctggtct taaagatgct
     1561 atggaaaatg gtgtcttagc aggttatcct ttaattgatg ttaaagctaa attatatgat
     1621 ggttcatacc atgatgtcga ttcatctgaa atggccttca aaattgctgc atcattagca
     1681 cttaaagaag ctgctaaaaa atgtgatcct gtaatcttag aaccaatgat gaaagtaact
```

APPENDIX 32

GenBank flat file for mutational changes in *fusA* gene for MRSA0810-10

```
//
LOCUS       JN597301               1740 bp    DNA     linear   BCT 18-JAN-2012
DEFINITION  Staphylococcus aureus strain MRSA0810-10 elongation factor G (fusA)
            gene, partial cds.
ACCESSION   JN597301
VERSION     JN597301
KEYWORDS    .
SOURCE      Staphylococcus aureus
  ORGANISM  Staphylococcus aureus
            Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE   1  (bases 1 to 1740)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Rifampicin and Fusidic Acid Resistance among Methiciilin-Resistant
            Staphylococcus aureus Strains from a Tertiary Hospital in Malaysia
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1740)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-AUG-2011) Institut of Biological Sciences, Faculty of
            Science, University of Malaya, Kuala Lumpur, Kuala Lumpur 50603,
            Malaysia
FEATURES             Location/Qualifiers
     source          1..1740
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                     /mol_type="genomic DNA"
                     /strain="MRSA0810-10"
                     /host="Homo sapiens"
                     /specimen_voucher="ST0810-10"
                     /db_xref="taxon:1280"
                     /country="Malaysia"
                     /collection_date="04-Oct-2008"
     gene            <1..>1740
                     /gene="fusA"
     CDS             <1..>1740
                     /gene="fusA"
                     /codon_start=1
                     /transl_table=11
                     /product="elongation factor G"
                     /protein_id="AEX93405"
                     /translation="RILYYTGRIHKIGETHEGASQMDWMEQEQDRGITITSATTTAAW
                     EGHRVNIIDTPGHVDFTVEVERSLRVLDGAVTVLDAQSGVEPQTETVWRQATTYGVPR
                     IVFVNKMDKLGANFEYSVSTLHDRLQANAAPIQLPIGAEDEFEAIIDLVEMKCFKYTN
                     DLGTEIEEIEIPEDHLDRAEEARASLIEAVAETSDELMEKYLGDEEISVSELKEAIRQ
                     ATTNVEFYPVLCGTAFKNKGVQLMLDAVIDYLPSPLDVKPIIGHRASNPEEEVIAKAD
                     DSAEFAALAFKVMTDPYVGKLTFFRVYSGTMTSGSYVKNSTKGKRERVGRLLQMHANS
                     RQEIDTVYSGDIAAAVGLKDTGTGDTLCGEKNDIILESMEFPEPVIHLSVEPKSKADQ
                     DKMTQALVKLQEEDPTFHAHTDEETGQVIIGGMGELYLDILVDRMKKEFNVECNVGAP
                     MVSYRETFKSSAQVQGKFSRQSGGRGQYGDVHIEFTPNETGAGFEFENAIVGGVVPRE
                     YIPSVEAGLKDAMENGVLAGYPLIDVKAKLYDGSYHDVDSSEMAFKIAASLALKEAAK
                     KCDPVILEPMMKVT"
ORIGIN
        1 cgtattcttt attacactgg ccgtatccac aaaattggtg aaacacacga aggtgcttca
       61 caaatggact ggatggagca agaacaagac cgtggtatta ctatcacatc tgctacaaca
      121 acagcagctt gggaaggtca ccgtgtaaac attatcgata cacctggaca cgtagacttc
      181 actgtagaag ttgaacgttc attacgtgta cttgacggag cagttacagt acttgatgca
      241 caatcaggtg ttgaacctca aactgaaaca gtttggcgtc aggctacaac ttatggtgtt
      301 ccacgtatcg tatttgtaaa caaaatggac aaattaggtg ctaacttcga atactctgta
      361 agtacattac atgatcgttt acaagctaac gctgctccaa tccaattacc aattggtgcg
      421 gaagacgaat tcgaagcaat cattgactta gttgaaatga aatgtttcaa atatacaaat
      481 gatttaggta ctgaaattga agaaattgaa attcctgaag accacttaga tagagctgaa
      541 gaagctcgtg ctagcttaat cgaagcagtt gcagaaacta gcgacgaatt aatggaaaaa
      601 tatcttggtg acgaagaaat ttcagtttct gaattaaaag aagctatccg ccaagctact
      661 actaacgtag aattctaccc agtactttgt ggtacagctt tcaaaaacaa aggtgttcaa
      721 ttaatgcttg acgctgtaat tgattactta ccttcaccac tagacgttaa accaattatt
      781 ggtcaccgtg ctagcaaccc tgaagaagaa gtaatcgcga aagcagacga ttcagctgaa
      841 ttcgctgcat tagcgttcaa agttatgact gacccttatg ttggtaaatt gacattcttc
      901 cgtgtgtatt caggtacaat gacatctggt tcatacgtta agaactctac taaaggtaaa
      961 cgtgaacgtg taggtcgttt attacaaatg cacgctaact cacgtcaaga aatcgatact
     1021 gtatactctg gagatatcgc tgctgcggta ggtcttaaag atacaggtac tggtgatact
     1081 ttatgtggtg agaaaaatga cattatcttg gaatcaatgg aattcccaga gccagttatt
     1141 cacttatcag tagagccaaa atctaaagct gaccaagata aaatgactca agctttagtt
     1201 aaattacaag aagaagaccc aacattccat gcacacactg acgaagaaac tggacaagtt
     1261 atcatcggtg gtatgggtga gctttactta gacatcttag tagaccgtat gaagaaagaa
     1321 ttcaacgttg aatgtaacgt aggtgctcca atggtttcat atcgtgaaac attcaaatca
     1381 tctgcacaag ttcaaggtaa attctctcgt caatctggtg gtcgtggtca atacggtgat
     1441 gttcacattg aattcacacc aaacgaaaca ggcgcaggtt tcgaattcga aaacgctatc
     1501 gttggtggtg tagttcctcg tgaatacatt ccatcagtag aagctggtct taaagatgct
     1561 atggaaaatg gtgtcttagc aggttatcct ttaattgatg ttaaagctaa attatatgat
     1621 ggttcatacc atgatgtcga ttcatctgaa atggccttca aaattgctgc atcattagca
     1681 cttaaagaag ctgctaaaaa atgtgatcct gtaatcttag aaccaatgat gaaagtaact
```

APPENDIX 33

GenBank flat file for mutational changes in *fusA* gene for MRSA0810-17

```
//
LOCUS       JN597302               1740 bp    DNA     linear   BCT 18-JAN-2012
DEFINITION  Staphylococcus aureus strain MRSA0810-17 elongation factor G (fusA)
            gene, partial cds.
ACCESSION   JN597302
VERSION     JN597302
KEYWORDS    .
SOURCE      Staphylococcus aureus
  ORGANISM  Staphylococcus aureus
            Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE   1  (bases 1 to 1740)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Rifampicin and Fusidic Acid Resistance among Methiciilin-Resistant
            Staphylococcus aureus Strains from a Tertiary Hospital in Malaysia
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1740)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-AUG-2011) Institut of Biological Sciences, Faculty of
            Science, University of Malaya, Kuala Lumpur, Kuala Lumpur 50603,
            Malaysia
FEATURES             Location/Qualifiers
     source          1..1740
                     /organism="Staphylococcus aureus"
                     /mol_type="genomic DNA"
                     /strain="MRSA0810-17"
                     /host="Homo sapiens"
                     /specimen_voucher="ST0810-17"
                     /db_xref="taxon:1280"
                     /country="Malaysia"
                     /collection_date="19-Oct-2008"
     gene            <1..>1740
                     /gene="fusA"
     CDS             <1..>1740
                     /gene="fusA"
                     /codon_start=1
                     /transl_table=11
                     /product="elongation factor G"
                     /protein_id="AEX93406"
                     /translation="RILYYTGRIHKIGETHEGASQMDWMEQEQDRGITITSATTTAAW
                     EGHRVNIIDTPGHVDFTVEVERSLRVLDGAVTVLDAQSGVEPQTETVWRQATTYGVPR
                     IVFVNKMDKLGANFEYSVSTLHDRLQANAAPIQLPIGAEDEFEAIIDLVEMKCFKYTN
                     DLGTEIEEIEIPEDHLDRAEEARASLIEAVAETSDELMEKYLGDEEISVSELKEAIRQ
                     ATTNVEFYPVLCGTAFKNKGVQLMLDAVIDYLPSPLDVKPIIGHRASNPEEEVIAKAD
                     DSAEFAALAFKVMTDPYVGKLTFFRVYSGTMTSGSYVKNSTKGKRERVGRLLQMHANS
                     RQEIDTVYSGDIAAAVGLKDTGTGDTLCGEKNDIILESMEFPEPVIHLSVEPKSKADQ
                     DKMTQALVKLQEEDPTFHAHTDEETGQVIIGGMGELYLDILVDRMKKEFNVECNVGAP
                     MVSYRETFKSSAQVQGKFSRQSGGRGQYGDVHIEFTPNETGAGFEFENAIVGGVVPRE
                     YIPSVEAGLKDAMENGVLAGYPLIDVKAKLYDGSYHDVDSSEMAFKIAASLALKEAAK
                     KCDPVILEPMMKVT"
ORIGIN
        1 cgtattcttt attacactgg ccgtatccac aaaattggtg aaacacacga aggtgcttca
       61 caaatggact ggatggagca agaacaagac cgtggtatta ctatcacatc tgctacaaca
      121 acagcagctt gggaaggtca ccgtgtaaac attatcgata cacctggaca cgtagacttc
      181 actgtagaag ttgaacgttc attacgtgta cttgacggag cagttacagt acttgatgca
      241 caatcaggtg ttgaacctca aactgaaaca gtttggcgtc aggctacaac ttatggtgtt
      301 ccacgtatcg tatttgtaaa caaaatggac aaattaggtg ctaacttcga atactctgta
      361 agtacattac atgatcgttt acaagctaac gctgctccaa tccaattacc aattggtgcg
      421 gaagacgaat tcgaagcaat cattgactta gttgaaatga aatgtttcaa atatacaaat
      481 gatttaggta ctgaaattga agaaattgaa attcctgaag accacttaga tagagctgaa
      541 gaagctcgtg ctagcttaat cgaagcagtt gcagaaacta gcgacgaatt aatggaaaaa
      601 tatcttggtg acgaagaaat ttcagtttct gaattaaaag aagctatccg ccaagctact
      661 actaacgtag aattctaccc agtactttgt ggtacagctt tcaaaaacaa aggtgttcaa
      721 ttaatgcttg acgctgtaat tgattactta ccttcaccac tagacgttaa accaattatt
      781 ggtcaccgtg ctagcaaccc tgaagaagaa gtaatcgcga aagcagacga ttcagctgaa
      841 ttcgctgcat tagcgttcaa agttatgact gacccttatg ttggtaaatt gacattcttc
      901 cgtgtgtatt caggtacaat gacatctggt tcatacgtta agaactctac taaaggtaaa
      961 cgtgaacgtg taggtcgttt attacaaatg cacgctaact cacgtcaaga aatcgatact
     1021 gtatactctg gagatatcgc tgctgcggta ggtcttaaag atacaggtac tggtgatact
     1081 ttatgtggtg agaaaaatga cattatcttg gaatcaatgg aattcccaga gccagttatt
     1141 cacttatcag tagagccaaa atctaaagct gaccaagata aaatgactca agctttagtt
     1201 aaattacaag aagaagaccc aacattccat gcacacactg acgaagaaac tggacaagtt
     1261 atcatcggtg gtatgggtga gctttactta gacatcttag tagaccgtat gaagaaagaa
     1321 ttcaacgttg aatgtaacgt aggtgctcca atggtttcat atcgtgaaac attcaaatca
     1381 tctgcacaag ttcaaggtaa attctctcgt caatctggtg gtcgtggtca atacggtgat
     1441 gttcacattg aattcacacc aaacgaaaca ggcgcaggtt tcgaattcga aaacgctatc
     1501 gttggtggtg tagttcctcg tgaatacatt ccatcagtag aagctggtct taaagatgct
     1561 atggaaaatg gtgtcttagc aggttatcct ttaattgatg ttaaagctaa attatatgat
     1621 ggttcatacc atgatgtcga ttcatctgaa atggccttca aaattgctgc atcattagca
     1681 cttaaagaag ctgctaaaaa atgtgatcct gtaatcttag aaccaatgat gaaagtaact
```

APPENDIX 34

GenBank flat file for mutational changes in *fusA* gene for MRSA08120-30

```
//
LOCUS       JN597303               879 bp    DNA     linear   BCT 18-JAN-2012
DEFINITION  Staphylococcus aureus strain MRSA0812-30 elongation factor G (fusA)
            gene, partial cds.
ACCESSION   JN597303
VERSION     JN597303
KEYWORDS    .
SOURCE      Staphylococcus aureus
  ORGANISM  Staphylococcus aureus
            Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE   1  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Rifampicin and Fusidic Acid Resistance among Methiciilin-Resistant
            Staphylococcus aureus Strains from a Tertiary Hospital in Malaysia
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-AUG-2011) Institut of Biological Sciences, Faculty of
            Science, University of Malaya, Kuala Lumpur, Kuala Lumpur 50603,
            Malaysia
FEATURES             Location/Qualifiers
     source          1..879
                     /organism="Staphylococcus aureus"
                     /mol_type="genomic DNA"
                     /strain="MRSA0812-30"
                     /host="Homo sapiens"
                     /specimen_voucher="ST0812-30"
                     /db_xref="taxon:1280"
                     /country="Malaysia"
                     /collection_date="28-Dec-2008"
     gene            <1..>879
                     /gene="fusA"
     CDS             <1..>879
                     /gene="fusA"
                     /codon_start=1
                     /transl_table=11
                     /product="elongation factor G"
                     /protein_id="AEX93407"
                     /translation="VMTDPYVGKLTFFRVYSGTMTSGSYVKNSTKGKRERVGRLLQMH
                     ANSRQEIDTVYSGDIAAAVGLKDTGTGDTLCGEKNDIILESMEFPEPVIHLSVEPKSK
                     ADQDKMTQALVKLQEEDPTFHAHTDEETGQVIIGGMGELHLDIKVDRMKKEFNVECNV
                     GAPMVSYRETFKSSAQVQGKFSRQSGGRGQYGDVHIEFTPNETGAGFEFENAIVGGVV
                     PREYIPSVEAGLKDAMENGVLAGYPLIDVKAKLYDGSYHDVDSSEMAFKIAASLALKE
                     AAKKCDPVILEPMMKVT"
ORIGIN
        1 gttatgactg acccttatgt tggtaaatta acattcttcc gtgtgtattc aggtacaatg
       61 acatctggtt catacgttaa gaactctact aaaggtaaac gtgaacgtgt aggtcgttta
      121 ttacaaatgc acgctaactc acgtcaagaa atcgatactg tatactctgg agatatcgct
      181 gctgcggtag gtcttaaaga tacaggtact ggtgatactt tatgtggtga gaaaaatgac
      241 attatcttgg aatcaatgga attcccagag ccagttattc acttatcagt agagccaaaa
      301 tctaaagctg accaagataa aatgactcaa gctttagtta aattacaaga agaagaccca
      361 acattccatg cacacactga cgaagaaact ggacaagtta tcatcggtgg tatgggtgag
      421 cttcacttag acatcaaagt agaccgtatg aagaaagaat tcaacgttga atgtaacgta
      481 ggtgctccaa tggtttcata tcgtgaaaca ttcaaatcat ctgcacaagt tcaaggtaaa
      541 ttctctcgtc aatctggtgg tcgtggtcaa tacggtgatg ttcacattga attcacacca
      601 aacgaaacag gcgcaggttt cgaattcgaa aacgctatcg ttggtggtgt agttcctcgt
      661 gaatacattc catcagtaga agctggtctt aaagatgcta tggaaaatgg tgttttagca
      721 ggttatcctt taattgatgt taaagctaaa ttatatgatg gttcatacca tgatgtcgat
      781 tcatctgaaa tggccttcaa aattgctgca tcattagcac ttaaagaagc tgctaaaaaa
      841 tgtgatcctg taatcttaga accaatgatg aaagtaact
```

APPENDIX 35

GenBank flat file for mutational changes in *fusA* gene for MRSA0811-25

```
//
LOCUS       JN597304               879 bp    DNA     linear   BCT 18-JAN-2012
DEFINITION  Staphylococcus aureus strain MRSA0811-25 elongation factor G (fusA)
            gene, partial cds.
ACCESSION   JN597304
VERSION     JN597304
KEYWORDS    .
SOURCE      Staphylococcus aureus
  ORGANISM  Staphylococcus aureus
            Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE   1  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Rifampicin and Fusidic Acid Resistance among Methiciilin-Resistant
            Staphylococcus aureus Strains from a Tertiary Hospital in Malaysia
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-AUG-2011) Institut of Biological Sciences, Faculty of
            Science, University of Malaya, Kuala Lumpur, Kuala Lumpur 50603,
            Malaysia
FEATURES             Location/Qualifiers
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                     /country="Malaysia"
                     /collection_date="21-Nov-2008"
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                     /gene="fusA"
     CDS             <1..>879
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                     ANSRQEIDTVYSGDIAAAVGLKDTGTGDTLCGEKNDIILESMEFPEPVIHLSVEPKSK
                     ADQDKMTQALVKLQEEDPTFHAHTDEETGQVIIGGMGELHLDIKVDRMKKEFNVECNV
                     GAPMVSYRETFKSSAQVQGKFSRQSGGRGQYGDVHIEFTPNETGAGFEFENAIVGGVV
                     PREYIPSVEAGLKDAMENGVLAGYPLIDVKAKLYDGSYHDVDSSEMAFKIAASLALKE
                     AAKKCDPVILEPMMKVT"
ORIGIN
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       61 acatctggtt catacgttaa gaactctact aaaggtaaac gtgaacgtgt aggtcgttta
      121 ttacaaatgc acgctaactc acgtcaagaa atcgatactg tatactctgg agatatcgct
      181 gctgcggtag gtcttaaaga tacaggtact ggtgatactt tatgtggtga gaaaaatgac
      241 attatcttgg aatcaatgga attcccagag ccagttattc acttatcagt agagccaaaa
      301 tctaaagctg accaagataa aatgactcaa gctttagtta aattacaaga agaagaccca
      361 acattccatg cacacactga cgaagaaact ggacaagtta tcatcggtgg tatgggtgag
      421 cttcacttag acatcaaagt agaccgtatg aagaaagaat tcaacgttga atgtaacgta
      481 ggtgctccaa tggtttcata tcgtgaaaca ttcaaatcat ctgcacaagt tcaaggtaaa
      541 ttctctcgtc aatctggtgg tcgtggtcaa tacggtgatg ttcacattga attcacacca
      601 aacgaaacag gcgcaggttt cgaattcgaa aacgctatcg ttggtggtgt agttcctcgt
      661 gaatacattc catcagtaga agctggtctt aaagatgcta tggaaaatgg tgttttagca
      721 ggttatcctt taattgatgt taaagctaaa ttatatgatg gttcatacca tgatgtcgat
      781 tcatctgaaa tggccttcaa aattgctgca tcattagcac ttaaagaagc tgctaaaaaa
      841 tgtgatcctg taatcttaga accaatgatg aaagtaact
```

APPENDIX 36

GenBank flat file for mutational changes in *fusA* gene for MRSA0812-33

```
//
LOCUS       JN597305               879 bp    DNA     linear   BCT 18-JAN-2012
DEFINITION  Staphylococcus aureus strain MRSA0812-33 elongation factor G (fusA)
            gene, partial cds.
ACCESSION   JN597305
VERSION     JN597305
KEYWORDS    .
SOURCE      Staphylococcus aureus
  ORGANISM  Staphylococcus aureus
            Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE   1  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Rifampicin and Fusidic Acid Resistance among Methiciilin-Resistant
            Staphylococcus aureus Strains from a Tertiary Hospital in Malaysia
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 879)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-AUG-2011) Institut of Biological Sciences, Faculty of
            Science, University of Malaya, Kuala Lumpur, Kuala Lumpur 50603,
            Malaysia
FEATURES             Location/Qualifiers
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                     /organism="Staphylococcus aureus"
                     /mol_type="genomic DNA"
                     /strain="MRSA0812-33"
                     /host="Homo sapiens"
                     /specimen_voucher="ST0812-33"
                     /db_xref="taxon:1280"
                     /country="Malaysia"
                     /collection_date="31-Dec-2008"
     gene            <1..>879
                     /gene="fusA"
     CDS             <1..>879
                     /gene="fusA"
                     /codon_start=1
                     /transl_table=11
                     /product="elongation factor G"
                     /protein_id="AEX93409"
                     /translation="VWTDPYVGKLTFFRVYSGTMTSGSYVKNSTKGKRERVGRLLQMH
                     ANSRQEIDTVYSGDIAAAVGLKDTGTGDTLCGEKNDIILESMEFPEPVIHLSVEPKSK
                     ADQDKMTQALVKLQEEDPTFHAHTDEETGQVIIGGMGELHLDIKVDRMKKEFNVECNV
                     GAPMVSYRETFKSSAQVQGKFSRQSGGRGQYGDVHIEFTPNETGAGFEFENAIVGGVV
                     PREYIPSVEAGLKDAMENGVLAGYPLIDVKAKLYDGSYHDVDSSEMAFKIAASLALKE
                     AAKKWDPVILKPMMKVT"
ORIGIN
        1 gtttggactg acccttatgt tggtaaatta acattcttcc gtgtgtattc aggtacaatg
       61 acatctggtt catacgttaa gaactctact aaaggtaaac gtgaacgtgt aggtcgttta
      121 ttacaaatgc acgctaactc acgtcaagaa atcgatactg tatactctgg agatatcgct
      181 gctgcggtag gtcttaaaga tacaggtact ggtgatactt tatgtggtga gaaaaatgac
      241 attatcttgg aatcaatgga attcccagag ccagttattc acttatcagt agagccaaaa
      301 tctaaagctg accaagataa aatgactcaa gctttagtta aattacaaga agaagaccca
      361 acattccatg cacacactga cgaagaaact ggacaagtta tcatcggtgg tatgggtgag
      421 cttcacttag acatcaaagt agaccgtatg aagaaagaat tcaacgttga atgtaacgta
      481 ggtgctccaa tggtttcata tcgtgaaaca ttcaaatcat ctgcacaagt tcaaggtaaa
      541 ttctctcgtc aatctggtgg tcgtggtcaa tacggtgatg ttcacattga attcacacca
      601 aacgaaacag gcgcaggttt cgaattcgaa aacgctatcg ttggtggtgt agttcctcgt
      661 gaatacattc catcagtaga agctggtctt aaagatgcta tggaaaatgg tgttttagca
      721 ggttatcctt taattgatgt taaagctaaa ttatatgatg gttcatacca tgatgtcgat
      781 tcatctgaaa tggccttcaa aattgctgca tcattagcac ttaaagaagc tgctaaaaaa
      841 tgggatcctg taatcttaaa accaatgatg aaagtaact
```

APPENDIX 37

GenBank flat file for mutational changes in *fusA* gene for MRSA0810-6

```
//
LOCUS       JN597306               799 bp    DNA     linear  BCT 18-JAN-2012
DEFINITION  Staphylococcus aureus strain MRSA0810-6 elongation factor G (fusA)
            gene, partial cds.
ACCESSION   JN597306
VERSION     JN597306
KEYWORDS    .
SOURCE      Staphylococcus aureus
  ORGANISM  Staphylococcus aureus
            Bacteria; Firmicutes; Bacillales; Staphylococcus.
REFERENCE   1  (bases 1 to 799)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Rifampicin and Fusidic Acid Resistance among Methiciilin-Resistant
            Staphylococcus aureus Strains from a Tertiary Hospital in Malaysia
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 799)
  AUTHORS   Lim,K.T., Hanifah,Y.A., Teh,C.S.J., Mohd Yusof,M.Y. and Thong,K.L.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-AUG-2011) Institut of Biological Sciences, Faculty of
            Science, University of Malaya, Kuala Lumpur, Kuala Lumpur 50603,
            Malaysia
FEATURES             Location/Qualifiers
     source          1..799
                     /organism="Staphylococcus aureus"
                     /mol_type="genomic DNA"
                     /strain="MRSA0810-6"
                     /host="Homo sapiens"
                     /specimen_voucher="ST0810-6"
                     /db_xref="taxon:1280"
                     /country="Malaysia"
                     /collection_date="01-Oct-2008"
     gene            <1..>799
                     /gene="fusA"
     CDS             <1..>799
                     /gene="fusA"
                     /codon_start=1
                     /transl_table=11
                     /product="elongation factor G"
                     /protein_id="AEX93410"
                     /translation="RILYYTGRIHKIGETHEGASQMDWMEQEQDRGITITSATTTAAW
                     EGHRVNIIDTPGHVDFTVEVERSLRVLDGAVTVLDAQSGVEPQTETVWRQATTYGVPR
                     IVFVNKMDKLGANFEYSVSTLHDRLQANAAPIQLPIGAEDEFEAIIDLVEMKCFKYTN
                     DLGTEIEEIEIPEDHLDRAEEARASLIEAVAETSDELMEKYLGDEEISVSELKEAIRQ
                     ATTNVEFYPVLCGTAFKNKGVQLMLDAVIDYLPSPLDVKPIIGHRASN"
ORIGIN
        1 cgtattcttt attacactgg ccgtatccac aaaattggtg aaacacacga aggtgcttca
       61 caaatggact ggatggagca agaacaagac cgtggtatta ctatcacatc tgctacaaca
      121 acagcagctt gggaaggtca ccgtgtaaac attatcgata cacctggaca cgtagacttc
      181 actgtagaag ttgaacgttc attacgtgta cttgacggag cagttacagt acttgatgca
      241 caatcaggtg ttgaacctca aactgaaaca gtttggcgtc aggctacaac ttatggtgtt
      301 ccacgtatcg tatttgtaaa caaaatggac aaattaggtg ctaacttcga atactctgta
      361 agtacattac atgatcgttt acaagctaac gctgctccaa tccaattacc aattggtgcg
      421 gaagacgaat tcgaagcaat cattgactta gttgaaatga aatgtttcaa atatacaaat
      481 gatttaggta ctgaaattga agaaattgaa attcctgaag accacttaga tagagctgaa
      541 gaagctcgtg ctagcttaat cgaagcagtt gcagaaacta gcgacgaatt aatggaaaaa
      601 tatcttggtg acgaagaaat ttcagtttct gaattaaaag aagctatccg ccaagctact
      661 actaacgtag aattctaccc agtactttgt ggtacagctt tcaaaaacaa aggtgttcaa
      721 ttaatgcttg acgctgtaat tgattactta ccttcaccac tagacgttaa accaattatt
      781 ggtcaccgtg ctagcaacc
//
```

APPENDIX 38
Standard Open Reading Frame BLAST search results for DNA sequence of the amplified enterotoxin type A (*sea*) from MRSA0811-1

```
  5 atctattattacaatgaaaaagctaaaactgaaaataaagagagt
    I Y Y Y N E K A K T E N K E S
 50 cacgatcaatttttacagcatactatattgtttaaaggctttttt
    H D Q F L Q H T I L F K G F F
 95 acagatcattcgtggtataacgatttattagtagattttgattca
    T D H S W Y N D L L V D F D S
140 aaggatattgttgataaatataaagggaaaaaagtagacttgtat
    K D I V D K Y K G K K V D L Y
185 ggtgcttattatggttatcaatgtgcgggtggtacaccaaacaaa
    G A Y Y G Y Q C A G G T P N K
230 acagcttgtatgtatggtggtgtaacgttacatgataataatcga
    T A C M Y G G V T L H D N N R
275 ttgaccgaagagaaaaaagtgccgatcaatttatggctagacggt
    L T E E K K V P I N L W L D G
320 aaacaaaatacagtacctttggaaacggttaaaacgaataagaaa
    K Q N T V P L E T V K T N K K
365 aatgtaactgttcaggagttggatcttcaagcaagacgttattta
    N V T V Q E L D L Q A R R Y L
410 caggaaaaatataatttatataactctgatgtttttgatgggaag
    Q E K Y N L Y N S D V F D G K
455 gttcagaggggattaatcgtgtttcatacttctacagaaccttcg
    V Q R G L I V F H T S T E P S
500 gttaattacgaatttatttg 519
    V N Y E F I
```

> gb|AAV84102.1| enterotoxin A [Staphylococcus aureus]
Length=261

Score = 353 bits (905), Expect = 4e-121, Method: Compositional matrix adjust.
Identities = 168/170 (99%), Positives = 169/170 (99%), Gaps = 0/170 (0%)

```
Query  1    IYYYNEKAKTENKESHDQFLQHTILFKGFFTDHSWYNDLLVDFDSKDIVDKYKGKKVDLY  60
            IYYYNEKAKTENKESHDQFLQHTILFKGFFTDHSWYNDLLVDFDSKDIVDKYKGKKVDLY
Sbjct  56   IYYYNEKAKTENKESHDQFLQHTILFKGFFTDHSWYNDLLVDFDSKDIVDKYKGKKVDLY  115

Query  61   GAYYGYQCAGGTPNKTACMYGGVTLHDNNRLTEEKKVPINLWLDGKQNTVPLETVKTNKK  120
            GAYYGYQCAGGTPNKTACMYGGVTLHDNNRLTEEKKVPINLWLDGKQNTVPLETVKTNKK
Sbjct  116  GAYYGYQCAGGTPNKTACMYGGVTLHDNNRLTEEKKVPINLWLDGKQNTVPLETVKTNKK  175

Query  121  NVTVQELDLQARRYLQEKYNLYNSDVFDGKVQRGLIVFHTSTEPSVNYEF  170
            NVTVQELDLQARRYLQEKYNLYNSDVFDGKVQRGLIVFHTSTEPSVNY+
Sbjct  176  NVTVQELDLQARRYLQEKYNLYNSDVFDGKVQRGLIVFHTSTEPSVNYDL  225
```

APPENDIX 39

Standard Open Reading Frame BLAST search results for DNA sequence of the amplified enterotoxin type C (*sec*) from MRSA0309-10

```
 81 atttcttttgaagtacaaactgataagaaaagtgtaacagctcaa
    I  S  F  E  V  Q  T  D  K  K  S  V  T  A  Q
126 gaactagacataaaagctaggaatttttttaattaataaaaaaaat
    E  L  D  I  K  A  R  N  F  L  I  N  K  K  N
171 ttgtatgagtttaacagttcaccatatgaaacaggatatataaaa
    L  Y  E  F  N  S  S  P  Y  E  T  G  Y  I  K
216 tttattgaaaataacggcaatacttttggtatgatatgat 256
    F  I  E  N  N  G  N  T  F  W  Y  D  M
```

> gb|AAP37185.1| enterotoxin sec variant [Staphylococcus aureus]
Length=222

Score = 121 bits (304), Expect = 6e-33, Method: Compositional matrix adjust.
Identities = 58/58 (100%), Positives = 58/58 (100%), Gaps = 0/58 (0%)

```
Query  1    ISFEVQTDKKSVTAQELDIKARNFLINKKNLYEFNSSPYETGYIKFIENNGNTFWYDM  58
            ISFEVQTDKKSVTAQELDIKARNFLINKKNLYEFNSSPYETGYIKFIENNGNTFWYDM
Sbjct  136  ISFEVQTDKKSVTAQELDIKARNFLINKKNLYEFNSSPYETGYIKFIENNGNTFWYDM  193
```

APPENDIX 40

Standard Open Reading Frame BLAST search results for DNA sequence of the amplified enterotoxin type G (*seg*) from MRSA0811-30

```
593 atgttgnatgctcaacccgatcctaaattagacgaactaaataaa
    M  L  X  A  Q  P  D  P  K  L  D  E  L  N  K
548 gtaagtgattataaaaataataagggaactatggtaatgtaatg
    V  S  D  Y  K  N  N  K  G  T  M  G  N  V  M
503 aatctttatacgtctccacctgttgaaggaagaggagttattaat
    N  L  Y  T  S  P  P  V  E  G  R  G  V  I  N
458 tctagacagtttttatctcatgatttaatttttccaattgagtat
    S  R  Q  F  L  S  H  D  L  I  F  P  I  E  Y
413 aagagttataatgaggttaaaactgaattagaaaatacagaatta
    K  S  Y  N  E  V  K  T  E  L  E  N  T  E  L
368 gctaacaattataaagataaaaaagtagacatttttggcgttcca
    A  N  N  Y  K  D  K  K  V  D  I  F  G  V  P
323 tatttttatacatgtataatacctaaatctgaaccggatataaac
    Y  F  Y  T  C  I  I  P  K  S  E  P  D  I  N
278 caaaattttggaggttgttgtatgtatggtggtcttacatttaat
    Q  N  F  G  G  C  C  M  Y  G  G  L  T  F  N
233 agttcagaaaatgaaagagataaattaattactgtacaggtaaca
    S  S  E  N  E  R  D  K  L  I  T  V  Q  V  T
188 atcgacaatagacaatcacttggatttacaataactacaaataag
    I  D  N  R  Q  S  L  G  F  T  I  T  T  N  K
143 aatatggttactattcaggaactagattacaaagcaagacactgg
    N  M  V  T  I  Q  E  L  D  Y  K  A  R  H  W
 98 ctcactaaagaaaaaagctatacgagtttgatggttctgcattt
    L  T  K  E  K  K  L  Y  E  F  D  G  S  A  F
 53 gaatctggatatataaaatttactgaaaagaacaatacaagtttt
    E  S  G  Y  I  K  F  T  E  K  N  N  T  S  F
  8 tggtttga 1
    W  F
```

> gb|EGL85846.1| enterotoxin type G [Staphylococcus aureus subsp. aureus 21305]
Length=258

Score = 404 bits (1038), Expect = 7e-141, Method: Compositional matrix adjust.
Identities = 194/194 (100%), Positives = 194/194 (100%), Gaps = 0/194 (0%)

```
Query  4    AQPDPKLDELNKVSDYKNNKGTMGNVMNLYTSPPVEGRGVINSRQFLSHDLIFPIEYKSY  63
            AQPDPKLDELNKVSDYKNNKGTMGNVMNLYTSPPVEGRGVINSRQFLSHDLIFPIEYKSY
Sbjct  25   AQPDPKLDELNKVSDYKNNKGTMGNVMNLYTSPPVEGRGVINSRQFLSHDLIFPIEYKSY  84

Query  64   NEVKTELENTELANNYKDKKVDIFGVPYFYTCIIPKSEPDINQNFGGCCMYGGLTFNSSE  123
            NEVKTELENTELANNYKDKKVDIFGVPYFYTCIIPKSEPDINQNFGGCCMYGGLTFNSSE
Sbjct  85   NEVKTELENTELANNYKDKKVDIFGVPYFYTCIIPKSEPDINQNFGGCCMYGGLTFNSSE  144

Query  124  NERDKLITVQVTIDNRQSLGFTITTNKNMVTIQELDYKARHWLTKEKKLYEFDGSAFESG  183
            NERDKLITVQVTIDNRQSLGFTITTNKNMVTIQELDYKARHWLTKEKKLYEFDGSAFESG
Sbjct  145  NERDKLITVQVTIDNRQSLGFTITTNKNMVTIQELDYKARHWLTKEKKLYEFDGSAFESG  204

Query  184  YIKFTEKNNTSFWF  197
            YIKFTEKNNTSFWF
Sbjct  205  YIKFTEKNNTSFWF  218
```

APPENDIX 41

Standard Open Reading Frame BLAST search results for DNA sequence of the amplified enterotoxin I (*sei*) from MRSA0805-10

```
  11 atagatttaaaaggcgtcacagataaaaatctacctattgcaaat
     I  D  L  K  G  V  T  D  K  N  L  P  I  A  N
  56 caactcgaattttcaacaggtaccaatgatttgatctcagaatct
     Q  L  E  F  S  T  G  T  N  D  L  I  S  E  S
 101 aataattgggacgaaataagtaaatttaaaggaaagaaactggat
     N  N  W  D  E  I  S  K  F  K  G  K  K  L  D
 146 atttttggcattgattataatggtccttgtaaatctaaatacatg
     I  F  G  I  D  Y  N  G  P  C  K  S  K  Y  M
 191 tttggaggggccactttatcaggacaatacttaaattctgctaga
     F  G  G  A  T  L  S  G  Q  Y  L  N  S  A  R
 236 aaaatccctattaatctttgggttaatggcaaacataaaacaatt
     K  I  P  I  N  L  W  V  N  G  K  H  K  T  I
 281 tctactgacaaaatagcaactaataaaaaactagtaacagctcaa
     S  T  D  K  I  A  T  N  K  K  L  V  T  A  Q
 326 gaaattgatgttaaattaaggagatatcttcaagaagaatacaat
     E  I  D  V  K  L  R  R  Y  L  Q  E  E  Y  N
 371 atatatggtcataataacactggtaaaggcaaagaatatggatat
     I  Y  G  H  N  N  T  G  K  G  K  E  Y  G  Y
 416 aaatctaaattttattcaggttttaataatgggaaagttttattt
     K  S  K  F  Y  S  G  F  N  N  G  K  V  L  F
 461 catttaaataatgaaaaatcattttcatatgatttgttttataca
     H  L  N  N  E  K  S  F  S  Y  D  L  F  Y  T
 506 ggagatggactgcctgtaaagtttttt 532
     G  D  G  L  P  V  K  F  F
```

> dbj|BAB85991.1| enterotoxin I [Staphylococcus aureus]
Length=218

Score = 349 bits (896), Expect = 3e-120, Method: Compositional matrix adjust.
Identities = 172/174 (99%), Positives = 172/174 (99%), Gaps = 0/174 (0%)

```
Query  1    IDLKGVTDKNLPIANQLEFSTGTNDLISESNNWDEISKFKGKKLDIFGIDYNGPCKSKYM  60
            IDLKGVTDKNLPIANQLEFSTGTNDLISESNNWDEISKFKGKKLDIFGIDYNGPCKSKYM
Sbjct  19   IDLKGVTDKNLPIANQLEFSTGTNDLISESNNWDEISKFKGKKLDIFGIDYNGPCKSKYM  78

Query  61   FGGATLSGQYLNSARKIPINLWVNGKHKTISTDKIATNKKLVTAQEIDVKLRRYLQEEYN  120
            FGGATLSGQYLNSARKIPINLWVNGKHKTISTDKIATNKKLVTAQEIDVKLRRYLQEEYN
Sbjct  79   FGGATLSGQYLNSARKIPINLWVNGKHKTISTDKIATNKKLVTAQEIDVKLRRYLQEEYN  138

Query  121  IYGHNNTGKGKEYGYKSKFYSGFNNGKVLFHLNNEKSFSYDLFYTGDGLPVKFF  174
            IYGHNNTGKGKEYGYKSKFYSGFNNGKVLFHLNNEKSFSYDLFYTGDGLPV F
Sbjct  139  IYGHNNTGKGKEYGYKSKFYSGFNNGKVLFHLNNEKSFSYDLFYTGDGLPVSFL  192
```

APPENDIX 42

Standard Open Reading Frame BLAST search results for DNA sequence of the amplified enfoliative toxin D (*etd*) from MRSA0806-13

```
304 atggctaaaaaagatccgactaaagttatattcacacctggttcg
    M  A  K  K  D  P  T  K  V  I  F  T  P  G  S
259 actaaaacagaagatggagtatataaaactccatatggacaatttt
    T  K  T  E  D  G  V  Y  K  T  P  Y  G  Q  F
214 gtagcagaagaaattaatgaacacccatatggacaaggaactgat
    V  A  E  E  I  N  E  H  P  Y  G  Q  G  T  D
169 ttgtctataattaaactaaaacccaataaagacggaaagtctgca
    L  S  I  I  K  L  K  P  N  K  D  G  K  S  A
124 ggtgatttaattccccctgcaaagattgcagattctatagatttg
    G  D  L  I  P  P  A  K  I  A  D  S  I  D  L
 79 caacaaggtgacaaaataagcttgctcggataccttataacttt
    Q  Q  G  D  K  I  S  L  L  G  Y  P  Y  N  F
 34 tctactaattctttatatagaagtgaaattgaaa 1
    S  T  N  S  L  Y  R  S  E  I  E
```

> dbj|BAC22944.1| exfoliative toxin D [Staphylococcus aureus]
gb|EGL88744.1| exfoliative toxin B [Staphylococcus aureus subsp. aureus 21305]
Length=281

Score = 208 bits (530), Expect = 2e-65, Method: Compositional matrix adjust.
Identities = 101/101 (100%), Positives = 101/101 (100%), Gaps = 0/101 (0%)

```
Query  1    MAKKDPTKVIFTPGSTKTEDGVYKTPYGQFVAEEINEHPYGQGTDLSIIKLKPNKDGKSA  60
            MAKKDPTKVIFTPGSTKTEDGVYKTPYGQFVAEEINEHPYGQGTDLSIIKLKPNKDGKSA
Sbjct  103  MAKKDPTKVIFTPGSTKTEDGVYKTPYGQFVAEEINEHPYGQGTDLSIIKLKPNKDGKSA  162

Query  61   GDLIPPAKIADSIDLQQGDKISLLGYPYNFSTNSLYRSEIE  101
            GDLIPPAKIADSIDLQQGDKISLLGYPYNFSTNSLYRSEIE
Sbjct  163  GDLIPPAKIADSIDLQQGDKISLLGYPYNFSTNSLYRSEIE  203
```

APPENDIX 43

Standard Open Reading Frame BLAST search results for DNA sequence of the amplified extracellular fibrinogen-binding protein (*efb*) from MRSA0812-17

```
382 ataggtattactacaactacaattgcgtcaacagcagatgcgagc
    I  G  I  T  T  T  T  I  A  S  T  A  D  A  S
337 gaaggatacggtccaagagaaaagaaaccagtgagtattaatcac
    E  G  Y  G  P  R  E  K  K  P  V  S  I  N  H
292 aatatcgtagagtacaatgatggtacttttaaatatcaatctaga
    N  I  V  E  Y  N  D  G  T  F  K  Y  Q  S  R
247 ccaaaatttaactcaacacctaaatatattaaattcaaacatgac
    P  K  F  N  S  T  P  K  Y  I  K  F  K  H  D
202 tataatattttagaatttaacgatggtacattcgaatatggtgca
    Y  N  I  L  E  F  N  D  G  T  F  E  Y  G  A
157 cgtccacaatttaataaaccagcagcgaaaactgatgcaactatt
    R  P  Q  F  N  K  P  A  A  K  T  D  A  T  I
112 aaaaaagaacaaaaattgattcaagctcaaaatcttgtgagagaa
    K  K  E  Q  K  L  I  Q  A  Q  N  L  V  R  E
 67 tttgaaaaaacacatactgtcagtgcacacagaaaagcacaaaag
    F  E  K  T  H  T  V  S  A  H  R  K  A  Q  K
 22 gcagtcaacttagttncgtn 1
    A  V  N  L  V  X  X
```

> gb|AEV78151.1| Extracellular fibrinogen-binding protein Efb [Staphylococcus aureus subsp. aureus M013]
Length=165

Score = 259 bits (661), Expect = 3e-86, Method: Compositional matrix adjust.
Identities = 124/125 (99%), Positives = 124/125 (99%), Gaps = 0/125 (0%)

```
Query  1    IGITTTTIASTADASEGYGPREKKPVSINHNIVEYNDGTFKYQSRPKFNSTPKYIKFKHD  60
            IGITTTTIASTADASEGYGPREKKPVSINHNIVEYNDGTFKYQSRPKFNSTPKYIKFKHD
Sbjct  16   IGITTTTIASTADASEGYGPREKKPVSINHNIVEYNDGTFKYQSRPKFNSTPKYIKFKHD  75

Query  61   YNILEFNDGTFEYGARPQFNKPAAKTDATIKKEQKLIQAQNLVREFEKTHTVSAHRKAQK  120
            YNILEFNDGTFEYGARPQFNKPAAKTD TIKKEQKLIQAQNLVREFEKTHTVSAHRKAQK
Sbjct  76   YNILEFNDGTFEYGARPQFNKPAAKTDVTIKKEQKLIQAQNLVREFEKTHTVSAHRKAQK  135

Query  121  AVNLV  125
             AVNLV
Sbjct  136  AVNLV  140
```

APPENDIX 44

Standard Nucloetide-nucleotide BLAST search results for DNA sequence of the amplified extracellular fibrinogen-binding protein (*fnbA*) from MRSA0812-17

> gb|JN848741.1| Staphylococcus aureus strain C290 fibronectin binding protein A (fnbA) gene, partial cds
Length=1089
Score = 287 bits (155), Expect = 2e-74
Identities = 156/157 (99%), Gaps = 0/157 (0%)
Strand=Plus/Minus

```
Query  16   CTANTTCAATCAGATTACTTTCAGTTGTATATTCTTTCGTATCTTCAACTGTTGTATGAT  75
            ||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  517  CTAATTCAATCAGATTACTTTCAGTTGTATATTCTTTCGTATCTTCAACTGTTGTATGAT  458

Query  76   CGCTCACTGCGCCAGTTACAATACCTTTTGTAGACTCTTCGTCAAATTCAACTAAGTTAG  135
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  457  CGCTCACTGCGCCAGTTACAATACCTTTTGTAGACTCTTCGTCAAATTCAACTAAGTTAG  398

Query  136  ACTCAGTAGTAACCTGACCACCACCTGGGTTTGTATC  172
            ||||||||||||||||||||||||||||||||||||
Sbjct  397  ACTCAGTAGTAACCTGACCACCACCTGGGTTTGTATC  361
```

APPENDIX 45

Standard Nucleotide-nucleotide BLAST search results for DNA sequence of the amplified gamma hemolysin (*hlg*) from MRSA0801-21

> gb|CP002110.1| Staphylococcus aureus subsp. aureus TCH60, complete genome
Length=2802675
Features in this part of subject sequence:
gamma hemolysin
gamma hemolysin
Score = 1439 bits (779), Expect = 0.0
Identities = 850/885 (96%), Gaps = 2/885 (0%)
Strand=Plus/Plus

```
Query  1       ATATCAATTCTGTCCTTTCACTTTGATTTCGTGTGTCTTCCAATTGACTTCATATTTCAC  60
               ||||||||||||||||||||||||||| ||||||||||||||||||||
Sbjct  842914  ATATCAATTCTGTCCTTTCACTTTGATTTCGTGAGTCTTCCAATTGACTTCATATTTCAC  842973
Query  61      AGTGTAGTTTCTATTTTTAAATGCATTATGGACTCTGTGACCATCTAAATAACTGTTGCC  120
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  842974  AGTGTAGTTTCTATTTTTAAATGCATTATGGACTCTGTGACCATCTAAATAACTGTTGCC  843033
Query  121     ATAATGTGTTGATCTTTTAATGGCATGAGTGACATCCATATTTCTTCCATAAGTGATTTC  180
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  843034  ATAATGTGTTGATCTTTTAATGGCATGAGTGACATCCATATTTCTTCCATAAGTGATTTC  843093
Query  181     AAATTCGCTCGTGTCGCCTGAACCTTTTTCATGAGATACTGTTGCGATAAATGAAGGGTT  240
               ||||||||||||||| |||||||||| ||||||||||||||||||||||||
Sbjct  843094  AAATTCGCTCGTGTCGCTTGAACCTTTTTCGTGAGATACTGTTGCGATAAATGAAGGGTT  843153
Query  241     AAATCCACTTTGCACAAGAGGTGGTAATTCGCTGTCTGGAACAAAATAATCTCTTGGATC  300
               |||||||||| |||||||||| |||||||||| |||||||||| |||||
Sbjct  843154  AAATCCACTTTGTACAAGAGGTGGTAACTCGCTGTCTGGAACGAAATAATCTCTAGGATC  843213
Query  301     TTTGCTATTTGGTTTGTATCCTACGAATAAATCACTATCAAATGCTGACTTTTGACCTGA  360
               ||| ||||  ||||||| ||||| ||||||| |||||||||||| |||||||||
Sbjct  843214  TTTACTATGAGGTTTGTAGCCTACAAATAAATCGCTATCAAATGCTGATTTTTGACCTGA  843273
Query  361     TGCAGTGACGAATGAATTCGCTTTGACACCCCATAAAACACTTTTTGAGTTTTGTTGTTC  420
               | ||||| |||||||||||||||| |||||||||||||||||||||||||||||||||
Sbjct  843274  TTCAGTGGCGAATGAATTCGCTTTGACGCCCCATAAAACACTTTTTGAGTTTTGTTGTTC  843333
Query  421     CACTTCACTGACATAATTTTGTTGTGTATAGCTAATCGATTTAGAGTAGTTAAAGGATCC  480
               ||||||| |||||||||||||||||||||||||||| |||||||| |||||
Sbjct  843334  TACTTCACTTACATAATTTTGTTGTGTATAGCTAATCGATTTAGAATAGTTAAATGATCC  843393
Query  481     ATTACCACCGAGTGATGGGGCTGATTGGAAATTACCGCCGATATTGTATCCTAATGTCTG  540
               |||||||| |||||||||||||||||||| || |||||||||||||||||
Sbjct  843394  ATTACCACCAAGTGATGGGGCTGATTGGAAATTACCACCAATATTGTATCCTAATGTCTG  843453
Query  541     GCTCACATTTGTAGATTCAATTTTATTTTTCGGTAAATAATTGATTAAAGAAACAT-TTG  599
               || || |||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  843454  ACTTACGTTTGTAGATTCAATTTTATTTTTCGGTAAATAATTGATTAAAGAAACATATTT  843513
Query  600     GATCATTTGTTTTTAACCCAATATTATATTGGAAGGGCCAACGCATAGATTTAATATGAT  659
               |||||||||| || ||||||||||||| ||||||||||||||||||||
Sbjct  843514  -ATCATTTGTTTTCAAACCAATATTATATTGGAATGGCCAACGCATAGATTTAATATGAT  843572
Query  660     TTGTATTTTTATAGTTATAATATGTTGTTCGAGAGCTAATGAATCCTTGCATCTTTAGGA  719
               |||||||||||||||||||||||||||||||||||||||||||||||||| ||
Sbjct  843573  TTGTATTTTTATAGTTATAATATGTTGTTCGAGAGCTAATGAATCCTTGCATCTTTAAGA  843632
Query  720     TCAAAGCATCTTTGTTATATTTTTTATCCTTCACAAAGTCGAATTGGATATTTTGAGTCA  779
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  843633  TCAAAGCATCTTTGTTATATTTTTTATCCTTCACAAAGTCGAATTGGATATTTTGAGTCA  843692
Query  780     CTCCCCATTTATTACTTGTTTTATCTTCTGTCCTTTTGATAATTTCTACATCGTTTCCTT  839
               | ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  843693  CGCCCCATTTATTACTTGTTTTATCTTCTGTCCTTTTGATAATTTCTACATCGTTTCCTT  843752
Query  840     TACCGATGTCTTCAGTATCATTGGCAGCTTTAGCATTTTCTAATA  884
               |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  843753  TACCGATGTCTTCAGTATCATTGGCAGCTTTAGCATTTTCTAATA  843797
```

APPENDIX 46

Standard Open Reading Frame BLAST search results for DNA sequence of the amplified intercellular adhesion protein A (*icaA*) from MRSA0801-21

```
 25 atgattgagaatttcaaacatgatccaaaacttggtgcagttaca
    M  I  E  N  F  K  H  D  P  K  L  G  A  V  T
 70 ggtaatcctagaattcgaaataagagttctattttaggtaaaatt
    G  N  P  R  I  R  N  K  S  S  I  L  G  K  I
115 caaacgatagaatatgcaagtttaattggctgtattaagcgaagt
    Q  T  I  E  Y  A  S  L  I  G  C  I  K  R  S
160 cagacacttgctggcgcagtcaatactatttcgggtgtcttcact
    Q  T  L  A  G  A  V  N  T  I  S  G  V  F  T
205 ctatttaaaaaaagtgcagttgtcgacgttggctactgggatact
    L  F  K  K  S  A  V  V  D  V  G  Y  W  D  T
250 gatatgattaccgaagatattgcagtttcttggaaattgcattta
    D  M  I  T  E  D  I  A  V  S  W  K  L  H  L
295 cgtggatatcgtattaagtatgaaccgcttgccatgtgttggatg
    R  G  Y  R  I  K  Y  E  P  L  A  M  C  W  M
340 ttggttccagaaacattgggaggtctttggaagcaacgcgtgaga
    L  V  P  E  T  L  G  G  L  W  K  Q  R  V  R
385 tgggctcaaggggacacgaagtattactacgagacttttttagc
    W  A  Q  G  G  H  E  V  L  L  R  D  F  F  S
430 acaatgaaaacgaaaaggtttcctttatatattttgatgtttgag
    T  M  K  T  K  R  F  P  L  Y  I  L  M  F  E
475 caaatcatctcaattttatgggtatatatagtgcttctatattta
    Q  I  I  S  I  L  W  V  Y  I  V  L  L  Y  L
520 ggctatttgttcataacagcaaacttcttagactatacatttatg
    G  Y  L  F  I  T  A  N  F  L  D  Y  T  F  M
565 acatatagtttttcaatatttctactatcatcatttactatgact
    T  Y  S  F  S  I  F  L  L  S  S  F  T  M  T
610 tttataaacgttattcaatttacagtcgcactctttattgatagt
    F  I  N  V  I  Q  F  T  V  A  L  F  I  D  S
655 cgctacgagaaaaagaatatggctggactcatatttgtaagttgg
    R  Y  E  K  K  N  M  A  G  L  I  F  V  S  W
700 tatccgacagtatactggattattacg 726
    Y  P  T  V  Y  W  I  I  T
```

> ref|ZP_05687633.1| intercellular adhesion protein A [Staphylococcus aureus A9635]
gb|EEV69097.1| intercellular adhesion protein A [Staphylococcus aureus A9635]
Length=412

Score = 480 bits (1236), Expect = 5e-168, Method: Compositional matrix adjust.
Identities = 233/234 (99%), Positives = 233/234 (99%), Gaps = 0/234 (0%)

```
Query  1    MIENFKHDPKLGAVTGNPRIRNKSSILGKIQTIEYASLIGCIKRSQTLAGAVNTISGVFT  60
            MIENFKHDPKLGAVTGNPRIRNKSSILGKIQTIEYASLIGCIKRSQTLAGAVNTISGVFT
Sbjct  147  MIENFKHDPKLGAVTGNPRIRNKSSILGKIQTIEYASLIGCIKRSQTLAGAVNTISGVFT  206

Query  61   LFKKSAVVDVGYWDTDMITEDIAVSWKLHLRGYRIKYEPLAMCWMLVPETLGGLWKQRVR  120
            LFKKSAVVDVGYWDTDMITEDIAVSWKLHLRGYRIKYEPLAMCWMLVPETLGGLWKQRVR
Sbjct  207  LFKKSAVVDVGYWDTDMITEDIAVSWKLHLRGYRIKYEPLAMCWMLVPETLGGLWKQRVR  266

Query  121  WAQGGHEVLLRDFFSTMKTKRFPLYILMFEQIISILWVYIVLLYLGYLFITANFLDYTFM  180
            WAQGGHEVLLRDFFSTMKTKRFPLYILMFEQIISILWVYIVLLYLGYLFITANFLDYTFM
Sbjct  267  WAQGGHEVLLRDFFSTMKTKRFPLYILMFEQIISILWVYIVLLYLGYLFITANFLDYTFM  326

Query  181  TYSFSIFLLSSFTMTFINVIQFTVALFIDSRYEKKNMAGLIFVSWYPTVYWIIT  234
            TYSFSIFLLSSFTMTFINVIQFTVALFIDSRYEKKNMAGLIFVSWYPTVYWII
Sbjct  327  TYSFSIFLLSSFTMTFINVIQFTVALFIDSRYEKKNMAGLIFVSWYPTVYWIIN  380
```

APPENDIX 47

Standard Open Reading Frame BLAST search results for DNA sequence of the amplified bone sialoprotein-binding protein (*sdrE*) from MRSA0812-17

```
593 atgcgctttgcagttgcacaaccagcagcagttgcttcaaacaat
    M  R  F  A  V  A  Q  P  A  A  V  A  S  N  N
548 gtaaatgatttaattaaagtgacgaagcaaacaatcaaagttggc
    V  N  D  L  I  K  V  T  K  Q  T  I  K  V  G
503 gatggtaaagataatgtggcagcagcgcatgacggtaaagatatt
    D  G  K  D  N  V  A  A  A  H  D  G  K  D  I
458 gaatatgatacagagtttacaattgacaataaagtcaaaaaaggc
    E  Y  D  T  E  F  T  I  D  N  K  V  K  K  G
413 gatacaatgacgattaattatgataagaatgtaattccttcggat
    D  T  M  T  I  N  Y  D  K  N  V  I  P  S  D
368 ttaacagataaaaatgatcctatcgatattactgatccatcagga
    L  T  D  K  N  D  P  I  D  I  T  D  P  S  G
323 gaggtcattgctaaaggaacatttgataaagcaactaagcaaatc
    E  V  I  A  K  G  T  F  D  K  A  T  K  Q  I
278 acatatacatttacagactatgtagataaatatgaagatataaaa
    T  Y  T  F  T  D  Y  V  D  K  Y  E  D  I  K
233 tcacgcttaactctatattcgtatattgataaaaaaacagttcca
    S  R  L  T  L  Y  S  Y  I  D  K  K  T  V  P
188 aatgagacaagtttgaatttaacatttgctacagcaggtaaagaa
    N  E  T  S  L  N  L  T  F  A  T  A  G  K  E
143 acaagccaaaatgtcactgttgattatcaagatccaatggtccat
    T  S  Q  N  V  T  V  D  Y  Q  D  P  M  V  H
 98 ggtgattcaaacattcaatctatctttacaaaattagatgaagat
    G  D  S  N  I  Q  S  I  F  T  K  L  D  E  D
 53 aagcaaactattgaacaacaaatttatgttaacccatga 15
    K  Q  T  I  E  Q  Q  I  Y  V  N  P  *
```

> ref|ZP_03565541.1| bone sialoprotein-binding protein [Staphylococcus aureus subsp. aureus str. JKD6009]
Length=797

Score = 387 bits (994), Expect = 3e-127, Method: Compositional matrix adjust.
Identities = 192/192 (100%), Positives = 192/192 (100%), Gaps = 0/192 (0%)

```
Query  1    MRFAVAQPAAVASNNVNDLIKVTKQTIKVGDGKDNVAAAHDGKDIEYDTEFTIDNKVKKG  60
            MRFAVAQPAAVASNNVNDLIKVTKQTIKVGDGKDNVAAAHDGKDIEYDTEFTIDNKVKKG
Sbjct  260  MRFAVAQPAAVASNNVNDLIKVTKQTIKVGDGKDNVAAAHDGKDIEYDTEFTIDNKVKKG  319

Query  61   DTMTINYDKNVIPSDLTDKNDPIDITDPSGEVIAKGTFDKATKQITYTFTDYVDKYEDIK  120
            DTMTINYDKNVIPSDLTDKNDPIDITDPSGEVIAKGTFDKATKQITYTFTDYVDKYEDIK
Sbjct  320  DTMTINYDKNVIPSDLTDKNDPIDITDPSGEVIAKGTFDKATKQITYTFTDYVDKYEDIK  379

Query  121  SRLTLYSYIDKKTVPNETSLNLTFATAGKETSQNVTVDYQDPMVHGDSNIQSIFTKLDED  180
            SRLTLYSYIDKKTVPNETSLNLTFATAGKETSQNVTVDYQDPMVHGDSNIQSIFTKLDED
Sbjct  380  SRLTLYSYIDKKTVPNETSLNLTFATAGKETSQNVTVDYQDPMVHGDSNIQSIFTKLDED  439

Query  181  KQTIEQQIYVNP  192
            KQTIEQQIYVNP
Sbjct  440  KQTIEQQIYVNP  451
```

APPENDIX 48

Standard Open Reading Frame BLAST search results for DNA sequence of the amplified panton-valentine leukocidin (*pvl*) from MRSA0805-10

```
  397 atgtctggacatgatccaaatttatttgttggatataaaccatat
      M  S  G  H  D  P  N  L  F  V  G  Y  K  P  Y
  352 agtcaaaatccgagagactattttgtgccagacaatgaattaccc
      S  Q  N  P  R  D  Y  F  V  P  D  N  E  L  P
  307 ccattagtacacagtggtttcaatccttcatttattgcaactgtt
      P  L  V  H  S  G  F  N  P  S  F  I  A  T  V
  262 tctcatgaaaaaggctcaggagatacaagtgaatttgaaataacg
      S  H  E  K  G  S  G  D  T  S  E  F  E  I  T
  217 tatggcagaaatatggatgttactcatgctactagaagaacaaca
      Y  G  R  N  M  D  V  T  H  A  T  R  R  T  T
  172 cactatggcaatagttatttagaaggatctagaatacacaacgca
      H  Y  G  N  S  Y  L  E  G  S  R  I  H  N  A
  127 tttgtaaacagaaattacacagttaaatatgaagtgaactggaaa
      F  V  N  R  N  Y  T  V  K  Y  E  V  N  W  K
   82 actcatgaaattaaagtgaaaggacataatga 50
      T  H  E  I  K  V  K  G  H  N  *
```

```
>        gb|AEO79837.1|  LukPVS [Staphylococcus aureus]
Length=119
 Score =  240 bits (612),  Expect = 1e-79, Method: Compositional matrix adjust.
 Identities = 115/115 (100%), Positives = 115/115 (100%), Gaps = 0/115 (0%)
Query  1    MSGHDPNLFVGYKPYSQNPRDYFVPDNELPPLVHSGFNPSFIATVSHEKGSGDTSEFEIT  60
            MSGHDPNLFVGYKPYSQNPRDYFVPDNELPPLVHSGFNPSFIATVSHEKGSGDTSEFEIT
Sbjct  5    MSGHDPNLFVGYKPYSQNPRDYFVPDNELPPLVHSGFNPSFIATVSHEKGSGDTSEFEIT  64

Query  61   YGRNMDVTHATRRTTHYGNSYLEGSRIHNAFVNRNYTVKYEVNWKTHEIKVKGHN  115
            YGRNMDVTHATRRTTHYGNSYLEGSRIHNAFVNRNYTVKYEVNWKTHEIKVKGHN
Sbjct  65   YGRNMDVTHATRRTTHYGNSYLEGSRIHNAFVNRNYTVKYEVNWKTHEIKVKGHN  119
```

APPENDIX 49

Standard Nucleotide-nucleotide BLAST search results for DNA sequence of the amplified *agr* type I (*agrB, agrD, agrC* gene) from MRSA0806-21

```
>        emb|AJ617710.1|  Staphylococcus aureus agrB gene (partial), agrD gene and agrC
gene (partial), strain gt54b-cp5
Length=1891
 Score =  723 bits (391),  Expect = 0.0
 Identities = 394/395 (99%), Gaps = 1/395 (0%)
 Strand=Plus/Minus
Query  19   tcccagtaataaaatcaaaaaataagttaaataatGTATTCATTTTAAGTCCTCCTTAAT  78
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  584  TCCCAGTAATAAAATCAAAAAATAAGTTAAATAATGTATTCATTTTAAGTCCTCCTTAAT  525

Query  79   AAAGAAAATAGGTAATAATGTAATAGCTTCTATTATGATGCCTAATTGAATGAATTGGGC  138
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  524  AAAGAAAATAGGTAATAATGTAATAGCTTCTATTATGATGCCTAATTGAATGAATTGGGC  465

Query  139  AAATGGCTCTTTGATGATAAGTGTGATAATGAAAAAGGTTAAACTAACAATAATCGCATA  198
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  464  AAATGGCTCTTTGATGATAAGTGTGATAATGAAAAAGGTTAAACTAACAATAATCGCATA  405

Query  199  ATAttttttCGTTTAATAAGTCGCACAGGAATGGGCTTCTTTTTAGTTGCTGCAGGAGC  258
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  404  ATATTTTTTTCGTTTAATAAGTCGCACAGGAATGGGCTTCTTTTTAGTTGCTGCAGGAGC  345

Query  259  ATATACTGAGATTATACCTAAAGAAATAACTGTTAAAATAATCATAATTAATGGATTAAT  318
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  344  ATATACTGAGATTATACCTAAAGAAATAACTGTTAAAATAATCATAATTAATGGATTAAT  285

Query  319  ATGAAAATTTACTATTACTAAAGGTAAGAGTATAAATAATAAAATACTTTCCACATAACA  378
            |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  284  ATGAAAATTTACTATTACTAAAGGTAAGAGTATAAATAATAAAATACTTTCCACATAACA  225

Query  379  CCAAAAGGAAGAAGGTGCATGTGCAC-ATGTGCAT  412
            |||||||||||||||||||| |||||||
Sbjct  224  CCAAAAGGAAGAAGGTGCATGTGCACCATGTGCAT  190
```

APPENDIX 50

Standard Nucleotide-nucleotide BLAST search results for DNA sequence of the amplified *agr* type II (*agrD, agrC, agrB* gene) from MRSA0812-36

> emb|AJ617719.1| Staphylococcus aureus agrD gene, agrC gene (partial) and agrB gene (partial), strain gt111-cp8
Length=1890
Score = 961 bits (520), Expect = 0.0
Identities = 523/525 (99%), Gaps = 0/525 (0%)
Strand=Plus/Minus

```
Query  1    ATGCTACACACTTTCTAAAATAACTTTTACTATTTGTCGTATAAATTCGTTAATTCNGCG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||| |||
Sbjct  714  ATGCTACACACTTTCTAAAATAACTTTTACTATTTGTCGTATAAATTCGTTAATTCAGCG  655
Query  61   GGTACTTTAGGTTCATCAAATAAACTGCTGCAAGCGTTTACGCCACCGACAATTCCGATT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  654  GGTACTTTAGGTTCATCAAATAAACTGCTGCAAGCGTTTACGCCACCGACAATTCCGATT  595

Query  121  GCTTTAGCTAATTTAATTATAAAATCAAAAAACATATTAACAAGTGTATTCATGATTAAT  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  594  GCTTTAGCTAATTTAATTATAAAATCAAAAAACATATTAACAAGTGTATTCATGATTAAT  535

Query  181  CCTCCTTAGGGaaaaaaaTAGGTAATAATGTAATAGATTCTACTAAAATTCCTAATAACA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  534  CCTCCTTAGGGAAAAAAATAGGTAATAATGTAATAGATTCTACTAAAATTCCTAATAACA  475

Query  241  TAAATTGAGCATAAAATGGATGTATTATTAAGGATAGAATCAAAACTAATAAATACATAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  474  TAAATTGAGCATAAAATGGATGTATTATTAAGGATAGAATCAAAACTAATAAATACATAA  415

Query  301  TTATAGATAAATACTTTTTACGTTTTACTAATTTTATTGGTATAGGTTGCTTTTTCGTTG  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  414  TTATAGATAAATACTTTTTACGTTTTACTAATTTTATTGGTATAGGTTGCTTTTTCGTTG  355

Query  361  CTGCAGGTGCATAAATAACAACGGAAATCAATCCTATAATAGATAAGGCCAGTAAATAAG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  354  CTGCAGGTGCATAAATAACAACGGAAATCAATCCTATAATAGATAAGGCCAGTAAATAAG  295

Query  421  TAAAATTAATGTCTATATTAATTAGAAAATAAGGTACAAATACAAATGTTAAAATACTTT  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  294  TAAAATTAATGTCTATATTAATTAGAAAATAAGGTACAAATACAAATGTTAAAATACTTT  235

Query  481  GTATGTAACAAAGTATCGAAGACTTTGCATGTGCACCATGTGCAT  525
            |||||||||||||||||||||| ||||||||||
Sbjct  234  GTATGTAACAAAGTATCGAAGACTTTGCATGAGCACCATGTGCAT  190
```

APPENDIX 51

Standard Nucleotide-nucleotide BLAST search results for DNA sequence of the amplified *agr* type III (*agrD, agrC, agrB* gene) from MRSA0806-13

> emb|AJ617720.1| Staphylococcus aureus agrD gene, agrC gene (partial) and agrB gene (partial), strain gt108-cp8
Length=1892
Score = 516 bits (279), Expect = 4e-143
Identities = 280/281 (99%), Gaps = 0/281 (0%)
Strand=Plus/Minus

```
Query  1    TTGGGTATATGGTTCTTTTACAAAAAGTGTGATAATGAAAAGAATTGTACTAATTATAAT  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  470  TTGGGTATATGGTTCTTTTACAAAAAGTGTGATAATGAAAAGAATTGTACTAATTATAAT  411
Query  61   TGAAAAATATCTTTTTTGCTTTACAAGCCTCGCTGGGATAGGCTTCTTCTTAGTGGCTGC  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  410  TGAAAAATATCTTTTTTGCTTTACAAGCCTCGCTGGGATAGGCTTCTTCTTAGTGGCTGC  351
Query  121  AGGTGCATATTTAATCACTACTCCAACACTTATTAATGCTAAAAACATCATTAGTGTTTC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  350  AGGTGCATATTTAATCACTACTCCAACACTTATTAATGCTAAAAACATCATTAGTGTTTC  291

Query  181  ATTAATATGAAAATGCAATACTAATAGAGGTAGCACAATAAAAAGTGTAATGCTTTCTAT  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  290  ATTAATATGAAAATGCAATACTAATAGAGGTAGCACAATAAAAAGTGTAATGCTTTCTAT  231

Query  241  ATAACACCAAAAAGATGAAGGTGCATGTGCANCATGTGCAT  281
            |||||||||||||||||||||| ||||||||
Sbjct  230  ATAACACCAAAAAGATGAAGGTGCATGTGCACCATGTGCAT  190
```

APPENDIX 52

New *spa* type (t6405) assigned from ridom *spa* server for MRSA0802-3

New | Reply | Reply all | Forward | Delete | Junk | Sweep | Mark as | Move to | Categories

AW: Spa-type Submissions

Back to messages

Alexander.Friedrich@ukmuenster.de Add to contacts
To q5thong@yahoomail.com, poly_lim@hotmail.com

12/3/2010
Reply

Dear colleague,
thank you for submitting the spa sequences:
For the following sequences a new spa type was assigned:

MRSA0802-3 t6405
MRSA0805-20 t6405
MRSA0801-16 t6405

The following sequences had a low reliability and were not accepted by the spa server. For assignment, please send me new fw/rev sequences.

Best wishes,
Alex Friedrich

SeqNet.org coordinator

PD Dr. med. A. W. Friedrich
Institute of Hygiene
University Hospital Münster
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Ridom Spa Server

SpaServer.ridom.de

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Database
Frequencies
Spa-types
Repeats
MLST Mapping

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Ridom SpaServer: spa-t6405

Spa-type:	t6405
Repeat succession:	15-12-16-02-25-17-24-25-17-24
Frequency:	0.00 %
Total strains:	3
Strain records:	3

Strain Records

Isolate ID	Isolation date	Submission date	Country	MRSA / MSSA	MLST	Association	Reliability	Submitter
MRSA0805-20	23-May-2008	09-Jun-2010	Malaysia	MRSA		colonization	excellent	
MRSA0802-3	11-Feb-2008	18-Mar-2010	Malaysia	MRSA		colonization	excellent	
MRSA0801-16	12-Jan-2008	12-Mar-2010	Malaysia	MRSA		unknown	excellent	

last modified: 27-Oct-2010

webmaster@ridom.de

APPENDIX 53

Multilocus query result for MLST type ST239

MLST
Multi Locus Sequence Typing

mlst.net | saureus.mlst.net

Thursday 8th March 2012

DATA ANALYSIS

DATABASES

- B.burgdorferi
- B.cereus
- B.henselae
- B.pseudomallei
- C.albicans
- C.glabrata
- C.krusei
- C.tropicalis
- C.jejuni
- C.neoformans var grubii
- E.coli
- E.faecalis
- E.faecium
- H.influenzae
- H.pylori
- Leptospira spp.
- M.catarrhalis
- N.meningitidis
- S.agalactiae
- **S.aureus**
- S.dysgalactiae
- S.enterica
- S.epidermidis
- S.pneumoniae
- S.pyogenes
- S.suis
- V.vulnificus

SUBMISSIONS

Staphylococcus aureus - Multiple Locus Query Results

Locus	Allele Number	Error Messages	Action
arcc	2	OK	None
aroe	3	OK	None
glpf	1	OK	None
gmk_	1	OK	None
pta_	4	OK	None
tpi_	4	OK	None
yqil	3	OK	None

arcc	2
aroe	3
glpf	1
gmk_	1
pta_	4
tpi_	4
yqil	3

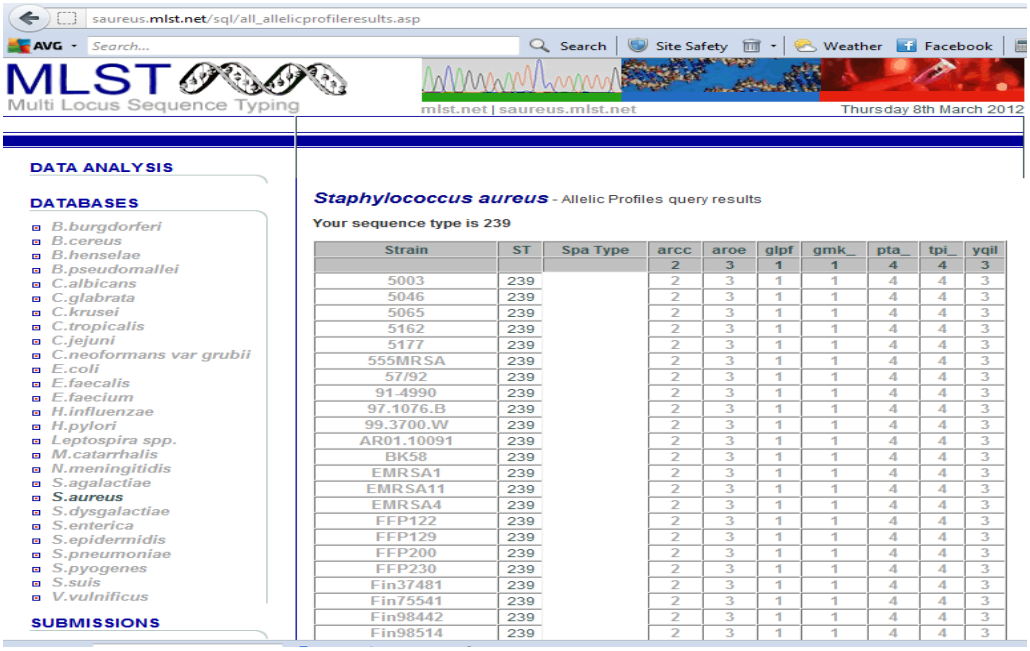
Submit data for allelic profile query

Exact or nearest match

Submit

APPENDIX 54

Allelic profile query for MLST type ST239



saureus.mlst.net/sql/all_allelicprofileresults.asp

MLST Multi Locus Sequence Typing

mlst.net | saureus.mlst.net

Thursday 8th March 2012

DATA ANALYSIS

DATABASES

- B.burgdorferi
- B.cereus
- B.henselae
- B.pseudomallei
- C.albicans
- C.glabrata
- C.krusei
- C.tropicalis
- C.jejuni
- C.neoformans var grubii
- E.coli
- E.faecalis
- E.faecium
- H.influenzae
- H.pylori
- Leptospira spp.
- M.catarrhalis
- N.meningitidis
- S.agalactiae
- **S.aureus**
- S.dysgalactiae
- S.enterica
- S.epidermidis
- S.pneumoniae
- S.pyogenes
- S.suis
- V.vulnificus

SUBMISSIONS

Staphylococcus aureus - Allelic Profiles query results

Your sequence type is 239

Strain	ST	Spa Type	arcc	aroe	glpf	gmk_	pta_	tpi_	yqil
			2	3	1	1	4	4	3
5003	239		2	3	1	1	4	4	3
5046	239		2	3	1	1	4	4	3
5065	239		2	3	1	1	4	4	3
5162	239		2	3	1	1	4	4	3
5177	239		2	3	1	1	4	4	3
555MRSA	239		2	3	1	1	4	4	3
57/92	239		2	3	1	1	4	4	3
91-4990	239		2	3	1	1	4	4	3
97.1076.B	239		2	3	1	1	4	4	3
99.3700.W	239		2	3	1	1	4	4	3
AR01.10091	239		2	3	1	1	4	4	3
BK58	239		2	3	1	1	4	4	3
EMRSA1	239		2	3	1	1	4	4	3
EMRSA11	239		2	3	1	1	4	4	3
EMRSA4	239		2	3	1	1	4	4	3
FFP122	239		2	3	1	1	4	4	3
FFP129	239		2	3	1	1	4	4	3
FFP200	239		2	3	1	1	4	4	3
FFP230	239		2	3	1	1	4	4	3
Fin37481	239		2	3	1	1	4	4	3
Fin75541	239		2	3	1	1	4	4	3
Fin98442	239		2	3	1	1	4	4	3
Fin98514	239		2	3	1	1	4	4	3

APPENDIX 55

New *dru* types assigned from *dru* server

To see messages related to this one, group messages by conversation.

Goering, Richard V. 14/1/2011

To Poly Lim, info@dru-typing.org, info@dru-typing.com, thong kwai lin Reply

Greetings!

These are excellent sequences and all of them are new types that have not been seen before so your data is extremely interesting. Below is a summary of your results:

0305-23	dt13l	5a-1a-3c-5b-3a-5b-3a-2g-2b-2g-2j-4e-3e
0307-10	dt13m	5a-1a-3c-5b-3a-2g-2b-5d-3a-2g-2j-4e-3e
0307-5	dt11al	5a-1a-3c-2g-2b-5b-3a-2g-2j-4e-3e
0308-23	dt15l	5a-1a-3c-5b-3a-5b-3a-2g-2b-5b-3a-3o-2j-3a-3e
0310-26	dt15m	5a-1a-3c-5b-3a-5b-3a-3o-2b-5b-3a-3o-2j-3a-3e
0408-33	dt7v	5a-1a-3c-2g-2j-4e-3e
0408-34	dt13n	5a-1a-3c-5b-3a-3o-2b-5b-3a-2g-2j-4e-3e
0705-13	dt15n	5a-1a-3c-5b-3a-5b-3a-2g-2b-5b-3a-3o-2b-3a-3e
0705-7	dt10aw	5a-2d-4a-0-3c-2g-2c-2g-3b-4e
0705-8	dt13o	5a-0-3c-5b-3a-2g-2b-5b-3a-2g-2j-4e-3e
0801-1	dt11am	5a-1a-3c-5b-3a-1a-3c-2g-2j-4e-3e
0802-14	dt13p	5a-1a-2d-5b-3a-2g-2b-5b-3a-2g-2j-4e-3e
0805-20	dt11an	5a-1a-3c-5b-3a-3o-2b-5b-3a-2f-3e
0807-7	dt13q	5a-1a-3c-2g-2b-5b-3a-5b-3a-2g-2j-4e-3e
0809-30	dt10ax	5a-1a-3c-5b-3a-2g-2b-2j-4e-3e
0809-32	dt12j	5a-1a-3c-5b-3a-1b-2g-2b-5b-2a-3o-3e
0812-30	dt12k	5a-1a-3c-5b-3a-5b-3a-2g-2b-3o-3e-3e
mrsa-8	dt14h	5a-2b-3c-5b-3a-5b-3a-2g-2b-5b-3a-2g-2j-4e

All of these new types will now be added to the dru server database (http://www.dru-typing.org).

PUBLICATIONS

1. **Lim, K. T**., Hanifah, Y. A., Mohd Yusof, M. Y., & Thong, K. L. (2010). Prevalence of mupirocin resistance in methicillin-resistant *Staphylococcus aureus* strains isolated from a Malaysian hospital. *Japanese Journal of Infectious Disease, 63*(4), 286-289.
2. **Lim, K. T**., Goering, R., Hanifah, Y. A., Yusof, M. Y. M., & Thong, K. L. (2011). Enhance discrimination of pandemic clone ST239-methicillin resistant *Staphylococcus aureus* in a tertiary hospital in Malaysia by *mec*-associated direct repeat unit and spa typing. *International Journal of Infectious Disease, 15* (Supplement 1), S52.
3. **Lim, K. T.,** Hanifah, Y. A., Mohd Yusof, M. Y., & Thong, K. L. (2012). Investigation of toxin genes among methicillin resistant *Staphylococcus aureus* strains isolated from a tertiary hospital in Malaysia. *Tropical Biomedicine, 29*(2), 1-8.
4. **Lim, K. T**., Hanifah, Y. A., Yusof, M. Y. M., & Thong, K. L. (2012). *ermA, ermC, tetM* and *tetK* are essential for erythromycin and tetracycline resistance among methicillin-resistant *Staphylococcus aureus* strains isolated from a tertiary hospital in Malaysia. *Indian Journal of Medical Microbiology, 30*(2), 203-207.
5. **Lim, K. T**., Hanifah, Y. A., Yusof, M. Y. M., Ito, T., & Thong, K. L. Comparison of methicillin-resistant *Staphylococcus aureus* strains isolated in 2003 and 2008 with an emergence of multidrug resistant ST22 SCC*mec* IV clone in a tertiary hospital, Malaysia. *Journal of Microbiology, Immnunology and Infections.* Accepted for publication.
6. **Lim, K. T**., Hanifah, Y. A., Yusof, M. Y. M., Goering, R., & Thong, K. L. Temporal changes in the genotypes of methicillin-resistant *Staphylococcus aureus* strains isolated from a tertiary Malaysian hospital based on MLST, *spa* and *mec*-

associated *dru* typing. *Diagnostic Microbiology and Infectious Disease.* 74, 106-112. doi:10.1016/j.diagmicrobio.2012.05.033

7. **Lim, K. T**., Abu Hanifah, Y., Mohd Yusof, M. Y., and Thong, K. L. (2012). Genetic diversity of Malaysian methicillin resistant Staphylococcus aureus based on virulotypes, pulsed-field gel electrophoresis and PCR RFLP of *coa* gene. *International Journal of Infectious Disease*, 16 (Supplement 1), Page E229. (http://dx.doi.org/10.1016/j.ijid.2012.05.837).
8. **Lim, K. T**., Hanifah, Y. A., Mohd Yusof, M. Y., and Thong, K. L. (2012). Characterisation of the virulence factors and genetic types of methicillin susceptible *Staphylococcus aureus* from patients and healthy individuals. *Indian Journal of Microbiology.* DOI 10.1007/s12088-012-0286-7.
9. **Lim, K. T**., Yeo, C. C., Suhaili, Z., and Thong, K. L. (2012). Comparison of methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* strains isolated from a tertiary hospital in the state of Terengganu, Malaysia. *Japanese Journal of Infectious Disease*. *In press.*

PRESENTATIONS

1. **Lim, K. T.**, Yasin, A. H., Mohd Yasim, M.Y., & Thong, K. L, 2012. Molecular evolution of methicillin resistant *Staphylococcus aureus* in a tertiary hospital. National Postgraduate Seminar, Research Management & Innovation Complex University of Malaya, Kuala Lumpur, 11th July 2012 (Oral Presentation).
2. **Lim, K. T**, Yasin, A. H, Mohd Yasim, M. Y. & Thong, K. L, 2012. Genetic diversity of Malaysian methicillin-resistant *Staphylococcus aureus* strains based on virulotypes, pulsed-field gel electrophoresis and PCR-RFLP of *coa* gene. 15th International Congress of Infectious Disease, Centara Grand & Bangkok

Convention Centre at Central World, Bangkok, Thailand, 13-16th June 2012 (Poster Presentation).

3. **Lim, K. T.,** Goering, R., Yasmin, A. H., Yasim, M. Y. M., & Thong, K. L. 2011. Enhanced discrimination of pandemic clone ST239- Methicillin-resistant *Staphylococcus aureus* in a tertiary hospital in Malaysia by *mec*-associated direct repeat unit and *spa* typing. The 5th Ditan International Conference of Infectious Disease, China National Convention Center, Beijing, China. 14-17th July 2011 (Poster Presentation) – Travel grant awarded
4. Thong, K. L., **Lim, K. T.,** Yasmin, A. H., & Yasim, M. Y. M. 2011. Distribution of virulence factors, *agr* groups, *spa* types and pulsotypes of *Staphylococcus aureus* isolates in Malaysia. 8th International Symposium on Antimicrobial Agents and Resistance, Seoul, Korea, 6-8th April 2011 (Poster Presentation).
5. **Lim, K. T.,** Yasmin, A. H., Yasim, M. Y. M., & Thong, K. L. 2010. Phenotypic and genetic characterization of methicillin-resistant *Staphylococcus aureus* isolates in Malaysia. 15th Biological Science Graduate Congress, University of Malaya, 15 – 17th December 20110 (Oral Presentation).
6. **Lim, K. T.,** Yasmin, A. H., Yasim, M. Y. M., & Thong, K. L. 2010. Prevalence of mupirocin resistance in methicillin resistant *Staphylococcus aureus* strains isolated from a Malaysian hospital. 1st AMDI-International Biohealth Science Conference (IBSC), Bayview Hotel, 28 – 30th Nov 2010 (Poster Presentation).
7. **Lim, K. T.,** Yasmin, A. H., Yasim, M. Y. M., & Thong, K. L. 2009. Distribution of SCC*mec* types and resistotypes of local methicillin resistant *Staphylococcus aureus* strains. International Congress of Malaysian Society for Microbiology 2009. Park Royal Hotel, Penang, 1 – 4th December 2009 (Poster Presentation).
8. **Lim, K. T.,** Yasmin, A. H., Yasim, M. Y. M., & Thong, K. L. 2009. Distribution of SCC*mec* types and resistotypes of local methicillin resistant *Staphylococcus aureus*

strains. 4th National Medical Microbiology Seminar, University of Putra Malaysia. 9th November 2009 (Poster Presentation).