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GENETIC AND PHENOTYPIC CHARACTERISATION OF CLINICAL METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN A MALAYSIAN HOSPITAL

Field of Study: Microbial Biotechnology

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the main bacterial pathogens responsible for a variety of nosocomial infections ranging from soft-tissue infections to bacteremia. Since most MRSA is often resistant to multiple antibiotics, this has raised a concern over the limited choice of antimicrobial-agents for treatment of life-threatening cases. The objectives of this study were to determine the antimicrobial resistance profiles, presence of resistance and virulence genes, to investigate the molecular epidemiology of MRSA and their evolution over a six-year period. The antibiograms of 188 MRSA strains isolated from UMMC were analyzed by disk-diffusion test and minimum inhibitory concentrations. The *agr* and SCC*mec* types, presence of resistance and virulence genes were determined by PCR, genetic diversity by PFGE, *coa*-RFLP, MLST, *spa* and *dru* typing and molecular evolution by MLST-*spa*-*dru* types. All the strains were sensitive to vancomycin. They showed high resistance (≥80%) towards ciprofloxacin, clindamycin, erythromycin and gentamicin. A significant increase (P<0.05) in resistance rates towards trimethoprim-sulfamethoxazole, netilmicin and tetracycline between 2003 and 2008 was observed. *blaZ* gene was detected in all strains whereas *ermA, aac(6')-aph(2"), tetM, ermC, tetK, ileS, 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. *blaZ, tetM, ermC, tetK, ileS2* and *mupA* genes were plasmid-encoded. Double mutations in *rpoB* gene were associated with high rifampicin-resistance while mutational change 461Leu/Lys in *fusA* gene was associated with high fusidic acid-resistance. The prevalence of *sea, sec* and *ica* among strains isolated in 2008 increased significantly (P<0.05) compared to 2003. *pvl* gene was detected in 2007 and 2008 strains. Three SCC*mec* types (SCC*mec* type III, 90%; SCC*mec* type IV, 9%; SCC*mec* V, 1%) and

three *agr* types (*agr* type-I, 97.5%; *agr* type-II, 1.2%; *agr* type-III, 0.6%) were observed. *coa*-RFLP, PFGE, MLST, *spa* and *dru* typing subtype the strains into 47 profiles, 85 PFPs, 10 MLST, 17 *spa* and 30 *dru* types, respectively. Some strains from six-years apart shared similar DNA profiles, indicating the persistence of a particular genotype. The predominant MLST type, ST239 (83.5%) was further distinguished to seven different *spa* and 26 different *dru* types, including 17 novel *dru* types. Maximum parsimony tree based on *dru* repeats revealed that 10 *dru* types (dt11am, dt13j, dt15n, dt13q, dt13n, dt13p, dt13f, dt13ao, dt12j, dt7v) shared similar MLST-*spa* type with dt13d, suggesting that they might have evolved from ST239-t037-dt13d. Clone ST239-t037-dt13g and 32 other MRSA clones being introduced in 2007-2008 had replaced ST239-t037-dt13d and nine MRSA clones present in 2003. In conclusion, the prevalence of resistance and virulence factors had increased over a six-year period. The association of resistance genes with mobile genetic elements possibly enhances the spread of resistant traits in MRSA. Correlation between DNA profiles (PFGE and *coa*-RFLP) and resistotypes was observed. ST239-t037-dt13d along with other MRSA clones in 2003 was replaced by ST239-t037-dt13g and other new emerging *spa* and *dru* types. The data from this study may act as a reference for monitoring mupirocin, rifampicin, fusidic acid and the prevalence of virulence among Malaysian MRSA strains over a longer period of time.

ABSTRAK

Methicillin-resistant *Staphylococcus aureus* (MRSA) merupakan salah sejenis bakteria utama yang bertanggungjawab untuk pelbagai jenis jangkitan 'nosocomial' termasuk jangkitan melibatkan tisu lembut dan bakteremia. Oleh kerana MRSA mempunyai daya ketahanan terhadap pelbagai antibiotik, ini menimbulkan kebimbangan terhadap pilihan antibiotik yang sedia-ada. Objektif kajian ini adalah untuk menentukan profil ketahanan antibiotik, kehadiran gen rintangan dan gen virulen, menyiasat molekular epidemiologi dan evolusi MRSA sepanjang tempoh enam tahun. Antibiogram 188 MRSA dijalankan melalui ujian cakera-resapan. *agr*, SCC*mec* dan kehadiran gen rintangan dan virulen ditentukan oleh PCR, genetik MRSA ditentukan melalui PFGE, *coa*-RFLP, MLST, *spa* dan *dru* serta evolusi MRSA ditentukan melalui MLST-*spa-dru*. Semua 'strain' bakteria adalah sensitif terhadap vancomycin dan mereka menunjukan kadar rintangan yang tinggi (≥ 80%) terhadap ciprofloxacin, clindamycin, erythromycin dan gentamicin. Peningkatan yang signifikan ($P < 0.05$) kepada kadar rintangan terhadap trimethoprim-sulfamehtoxazole, netilmicin dan tetracycline di antara tahun 2003 and 2008 diperolehi. Gen *blaZ* hadir dalam semua 'strain' manakala *ermA, aac(6')-aph(2"), tetM, ermC, tetK, ileS, msrA* and *mupA* hadir dalam 157(84%), 156(83%), 92(49%), 40(21%), 39(21%), 10(5%), 4(2%) and 2(1%) 'strain'. Gen *blaZ, tetM, ermC, tetK, ileS2* dan *mupA* adalah dikod pada plasmid. Mutasi berganda pada gen *rpoB* sering dikaitkan dengan kadar rintangan rifampicin yang tinggi manakala perubahan 461Leu/Lys pada gen *fusA* dikaitkan dengan kadar ringtangan fusidic asid yang tinggi. Kadar kehadiran gen *sea, sec* dan *ica* pada strain tahun 2008 telah meningkat secara mendadak ($P < 0.05$) berbanding dengan 'strain' tahun 2003. Gen *pvl* hadir dalam 'strain' yang diperolehi pada tahun 2007 dan 2008. Tiga jenis SCC*mec* (SCC*mec* taip III, 90%; SCC*mec* taip IV, 9%; SCC*mec* taip V, 1%) dan tiga

jenis *agr* (*agr* taip-I, 97.5%; *agr* taip-II, 1.2%; *agr* taip III, 0.6%) telah diperolehi. ‘Subtyping’ *coa*-RFLP, PFGE, MLST, *spa* dan *dru* pada 188 ‘strain’ mewujudkan 47 profil, 85 PFPs, 17 *spa* dan 30 *dru*. Klon dominan MLST ST239 (83.5%) boleh dibahagikan kepada tujuh jenis *spa* dan 26 jenis *dru* yang berlainan termasuk 17 *dru* yang novel. ‘Maksimum parsimony tree’ yang berdasarkan *dru* menunjukkan 10 taip *dru* (dt11am, dt13j, dt15n, dt13q, dt13n, dt13p, dt13f, dt13ao, dt12j dan dt7v) mempunyai taip MLST-*spa* yang sama dengan dt13d, mencadangkan bahawa mereka mungkin berkembang dari ST239-t037-dt13d. Klon ST239-t037-dt13g dan 32 jenis MRSA klon yang diperkenalkan pada tahun 2007 dan 2008 telah mengantikan ST239-t037-dt13d dan sembilan klon MRSA yang hadir pada tahun 2003. Sebagai kesimpulan, prevalen faktor rintangan dan virulen telah meningkat sepanjang tempoh enam tahun. Hubungan gen rintangan dengan unsur genetik mudah alih mungkin meningkatkan penyebaran ciri-ciri penahanan MRSA. Korelasi antara profil DNA (PFGE dan *coa*-RFLP) dan ‘resistotypes’ diperhatikan. ST239-t037-dt13d besama dengan klon MRSA pada tahun 2003 telah digantikan oleh ST239-t037-dt13g dan *spa-dru* yang baru. Data dari kajian ini boleh dijadikan rujukan untuk memantau kadar rintangan mupirocin, rifampicin, fusidic acid and virulen di kalangan ‘strain’ MRSA Malaysia.

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List of Symbols and Abbreviations

β	-	beta
bp	-	base pair
CA-MRSA	-	community-acquired methicillin resistant *Staphylococcus aureus*
CN	-	gentamicin
°C	-	Degree Celsius
CIP	-	ciprofloxacin
DA	-	clindamycin
ddH_2O	-	double deonised water
dru	-	direct repeat unit
ERY	-	erythromycin
FD	-	fusidic acid
g	-	gravity
g/l	-	gram per litre
HA-MRSA	-	hospital acquired methicillin-resistant *Staphylococcus aureus*
kDA	-	kilo-Dalton
kV	-	kilovolts
LZD	-	linezolid
M	-	molarity
min	-	minute
mg/ml	-	milligram per millilitre
ml	-	millilitre
MLST	-	multilocus sequence typing
mm	-	millimetre
mM	-	milimolar
MRSA	-	methicillin-resistant *Staphylococcus aureus*
MUP	-	mupirocin

NaCl	-	sodium chloride
ng	-	nanogram
NET	-	netilmicin
Ω	-	ohm
PCR	-	polymerase chain reactions
PFGE	-	pulsed-field gel electrophoresis
Pg	-	pictogram
RF	-	rifampicin
RFLP	-	restriction fragment length polymorphism
rpm	-	revolutions per minute
%	-	percent
sec	-	second
SXT	-	trimethoprim-sulfamethoxazole
TEC	-	teicoplanin
TET	-	tetracycline
U	-	unit
µF	-	microfarad
µg	-	microgram
µM	-	micomolar
µl	-	microlitre
UV	-	ultraviolet
V	-	volt
VA	-	vancomycin
V/cm	-	volt per centimetre
VISA	-	vancomycin intermediate *Staphylococcus aureus*
VRSA	-	vancomycin resistant *Staphylococcus aureus*

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