lanes in which limited numbers of PCR products were produced were not included in the analysis.

Normalizations were done on the positions of bands on each gel by using the 1kb ladder from 300bp to 5000bp as an external reference standard. Multiple gels were then compared using the normalization with the same set of external standards. DNA fragments less than 300bp long were tended to be indistinct and not used in analyses. Dendrograms were constructed by using the uweighted pair group method with arithmetic means (UPGMA) tree building method. The dendrograms were used to evaluate the strain diversity of the *E. coli* from each sample. The most relevant clusters in the dendograms were determined by calculating the similarity cutoff value that produced the highest point-bisectional correlation.

CHAPTER 4: RESULTS

4.1 Sampling and isolation of total coliform and *E. coli*

Collections of water samples for the study were done from 15th May 2008 until 20th May 2008 in areas around Bachok, Kelantan. Water samples were collected from 50 sites around Bachok, Kelantan. Detailed sample site coordinates and description of 50 samples collected from various water sources are listed in Table 4.1a (Appendixes). The salinity and other particulars were obtained for 15 samples only (Table 4.1b, Appendixes).

For isolation of *E. coli*, Chromocult Coliform agar (CCA) and Eosin Methylene Blue agar (EMB) were used as differential and selective plating media. Dark blue to violet colonies on CCA plates (Figure 4.1a) were considered to be presumptive *E. coli* isolates. Colonies with greenish metallic sheen (Figure 4.1b) on EMB agar plates were considered to be *E. coli*.

Total coliforms were recovered in all the 50 samples with an average density of 1×10^8 cfu/100mL. While *E. coli* were recovered in only 44 samples (average density of 1×10^6 cfu/100mL) and were not present in 6 coastal water samples. Highest concentrations of *E. coli* (up to 5.2×10^8 cfu/100mL) were observed for river water samples collected near the jetty area and lowest in coastal water samples (1×10^2 cfu/100mL) (Table 4.1c).

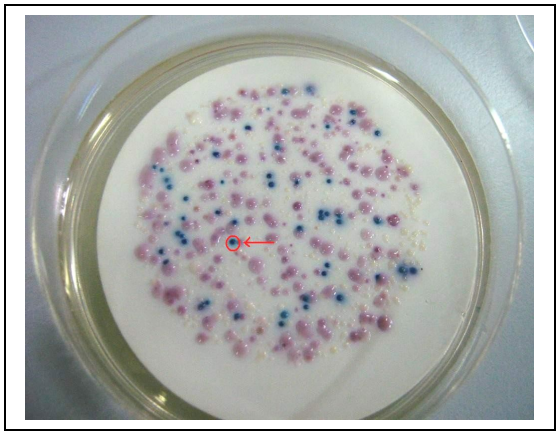


Fig. 4.1a *E. coli* produce dark blue to violet colonies on CCA plates (arrow). Total coliform produce salmon to red colonies.

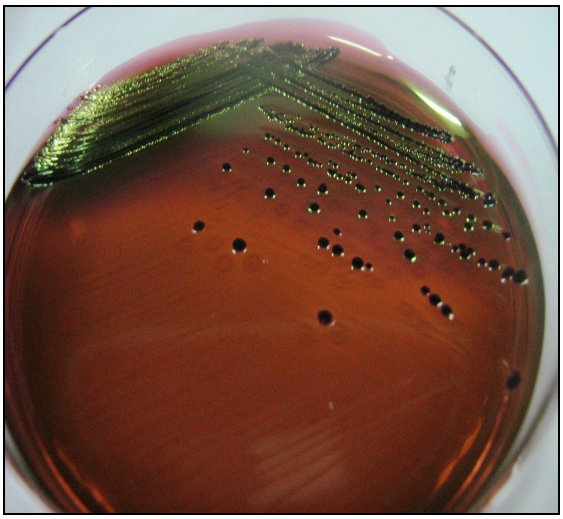


Figure 4.1b *E. coli* produce greenish metallic sheen colonies on EMB agar plates.

Table 4.1c Colony count of total coliform and *E. coli* for fifty samples at two different holding time

Sample	Source	Colony counts for Isolation within 6hours of sampling		Colony counts for isolation after 1 week of sampling	
		Total coliform (cfu/100ml)	*E. coli* (cfu/100ml)	Total coliform (cfu/100ml)	*E. coli* (cfu/100ml)
1	Kg Pak Mayong jetty	4.1×10^{10}	3.7×10^{6}	1.9×10^{8}	3.3×10^{3}
2	Near Kg Pak Mayong jetty	5.5×10^{10}	5.8×10^{6}	5.0×10^{8}	1.8×10^{3}
3	Kg Siam river	5.0×10^{8}	9.8×10^{6}	8.2×10^{7}	1.6×10^{4}
4	Kg Lembah river	4.2×10^{8}	1.3×10^{7}	3.3×10^{6}	5.3×10^{5}
5	Kg Ajin river	2.2×10^{8}	3.6×10^{6}	6.4×10^{6}	4.0×10^{4}
6	Kg Ajin river	2.5×10^{8}	4.9×10^{6}	4.1×10^{6}	8.2×10^{3}
7	Kg Cerang Ruku	2.3×10^{8}	3.0×10^{6}	3.5×10^{5}	3.9×10^{2}
8	Sg Cerang Ruku	8.1×10^{8}	8.7×10^{6}	1.3×10^{6}	7.1×10^{4}
9	Near the Aquaculture Cage	6.0×10^{8}	3.6×10^{6}	6.5×10^{6}	2.0×10^{4}
10	Near the Aquaculture Cage	4.6×10^{8}	6.1×10^{6}	3.5×10^{6}	1.3×10^{4}
11	Canal water	2.4×10^{8}	2.1×10^{6}	1.7×10^{6}	3.5×10^{4}
12	Canal water	6.1×10^{8}	3.6×10^{6}	9.8×10^{5}	7.1×10^{2}
13	Canal water	5.5×10^{8}	2.7×10^{6}	2.2×10^{6}	1.5×10^{4}
14	Mouth of canal	4.0×10^{8}	2.8×10^{6}	1.3×10^{6}	4.6×10^{4}
15	Opposite Kg Mayong jetty	3.6×10^{10}	3.3×10^{6}	2.9×10^{7}	7.4×10^{3}
16	River in mangrove (muddy)	6.2×10^{8}	1.5×10^{7}	5.0×10^{6}	5.0×10^{5}
17	Pantai irama river mouth	2.8×10^{8}	1.2×10^{7}	6.2×10^{6}	2.2×10^{5}
18	Kuala Melawi river mouth	3.3×10^{8}	5.4×10^{6}	2.0×10^{6}	4.2×10^{4}
19	Kg Tok Bali	1.2×10^{5}	1.7×10^{3}	5.5×10^{3}	5.5×10^{1}
20	Kuala Semerak	1.2×10^{2}	-	6.2×10^{1}	-
21	Kg Dalam	5.3×10^{2}	-	4.6×10^{2}	-
22	Tok Bali	1.0×10^{2}	-	1.3×10^{1}	-
23	Sg Gali (jetty)	1.9×10^{8}	9.1×10^{6}	$2.5x\ 10^{6}$	5.4×10^{3}
24	Sg Gali (near river mouth)	2.2×10^{8}	8.0×10^{5}	7.2×10^{6}	3.0×10^{3}
25	Opposite Kg Ajin river	1.6×10^{10}	2.5×10^{8}	3.9×10^{7}	8.8×10^{5}
26	Opposite Kg Ajin river	3.8×10^{10}	1.9×10^{7}	2.6×10^{8}	2.2×10^{5}
27	Temasu Telok Lubok	4.3×10^{10}	1.5×10^{8}	1.5×10^{7}	6.7×10^{4}

28	Sg Tasek	6.5×10^{9}	8.3×10^{7}	3.3×10^{7}	5.1×10^{5}
29	Tali air	5.3×10^{9}	2.4×10^{7}	4.1×10^{7}	5.8×10^{5}
30	Sg Tasek (mid river)	4.1×10^{10}	7.4×10^{7}	1.5×10^{8}	4.5×10^{4}
31	upper Sg Tasek	4.4×10^{8}	2.6×10^{6}	3.7×10^{6}	4.5×10^{4}
32	Near Bkt Awang	3.7×10^{8}	4.8×10^{6}	2.1×10^{6}	2.6×10^{4}
33	Near Kg Tok Luchar	1.5×10^{8}	5.2×10^{7}	8.2×10^{6}	8.1×10^{5}
34	Between Bachok & Sudara	3.4×10^{9}	3.4×10^{7}	1.2×10^{7}	1.0×10^{5}
35	Sg Rekang	2.0×10^{8}	1.6×10^{7}	5.6×10^{6}	7.1×10^{4}
36	Sg Dua (IOES)	1.6×10^{8}	3.6×10^{6}	1.1×10^{6}	2.3×10^{4}
37	Sg Limau Nipis	5.8×10^{8}	2.3×10^{6}	$5.1x\ 10^{6}$	2.4×10^{4}
38	Jeram Pasu	3.2×10^{8}	2.8×10^{6}	2.0×10^{5}	4.4×10^{3}
39	Jeram Linang	3.8×10^{8}	1.5×10^{6}	3.5×10^{6}	6.0×10^{4}
40	Kg Rekang	1.5×10^{8}	1.2×10^{7}	9.6×10^{5}	5.3×10^{5}
41	Kuala Rekang	6.6×10^{3}	-	2.7×10^{2}	-
42	Kg Sg Dua	5.1×10^{4}	-	4.5×10^{3}	-
43	Tok Bali	5.3×10^{3}	-	7.6×10^{1}	-
44	Muara	5.6×10^{8}	8.5×10^{6}	2.9×10^{6}	3.2×10^{4}
45	Hutan Lipur Bkt Bakar Semerak	$1.9x\ 10^{6}$	1.8×10^{4}	$2.0x\ 10^{4}$	4.5×10^{2}
46	Sg Pengkalan Datu	2.6×10^{8}	5.5×10^{6}	2.4×10^{6}	1.1×10^{4}
47	Kuala Senok	1.6×10^{7}	2.1×10^{4}	1.2×10^{6}	-
48	Sg Senok	8.0×10^{6}	1.9×10^{4}	4.1×10^{4}	2.9×10^{2}
49	Pantai Sabak	2.1×10^{6}	1.2×10^{4}	2.2×10^{4}	-
50	Kuala Kemasin	7.3×10^{7}	2.4×10^{5}	3.0×10^{5}	-
	Highest Colony Count				
	Lowest Colony Count				

4.2 Correlation between total coliform and *E. coli*

Correlation coefficients were calculated to determine whether significant relationships existed between total coliform and *E. coli* concentration. Pearson correlations were used to determine relationships between the colony counts. The correlation coefficient for the regression comparing the count of total coliform and *E. coli* was 0.767 (Table 4.2a). According to the results obtained, the presence of total coliform was significantly correlated to *E. coli* ($p<0.05$) (Figure 4.2a). Thus, total coliform were proven to be a good indicator of microbiological water quality in this study. Many authors have reported the usefulness of coliform as indicator for the detection of waterborne disease outbreaks (Rompre *et al*., 2002).

Table 4.2a Pearson correlation of *E. coli* and total coliform.

		Total coliform	*E. coli*
Total coliform	Pearson Correlation	1	.767(**)
	Sig. (2-tailed)		.000
	No. of samples	50	44
E. coli	Pearson Correlation	.767(**)	1
	Sig. (2-tailed)	.000	
	No. of samples	44	44

** Correlation is significant at the 0.01 level (2-tailed)

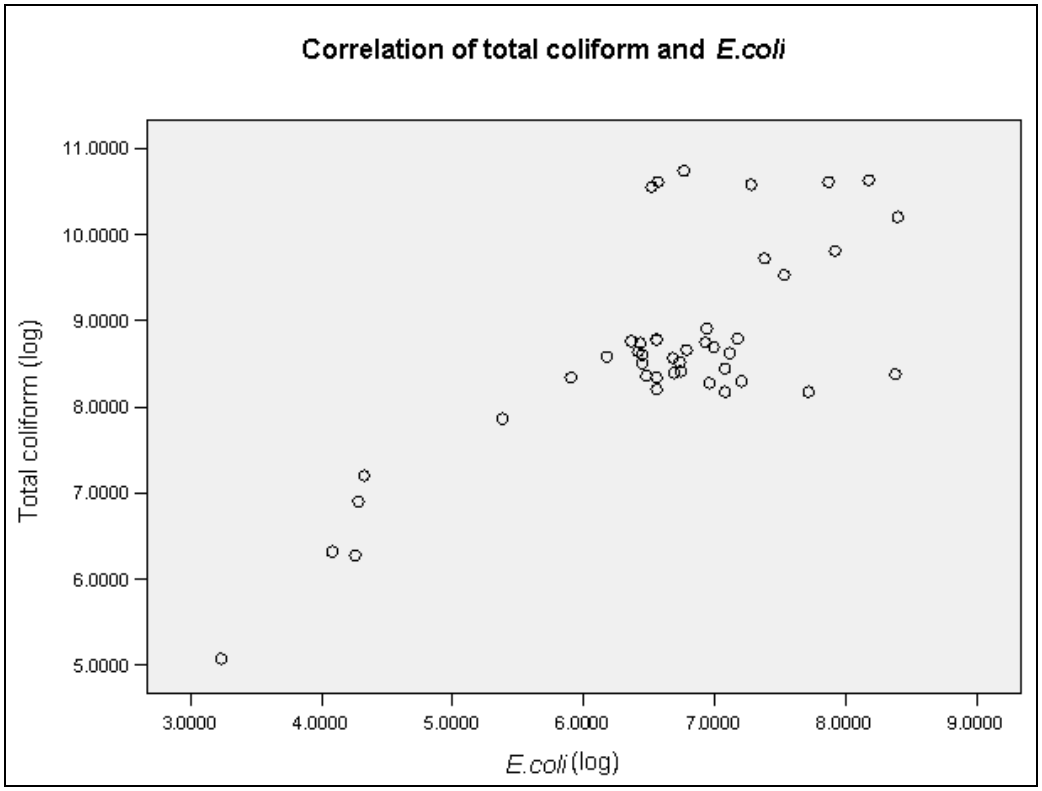


Fig. 4.2a Correlation of total coliform and *E. coli* ($p<0.05$)

4.2.1 Comparison of total coliform and *E. coli* colony counts isolated at different holding time

The comparison of colony count of total coliform and *E. coli* at different holding time shows an obvious drop of microbial population in samples analyzed after 1 week of collection (Figure 4.2b). Initial counts of total coliforms from the samples ranged from 1×10^2 cfu/100mL to 1×10^{10} cfu/100mL and *E. coli* from 1×10^2 cfu/100mL to 1×10^6 cfu/100mL. After one week of storage at 4°C the colony counts dropped to an average density of 1×10^6 cfu/100mL for total coliform and 1×10^4 cfu/100mL for *E. coli*. An average loss of microbial population after 1 week of holding period was 40% for both total coliform and *E. coli*.

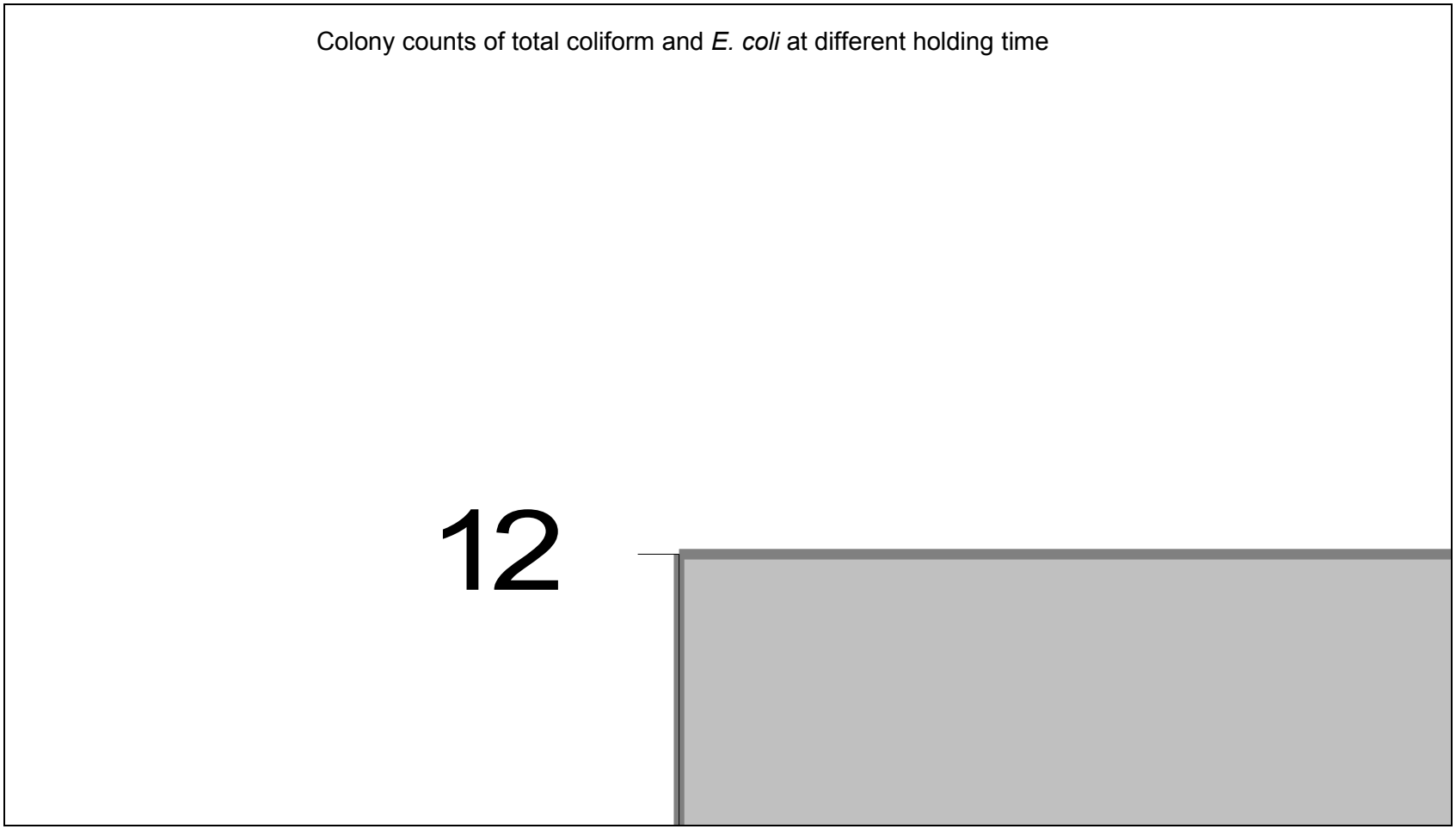


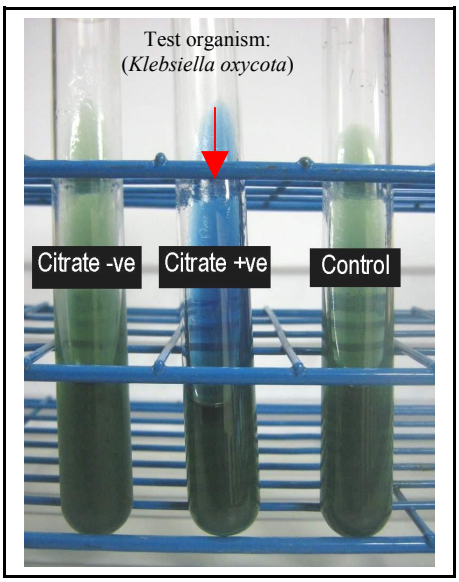
Fig. 4.2b Comparison of colony count of total coliform and *E. coli* isolated at different holding time

4.3 Identification and confirmation of *E. coli* isolates

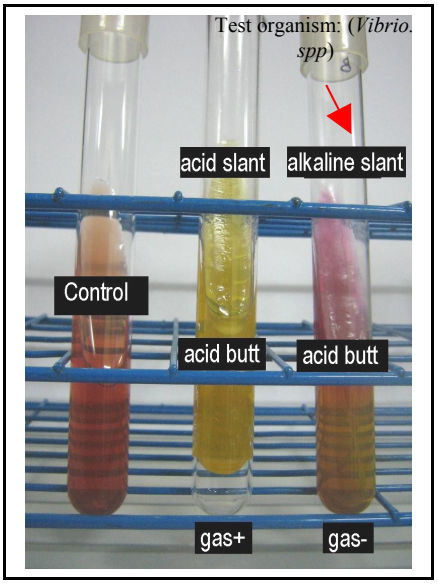
4.3.1 Biochemical tests

The *E. coli* isolates were screened by biochemical tests according to IMViC pattern (Indole production, Methyl Red (MR) test, Voges-Proskauer (VP) test, citrate utilization on Simmon's citrate medium) and utilization of carbohydrate on TSI agar as described by Edwards and Ewing (1972).

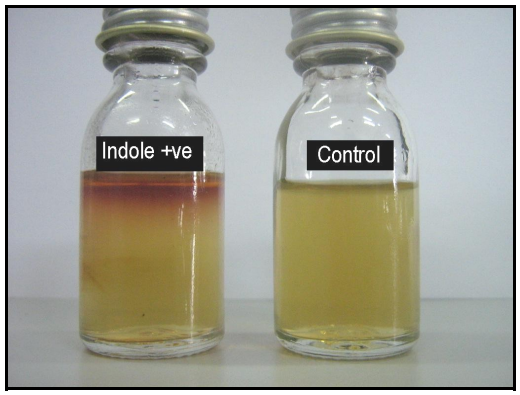
From the 44 non repeat *E. coli* isolates, 39 isolates were positive by IMViC methods. The biochemical behavior of the isolates positive for *E. coli* revealed that they all were positive for methyl red and indole production while negative for VP test and citrate utilization (Figure 4.3a to Figure 4.3d). However among the 39 isolates, 2 isolates were citrate positive (EC6 and EC2). In TSI tubes, all the isolates produce an acid butt, an acid slant, no production of H_2S and produced gas.



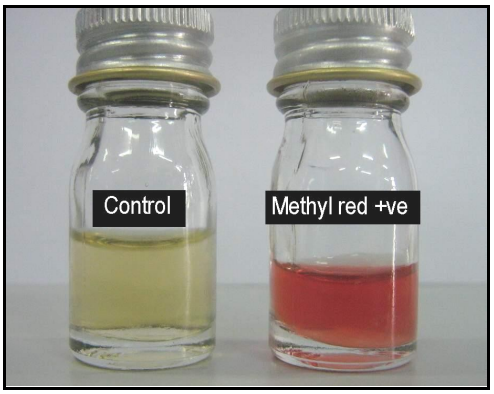
4.3a



4.3b



4.3c



4.3d

Fig. 4.3a *E. coli* negative for citrate utilization

4.3b *E. coli* in TSI tubes produce an acid butt, an acid slant and gas

4.3c *E. coli* was positive for indole production

4.3d *E. coli* was positive for methyl red test

4.3.2 Confirmation by using API 20E

The API-20E test strips contained 20 separate test compartments which are dehydrated. A bacterial suspension was used to rehydrate the wells. A profile number was determined from the sequence of + and – test results and checked in a software for a correlation between the numbers and bacterial species.

E. coli strains that were positive based on IMVic, tested using API 20E kit and out of 39 non repeat strains, 29 strains gave positive results. The results are as shown in Table 4.3a. Other tested isolates showed positive result for the detection of *Klebsiella* spp. However, these isolates showed the same characteristic as *E. coli* in biochemical tests.

Table 4.3a Results for API 20E test

Samples	Code	Identity	% of Identity
EC45 (a)	5144552	Escherichia coli	99.9%
EC23(c)	5104572	Escherichia coli	98.4%
EC1 (c)	5144572	Escherichia coli	97.3%
EC18 (d)	5104172	Escherichia coli I	81.0%
EC 15 (b)	5035773	*Klebsiella pneumoniae*	97.7%
EC 47 (a)	5235773	Klebsiella pneumoniae	97.6%
EC 25 (b)	5275773	*Klebsiella oxycota*	98.4%
EC 9 (c)	5275773	*Klebsiella oxycota*	98.4%

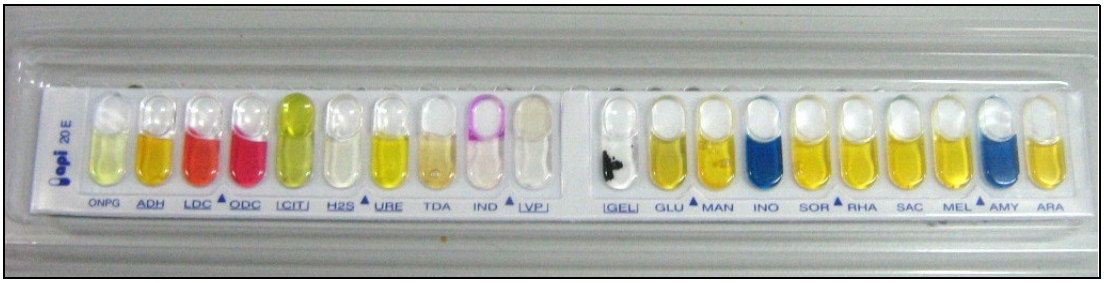


Fig. 4.3e Representative result for identification of *E. coli* using API 20E.

4.3.3 Optimization of monoplex PCR using lower primer concentration

Even though the conditions used for the monoplex PCR assays were well optimized by a previous student, unspecific bands and intense primer dimer were observed in the gel obtained (Figure 4.3f). Thus, concentration of primer was reduced to obtain a better result. Primer concentration was adjusted from 0.22µM to 0.1µM.

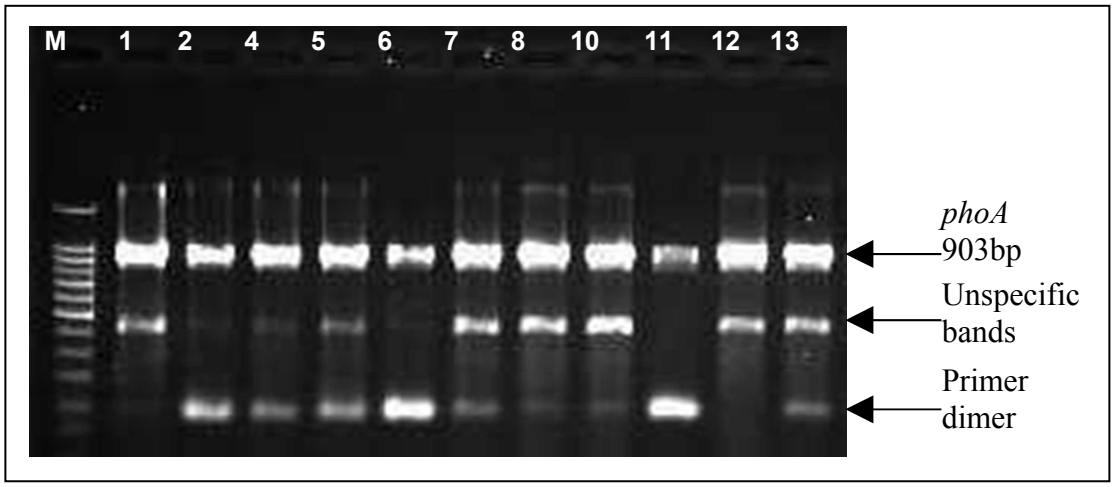


Fig. 4.3f Representative gel of monoplex PCR for *phoA* gene detection of 11 presumptive *E. coli* isolates.

Lane M, 100bp DNA ladder; Lane 1-13, *E. coli* isolates from respective samples.

4.3.4 Confirmation by using monoplex PCR assay

Monoplex PCR assay for detecting the *E. coli* housekeeping gene (*phoA*) was performed on all the screened presumptive *E. coli* as a final confirmation step using an

optimized condition (Figure 4.3g). All the 29 *E. coli* strains which were positive for API test, also showed positive results for the presence of *phoA* gene.

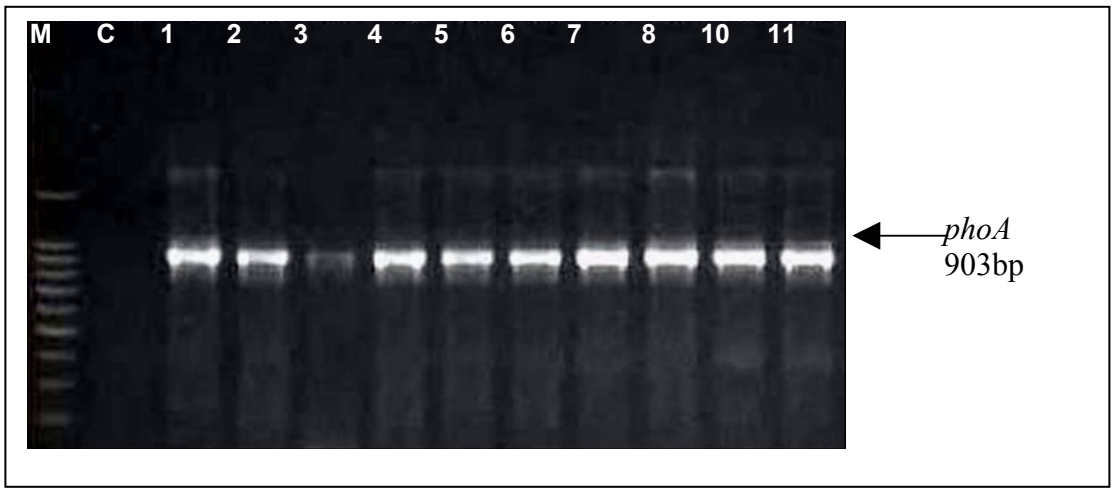


Fig. 4.3g Representative gel of monoplex PCR for *phoA* gene detection of 10 presumptive *E. coli* isolates.

Lane M, 100bp DNA ladder; Lane C, negative control; Lane 1-11, *E. coli* isolates from respective samples.

Table 4.3b Summary of *E. coli* recovery from different confirmation stages

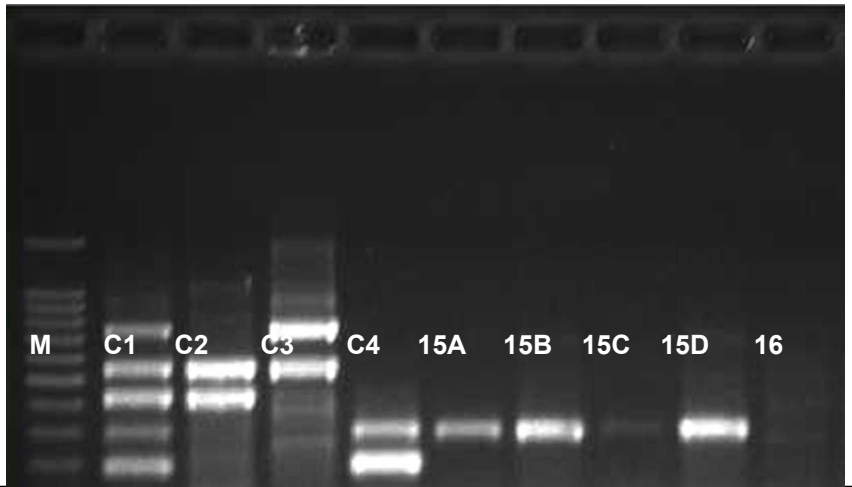
Sample	Biochemical test						API 20E	PCR (*phoA* gene)
	TSI	Indole	Citrate	Methyl red	Voges	Final Biochemical tests results		
EC1	acid/acid g+	+	-	+	-	+	+	+
EC2	acid/acid g+	+	+	+	-	-	+	+
EC3	acid/acid g+	+	-	+	-	+	-	-
EC4	acid/acid g+	+	-	+	-	+	+	+
EC5	acid/acid g+	+	-	+	-	+	+	+
EC6	acid/acid g+	+	+	+	-	-	+	+
EC7	acid/acid g+	+	-	+	-	+	+	+
EC8	acid/acid g+	+	-	+	-	+	+	+
EC9	acid/acid g+	-	+	+	+	-	-	-
EC10	acid/acid g+	+	-	+	-	+	+	+
EC11	acid/acid g+	+	-	+	-	+	+	+
EC12	acid/acid g+	+	-	+	-	+	+	+
EC13	acid/acid g+	+	-	+	-	+	+	+
EC14	acid/acid g+	+	-	+	-	+	+	+
EC15	acid/acid g+	+	-	+	-	+	+	+
EC16	acid/acid g+	+	-	+	-	+	+	+
EC17	acid/acid g+	-	-	+	+	-	-	-
EC18	acid/acid g+	+	-	+	-	+	+	+
EC19	acid/alkaline	+	-	+	-	-	-	-
EC20	acid/alkaline	+	-	+	-	-	-	-
EC21	acid/acid g+	+	-	+	-	+	-	-
EC22	acid/acid g+	+	-	+	-	+	-	-
EC23	acid/acid g+	+	-	+	-	+	+	+
EC24	acid/acid g+	+	-	+	-	+	+	+
EC25	acid/acid g+	+	-	+	-	+	-	-
EC26	acid/acid g+	+	-	+	-	+	-	-
EC27	acid/acid g+	+	-	+	-	+	-	-

EC28	acid/acid g+	+	-	+	-	+	-	-
EC29	acid/acid g+	+	-	+	-	+	+	+
EC30	acid/acid g+	+	-	+	-	+	+	+
EC31	acid/acid g+	+	-	+	-	+	+	+
EC32	acid/acid g+	+	-	+	-	+	+	+
EC33	acid/acid g+	+	-	+	-	+	+	+
EC34	acid/acid	+	+	+	-	-	-	-
EC35	acid/acid g+	+	-	+	-	+	-	-
EC36	acid/acid g+	+	-	+	-	+	+	+
EC37	acid/acid g+	+	-	+	-	+	-	-
EC38	acid/acid g+	+	-	+	-	+	+	+
EC39	acid/acid g+	+	-	+	-	+	+	+
EC40	acid/acid g+	+	-	+	-	+	-	-
EC41	acid/acid g+	+	+	+	+	-	-	-
EC42	acid/acid g+	+	-	+	-	+	-	-
EC43	acid/acid g+	+	-	+	-	+	-	-
EC44	acid/acid g+	+	-	+	-	+	+	+
EC45	acid/acid g+	+	-	+	-	+	+	+
EC46	acid/acid g+	+	-	+	-	+	+	+
EC47	acid/alkaline	+	-	+	+	-	-	-
EC48	acid/acid g+	+	-	+	-	+	+	+
EC49	acid/acid	+	-	+	+	-	-	-
EC50	acid/acid g+	-	+	+	-	-	-	-

4.4 Virulence gene detection

The multiplex PCR assay was designed to detect six virulence genes in pathogenic *E. coli* by five sets of primers (ST1, LT1, LT2, VT and AE). The assay was validated by testing positive control strains and was performed on confirmed *E. coli* strains. Bacterial strains used as positive control strains were *E. coli* SA53, known LT2 and VT positive isolate, *E. coli* ATCC 35401, known LT1 and ST positive isolate and *E. coli* 0157, known VT and *eaeA* positive isolate (Kong *et al*, 1999).

In this study, *E. coli* positive for the LT1 gene which produces LT (heat-labile) toxin was detected in 1 (EC15) of the 29 strains (Figure 4.4a). *E. coli* that produces this toxin has been reported as enterotoxigenic *E. coli* (ETEC) which is an important cause of diarrhea in infants and travellers in underdeveloped countries or regions of poor sanitation.



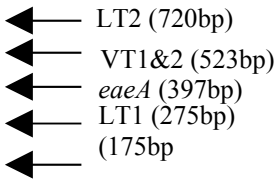


Fig. 4.4a Representative gel showing multiplex PCR using 5 sets of primers.

Lane M, 100bp DNA ladder; Lane C1, positive control (DNA mixture of all three reference strains); Lane C2, *E. coli* 0157; Lane C3, *E. coli* SA53; Lane C4, *E. coli* ATCC 35401; Lane 15A, 15B, 15C & 15D, EC15; Lane 16, EC16.

4.4.1 Detection of *E. coli* 0157:H7 by selective plating

Sorbitol MacConkey agar, selective medium for isolation and differentiation of serotype 0157:H7 was used for the easy detection of the pathogenic strain of *E. coli* 0157:H7 even though the multiplex PCR includes the detection of this pathogenic strain. The serotype 0157:H7 ferments sorbitol slowly or not at all, while other *E. coli* strains ferment sorbitol. The colourless colonies produce by *E. coli* 0157:H7 distinguishes these strains from normal *E. coli* that produces pink colonies. In this study, the results showed that none of the *E. coli* isolates showed positive result for *E. coli* 0157:H7 as this strain was not present in the studied isolates which also agree to the result obtained by multiplex PCR.

4.5 Antimicrobial susceptibility test of *E. coli* strains

On the basis of antibiotic susceptibility, only the ETEC strain was multiple resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole (Figure 4.5a). The rest of the 28 *E. coli* strains were susceptible to all tested antibiotics.

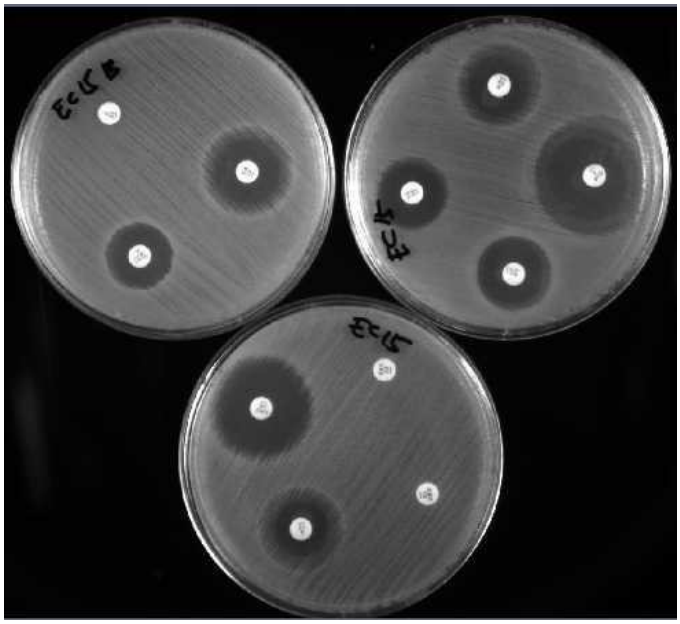


Fig. 4.5a Antimicrobial susceptibility test plates of sample 15 which was resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole.

4.6 Genetic diversity of *E. coli* using PCR based subtyping

4.6.1 Optimization of REP-PCR using different primers

The optimization of REP-PCR was carried out using four different primers to identify primer that gives discernible banding patterns for the *E. coli* strains. Out of the 4 primers, only primer REP1R(b) was the most useful as multiple bands were generated.

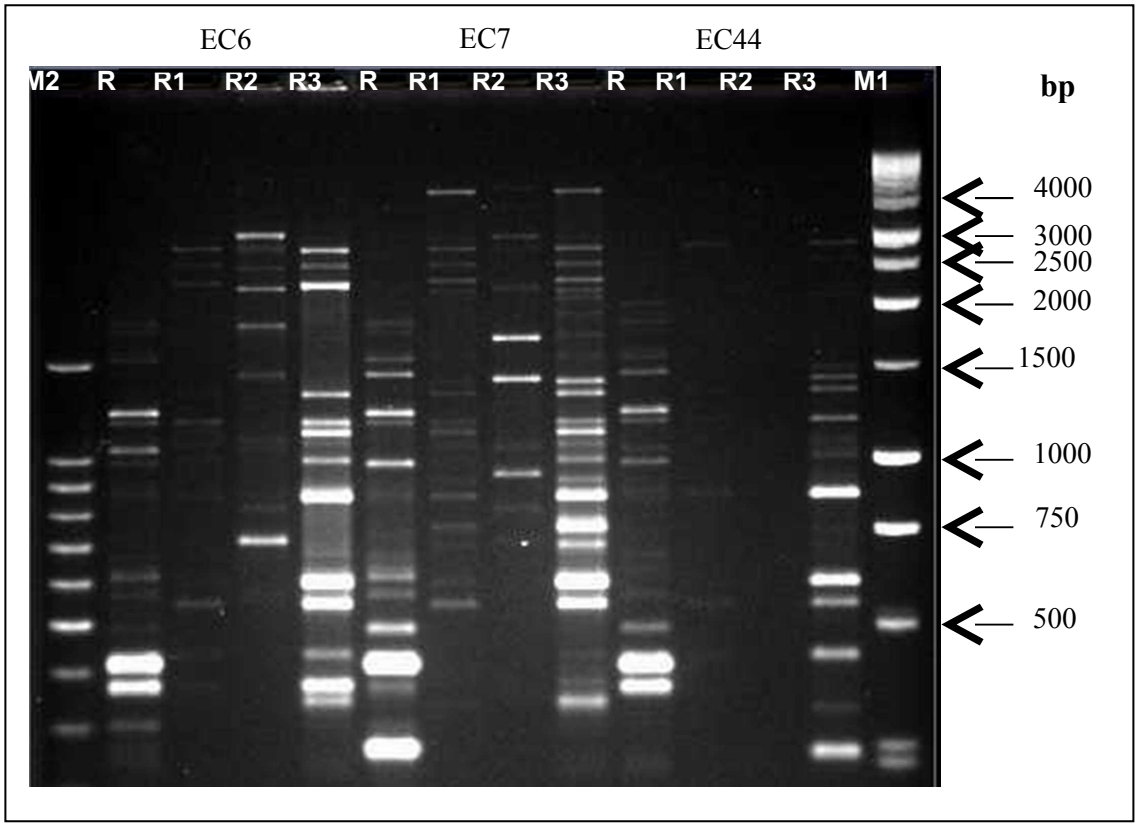


Fig. 4.6a Representative gel showing REP-PCR for three isolates (EC6, EC7, EC44) using four different types of primers. Lane M1, 1kb DNA ladder; Lane M2, 100bp DNA ladder; Lane R, primer REP; Lane R1, primer REP1R(a); Lane R2, primer REP2I; Lane R3, primer REP1R(b).

4.6.2 REP-PCR of isolates

The analysis of genomic diversity of 29 *E. coli* isolates by REP-PCR generated 27 patterns (F=0.26-1.0). The REP-PCR profiles were reproducible and the multiple DNA fingerprints showed that the *E. coli* isolates were genetically diverse. The REP-PCR of genomic DNA generated multiple DNA fragments in sizes ranging from 300bp to 4kb. Based on the results, each isolate produced REP profiles consisted of 10 to 20 visible bands. All the *E. coli* strains tested in REP-PCR assay produced two common DNA bands in the size of 550bp and 600bp. While, amplification bands of 850bp and 1.4kb were common to the REP- PCR fingerprints of majority of the *E. coli* isolates. A typical banding pattern of *E. coli* strains from different samples is shown in Fig. 4.6b. *E. coli* isolated from nearby sampling locations exhibited highly similar REP-PCR patterns.

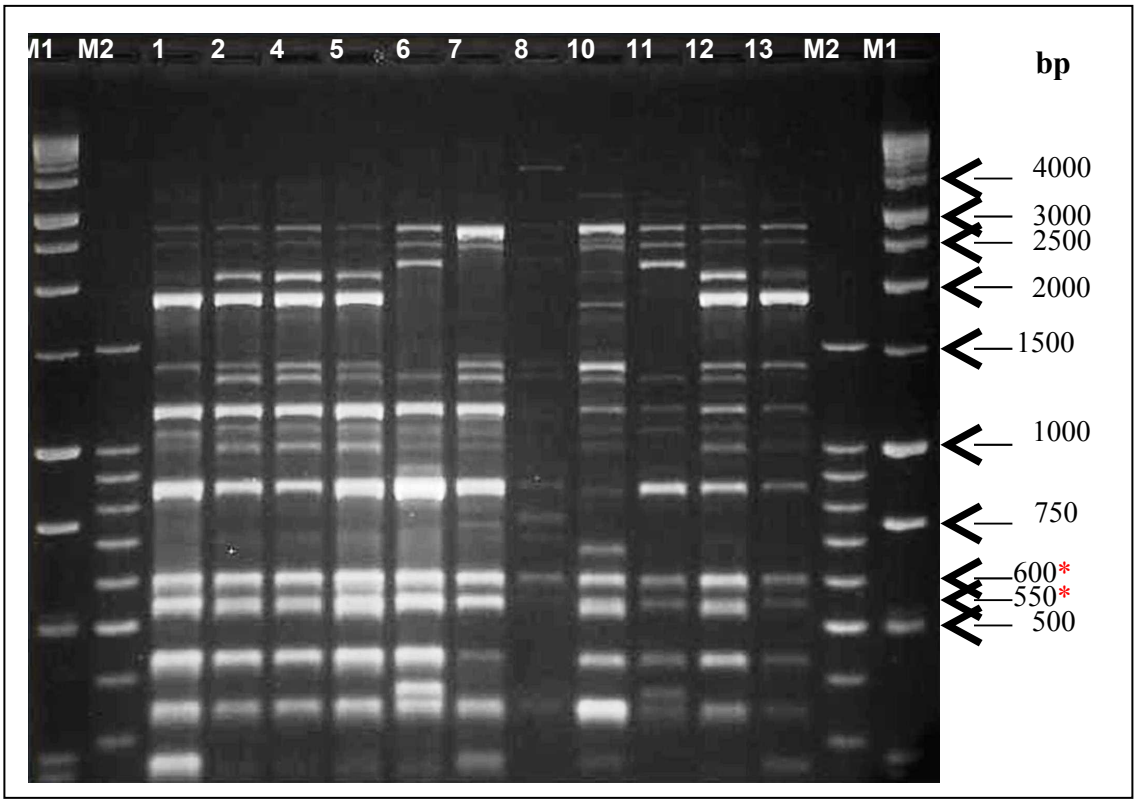


Fig. 4.6b Representative DNA fingerprints of 11 *E. coli* isolates generated by REP1R(b) primer. Lane M1: 1kb DNA ladder; Lane M2: 100bp DNA ladder; Lane 1-13: *E. coli* isolates from respective samples. The consistent bands at 550bp and 600bp (marked with red asterisk) were observed in all the *E. coli* strains.

4.6.3 Cluster analysis of *E. coli* isolates

The cluster analysis of the DNA fingerprints using a similarity coefficient of 80% showed 4 separate clusters (A, B, C and D) (Fig. 4.6c). Cluster A which was the major cluster was subdivided into two clusters (A1 and A2). Cluster A1 consisted of 4 isolates from jeram waters and river waters. Cluster A2 includes isolates collected around Kg Pak Mayong. The isolates of cluster A were from locations where the waterways were interconnected. Thus, clustering of these isolates was probably due to

the geographical locations which are interconnected. Kon *et al*. (2007) reported that different clusters of strains from the same sampling hole were closely related due to deposition of *E. coli* from a single source.

The ETEC strain (EC15) was clustered in cluster A as this cluster consisted of isolates retrieved around the area of jetty where the ETEC strain was detected. This could be the reason for the ETEC strain for harboring a similar fingerprint profile with cluster A isolates. Furthermore, isolates from the same water source were clustered together such as canal water samples and lake water samples in cluster D and cluster A. This indicates that there are similarities in the fingerprint pattern of strains from the same water sources. Two isolates from different places, Sg. Tok Bali (EC44) and Sg. Senok (EC48) were indistinguishable as they harbor the same fingerprint patterns. Isolates from Kg. Lembah river (EC4) and Kg Pak Mayong Jetty (EC2) also harbor the same fingerprint patterns. Isolates from irrigation water, Mangrove River and Sg.Tasek appeared in separate clusters. These isolates had their unique fingerprint patterns which differed from the others. Overall, analysis of the dendrogram shows that the rep-PCR system successfully distinguished among the isolates.

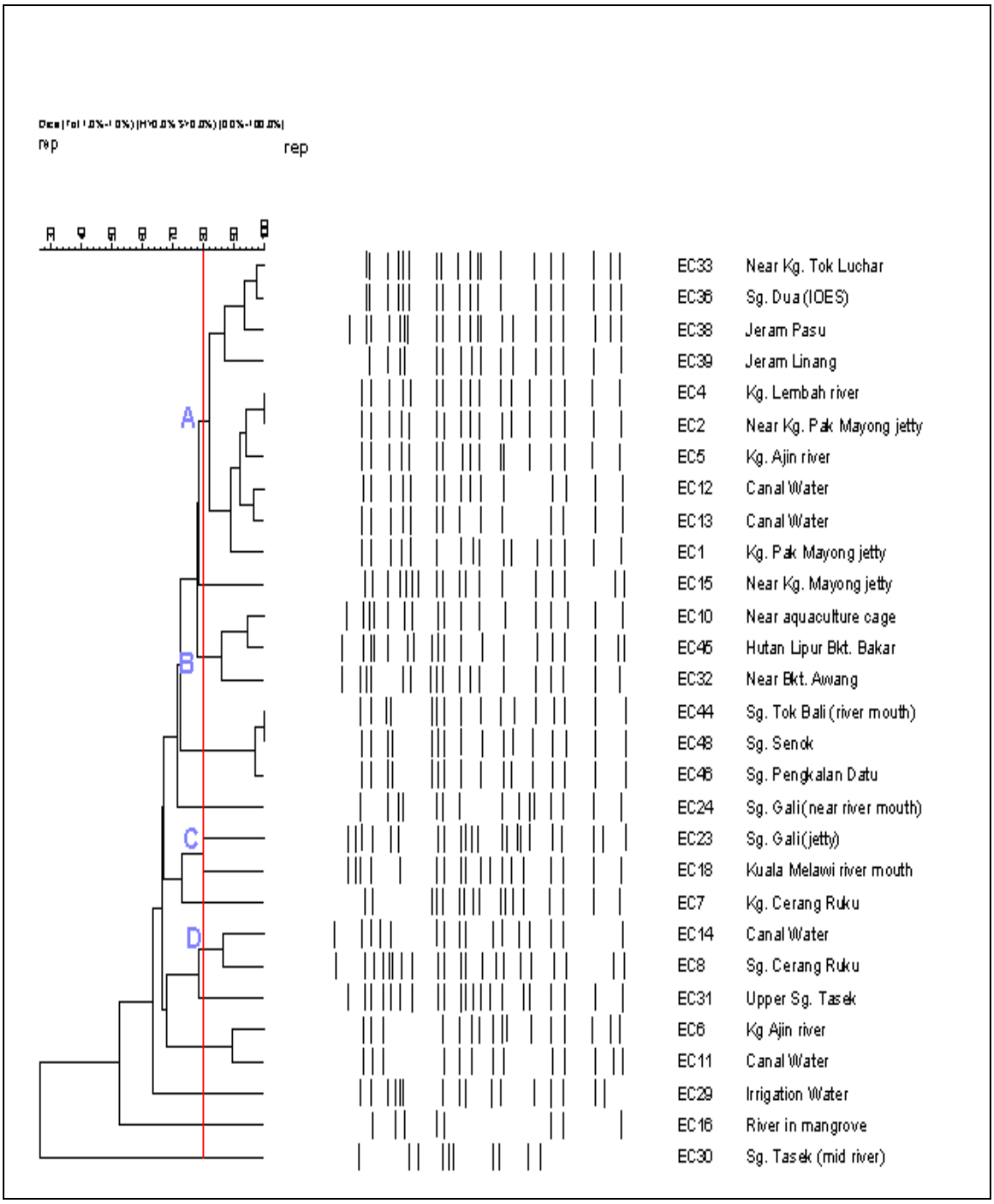


Fig. 4.6c Dendrogram showing the result of cluster analysis of the REP-PCR patterns from 29 isolates of environmental *E. coli* generated with GelCompar software by the UPGMA method. The different fingerprint patterns and location of samples are indicated. A to D represent the different clusters based on 80% similarity.

4.6.3 Comparison of REP-PCR of *E. coli* isolates from clinical and food samples

Additional strains from human and food samples were included to access the diversity of the *E. coli* strains from various sources. REP-PCR assay was carried out for 10 clinical and 10 food *E. coli* strains using REP1R(b) primer. The source and origin of the strains is as stated in Table 4.6a. The REP-PCR generated 46 patterns (F=0.45-1.0) out of 49 *E. coli* isolates. From the results, the REP-PCR of genomic DNA of *E. coli* isolates from different sources generated multiple DNA fragments in sizes ranging from 300bp to 4kb (Figure 4.6d). Each isolates produced genomic profiles consisted of 10 to 15 visible bands which were lesser than bands observed in environmental isolates. Both the clinical and food *E. coli* strains tested in REP-PCR assay also produced two common DNA bands in the size of 550bp and 600bp as observed in environment isolates. A typical banding pattern of clinical and food *E. coli* strains shown in Figure 4.6d.

Table 4.6a Sources and origin of clinical and food sample used

No	Strain	Source	Origin	Year of isolation
1	ECF1	Food	Chicken satay	2007
2	ECF2	Food	Chicken satay	2007
3	ECF3	Food	Beef satay	2007
4	ECF4	Food	Beef satay	2007
5	ECF5	Food	Beef satay	2007
6	ECF6	Food	Mutton satay	2007
7	ECF7	Food	Chicken liver	2007
8	ECF8	Food	Beef rendang	2007
9	ECF9	Food	Gizzard	2007
10	ECF10	Food	Ulamraja	2007
11	ECC1	Human	Bile, JB	2004

12	ECC2	Human	Aspirate, JB	2004
13	ECC3	Human	Wound swab, JB	2004
14	ECC4	Human	Aspirate, JB	2004
15	ECC5	Human	Blood, JB	2004
16	ECC6	Human	Urine, JB	2004
17	ECC7	Human	Triple lumen tip	2004
18	ECC8	Human	Blood, JB	2004
19	ECC9	Human	Urc tip, Kelantan	2004
20	ECC10	Human	Aspirate, JB	2004

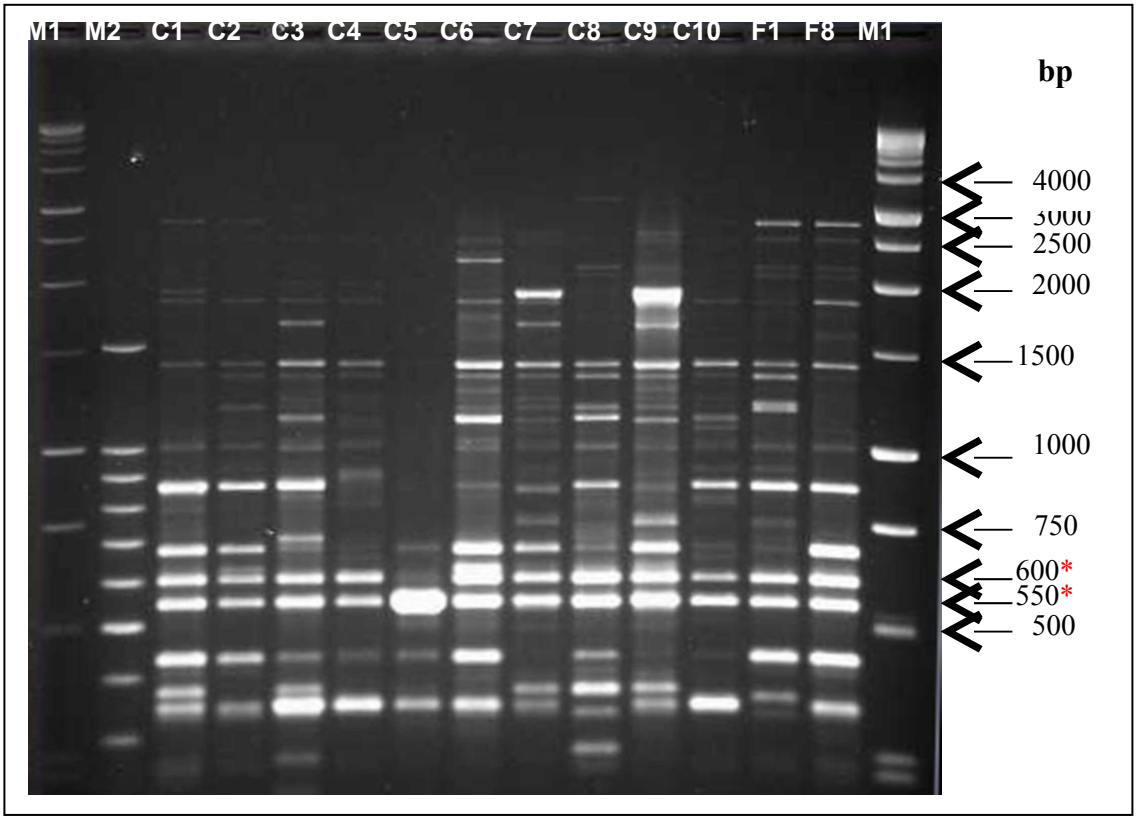


Fig. 4.6d Representative DNA fingerprint of clinical and food *E. coli* isolates generated by REP 1R(b) primer. Lane M1: 1kb DNA ladder; Lane M2: 100bp DNA ladder; Lane C1-10, clinical *E. coli* isolates; Lane F1 & F8, food *E. coli* isolates The consistent bands at 550bp and 600bp (marked with red asterisk) were observed in both clinical and food *E. coli* strains.

4.6.4 Cluster analysis of *E. coli* from different sources

The cluster analysis of the genomic fingerprints using a similarity coefficient of 70% showed 9 separate major clusters (A, B, C, D, E, F, G, H and I) (Figure 4.6e). Cluster B which was the major cluster was subdivided into two clusters (B1 and B2). Cluster A and B consisted of 21 of the 29 environment *E. coli* strains. Clusters A and B also included two food isolates, chicken liver and beef rendang (ECF1 and ECF8). Majority of the clinical isolates fell into a single cluster, as did the majority of the food isolates. Three isolates, from food isolate ulamraja (ECF10), Sg. Tasek (EC30) and Mangrove water (EC16) differed significantly from other isolates due to the unique fingerprint patterns. Environment *E. coli* isolates were genetically more diverse as compared to the clinical isolates. The genetic heterogeneity between the isolates obtained in natural environments may be due to differences between sampling sites, particularly when the sampling site is subject to high contamination, from diverse sources.

In this study, the REP-PCR has been proven to be a useful method for the comparison study of *E. coli* population from different sources. Dombek *et al.* (2000) also reported the use of REP-PCR to differentiate *E. coli* strains isolated from human and animal sources and their results indicate that the dendrogram have been useful for separating isolates into human and nonhuman source groups.

Although the observed diversity of *E. coli* population in this study was dependent upon the typing method, the trend in diversity of environment > food > clinical was consistent. The unstable and diverse nature of *E. coli* observed in this study has

practical implications with respect to its use as an indicator of water quality. Overall, dendrogram generated by combining REP-PCR results obtained from different *E. coli* sources showed large degree of genetic heterogeneity among the *E. coli* strains. There are possible explanations behind these wide patterns of diversity. Multiple isolates obtained from the same sample location, the same sample type and sampling time frame showed genetic diversity. Under a simplistic scenario, the isolates could have originated from entirely different sources.

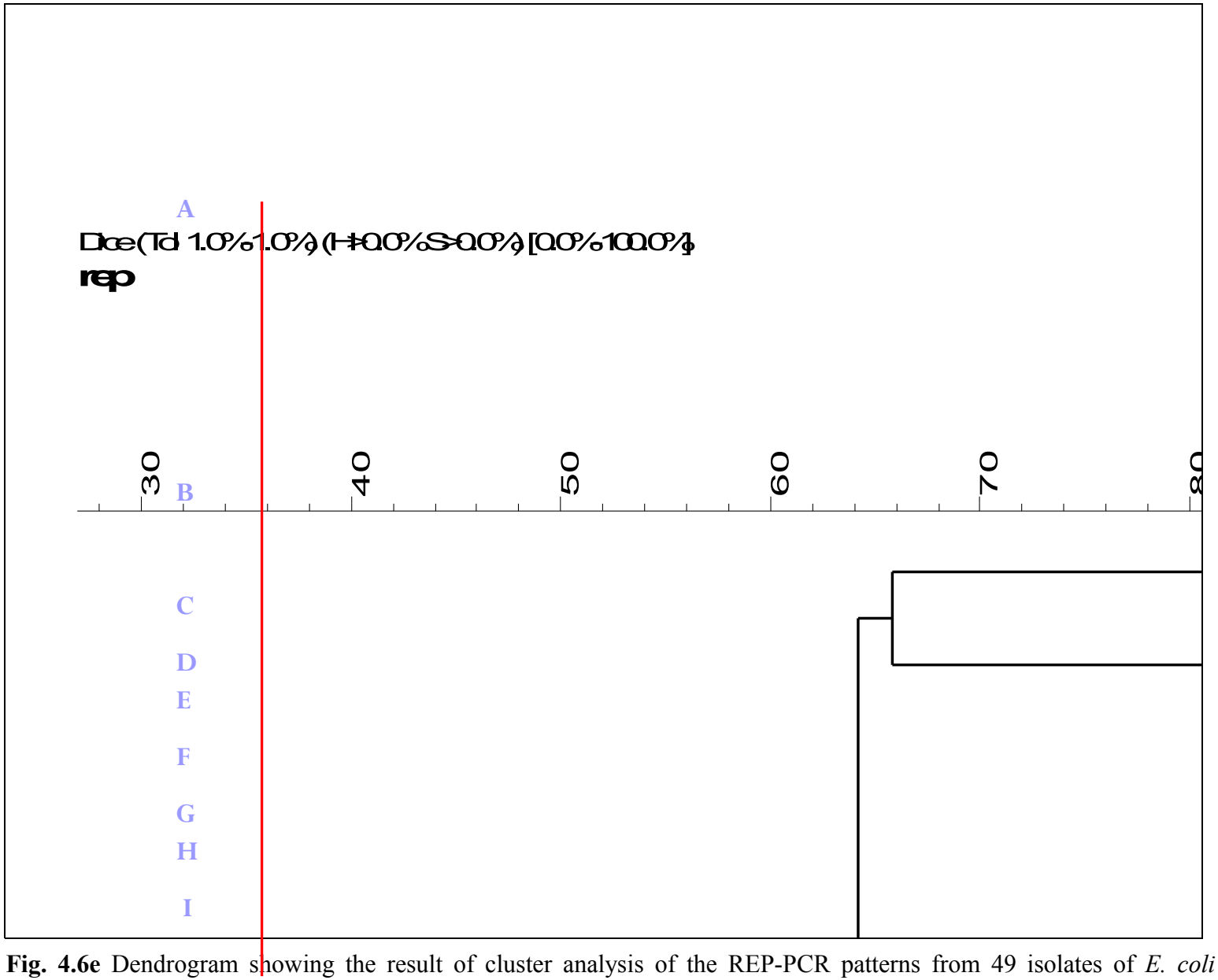


Fig. 4.6e Dendrogram showing the result of cluster analysis of the REP-PCR patterns from 49 isolates of *E. coli* generated with GelCompar software by the UPGMA method The different fingerprint patterns and location of samples are indicated. A to I represent the different clusters based on 70% similarity.