DNA profiling among egg and beef meat isolates of *Escherichia coli* by enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) and random amplified polymorphic DNA-PCR (RAPD-PCR)

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Abstract: Forty three (n=43) genomic DNA of *Escherichia coli* (11 isolates from eggs and 32 isolates from imported beef meats) were characterized by shiga toxin 1 (stx1), enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) and random amplified polymorphic DNA-PCR (RAPD-PCR) analyses. In the shiga toxin 1 (*stx*1) gene detection with primer stx 1F (5'-TTCTTCGGTATCCTATTCCC-3') and stx 1R (5'-CTGTCACAGTAACAACCGT-3'), 9 *E. coli* of beef meats isolates were positive toward sxt1 gene. The results of the ERIC-PCR and RAPD-PCR were analyzed using GelCompar II software. ERIC-PCR with primer ERIC1 (5'-CACTTAGGGGTCCTCGAATGTA -3') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') discriminated the *E. coli* into 6 clusters and 10 single isolates at 80% similarity. RAPD-PCR with primer Gen8 and Gen9, produced 10 clusters and 15 single isolates and 12 clusters and 14 single isolates of 80%, respectively. These results demonstrated that both ERIC-PCR and RAPD-PCR are useful and suitable tools for molecular typing of those isolates examined.

Keywords*:* DNA profiling, egg and beef meat isolates of *Escherichia coli*, ERIC-PCR and RAPD-PCR

Introduction

Escherichia coli is known as common microorganism exist in the intestine tract of human and warm-blooded animals. Most strains of *E. coli* are harmless and as part of the normal intestinal microflora of man, mammal and birds (Sahilah, 1997). They are opportunistic pathogens of human and animal. However, the *E. coli* associated with shiga-like toxin gene which are originated from *Shigella dysenteriae*, they would belong to either shigatoxigenic group of *E. coli* (STEC), enterohemorrhagic *E. coli* (EHEC) serotype O157:H7, or other enterhaemorrhagic *E. coli* (Beutin, 2006).

The shiga toxin-producing *E. coli* (STEC) are among the most important of food-borne disease. They are responsible for human gastrointestinal disease, including watery or bloody diarrhea and hemorrhagic colitis (HC). The majority of human infections is correlated with the consumption of locally contaminated food, particularly undercooked ground beef and unpasteurized milk (Rey *et al.*, 2006; *Caro and Garcia-Armesto, 2007*). While, the *E. Coli* O157:H7 has been established that cattle is the major reservoir of *E. coli* O157:H7 with outbreaks of disease are associated with consumption of foods of bovine origins and related products (Griffin, 1995; Pruimboom-Brees *et al., 2000*). The prevalence of *E. coli* O157:H in poultry has also been reported (Doyle and Schoeni, 1987). The *E. coli* O157:H7 often produces two antigenically distinct types of shiga toxin (*Stx)*, shiga toxin (*stx*1) and shiga toxin 2 (*stx*2) (Paton and Paton, 2002; Wang *et al*., 2002). Though, no outbreak has been reported in Malaysia related to *E. coli* shiga toxin-producing *E. coli* (STEC) or *E. coli* O157:H7 , those serotype have been detected in clinical (Son *et al*., 1996) samples as well as beef samples (Son *et al.,* 1998a; Sahilah *et al.,* 2010). The prevalence of *E. coli* shiga toxin-producing (STEC) or *E. coli* O157:H7 in beef meat samples in local market are rather alarming due to health public concern and it can serve as a vehicle for the transmission of disease to man.

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In this study we examined the shiga-toxin genes (Fagan *et al*., 1999) and genotypic characteristics among egg and beef meat of *E. coli* isolates using enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) and Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) fingerprinting. The typing of *E. coli* associated with the exposure to *E. coli* shiga toxin-producing (STEC) or *E. coli* O157:H7 is of considerable importance in the surveillance of possible public health risk as a research tool for predictive value in epidemiological control. It also allows the identification of the presence of virulent strains and changing pattern of these bacteria to be monitored which may be achieved by using polymerase chain reaction (PCR)-based technique.

Various DNA profiling techniques have been developed and applied to members of diverse bacterial genera based on PCR-based methods. These includes enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR)(Gillings and Holley, 1997; Dell-Costa *et al.,* 1998; Da Silveira *et al.,* 2002; Wieczorek *et al*., 2004; Sahilah *et al*., 2005; Casarez *et al*., 2007; Lindsay and Paul, 2006; Sahilah *et al.,* 2008)**,** random amplified polymorphic DNA-PCR (RAPD-PCR) (Sahilah *et al.,* 2003; Sahilah *et al*., 2010), amplified restriction fragment length polymorphism (AFLP), plasmid profiling and pulsed field gel electrophoresis (PFGE) (Sahilah *et al.,* 2003). These approaches are very helpful to investigate the genetic diversity and epidemiological relationships of the bacteria.

Materials and Methods

Escherichia coli

Forty (n=43) bacterial isolates (n=11; *Escherichia coli* of egg isolates ECE1- ECE11 and *E. coli* of imported beef isolates ECB1-ECB32) were obtained from the laboratory of Food Science, Universiti Kebangsaan Malaysia, Bangi Selangor. *E. coli* isolates were isolated from local retail eggs in Selangor area and imported beef meats.

Preparation of whole-cell DNA for PCR

DNA extraction was done involving boiling, chilling and centrifugation (Jothikumar and Griffiths, 2002; Sahilah *et al*., 2010). The cells were grown in 1.5 ml of Lauria-Bertani (LB)(tryptone, 4.0 g/L, yeast extract, 5.0g/L, sodium chloride, 10.0 g/L) at 35 °C for 20 h were harvested and centrifuged at 12,000 rpm for 1 min. The supernatant was discarded. The pellet was then washed with 1.0 ml sterile distilled water and vortex. Then, it was boiled at 97 °C for 10 min and immediately was kept frozen at -20°C for

10 min. The tube was centrifuged at 10,000 rpm for 3 min. The supernatant was used as a template.

Detection of shiga toxin gene

The preliminary study was carried out to determine the presence of s*tx* gene using a pair of primer as described by Fagan *et al*. (1999). These primers were designed to detect the presence of *stx*1 gene. The primer sequences used were stx 1F (5'-TTCTTCGGTATCCTATTCCC-3'), and stx 1R (5'-CTGTCACAGTAACAACCGT-3'). The detection assay was performed in a 25 µl volume containing 5.0 µl of 5 × PCR buffer (250 mM KCl, 50 mM Tris-HCl (pH 9.1) and 0.1% Triton™X-100), 1.0 µl of 35 mM dNTPs (Vivantis, MY) 1.0 µl of each 10 pmol primer stx 1F, stx 1R, 0.3 µl of 1.0 units of *Taq* DNA polymerase (Vivantis, MY), 11.70 µl of sterile ultrapure deionized water and 5.0 µl of 20 ng DNA template. A negative-DNA control was performed by adding 1 µl of sterile ultrapure deionized water. Amplification was performed in Eppendorf thermal-cycler (Eppendorf, Germany) with a temperature program consisting of the initial denaturation at 95°C for 9 min followed by 30 cycles of denaturation at 94°C for 30 second, annealing for 30 second at 72°C and polymerization at 72°C for 45 second. Final elongation was at 72°C for 7 min (Bopp *et al.,* 2003). The amplification products were analyzed by electrophoresis in a 1.5% agarose in 1X TAE (40 mM Tris-OH, 20mM acetic acid and 1mM of EDTA; pH 7.6) at 90 V for 70 min. Gels were stained with ethidium bromide. The amplified fragments were visualized with UV transilluminator (Alpha Innotech, USA). The 123 bp DNA ladder (Sigma, USA) was used as a DNA size marker.

Enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR)

The primer used were ERIC1R (5'-CACTTAGGGGTCCTCGAATGTA -3') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') as described by Versalovic *et al.,* (1991). PCR amplification reactions consisted of 25 µl volume containing 5 µl of 20 ng of genomic DNA, 2.5 µl 10X PCR buffer (500 mM KCl, 100 mM Tris-HCl (pH 9.1) and 0.1% Triton™X-100) (Vivantis, MY), 0.5 µl of 5 unit *Taq* polymerase (Vivantis, MY), 2.5 µl of 5 µM each of the forward and reverse primers, 1µl of 50mM MgCl$_2$ (Vivantis, MY) and 0.5 µl of dNTPs and 10.5 sterile ultrapure deionized water. Amplification was performed in Eppendorf thermal-cycler (Eppendorf, Germany) with a temperature program consisting of the initial denaturation at 95 °C for 1 min followed by 35 cycles of denaturation at 92 °C for 45 second,

annealing for 1 min at 52 °C and polymerization at 70 °C for 20 min. Final elongation was at 70 °C for 20 min (Sahilah *et al*., 2000). The amplification products were analyzed by electrophoresis in a 1.5 % agarose in 1X TAE (40 mM Tris-OH, 20 mM acetic acid and 1mM of EDTA; pH 7.6) at 90 V for 70 min. Gels were stained with ethidium bromide. The amplified fragments were visualized with UV transilluminator (Alpha Innotech, USA). The 123 bp DNA ladder (Sigma, USA) was used as a DNA size marker.

Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR)

The discriminatory ability and stability of RAPD-PCR fingerprinting were tested in a preliminary study against a panel of 2 different bacterial isolates of *E. coli* (Sahilah, 2002) with 10-mer random primers (Vivantis,My).PrimerGen8(5'-GGAAGACAAC-3') and Gen9 (5'-AGAAGCGATG-3') showed the greatest stability and discriminatory ability among the *E. coli* isolates, and was therefore used in this study. The RAPD-PCR fingerprinting assay was performed in a 25 µl volume containing 2.5 µl of 10× PCR buffer (500 mM KCl, 100 mM Tris-HCl (pH 9.1) and 0.1% Triton™X-100), 1.5 µl 50 mM MgCl$_2$, 0.5 µl of 10 mM dNTPs (Vivantis, MY), 1.0 µl of 100 mM primer (Either GEN8 or GEN9), 0.4 µl of 5 units of *Taq* DNA polymerase (Vivantis, My), 13.1 µl deionized water and 6 µl of 10 ng DNA template. A negative-DNA control was performed by adding 1 µl of sterile ultrapure deionized water. Amplification was performed in Eppendorf thermal-cycler (Eppendorf, Germany) with a temperature program consisting of the initial denaturation at 94 °C for 5 min followed by 45 cycles of denaturation at 94 °C for 1 min, annealing for 1 min at 35 °C and polymerization at 72 °C for 2 min. Final elongation was at 72 °C for 7 min (Sahilah *et al.,* 2010). The amplification products were analyzed by electrophoresis in a 1.5% agarose in 1.0 X TAE (0.1 M Tris, 0.1 M Boric acid, 0.1 mM EDTA) at 100 V for 40 minutes. Gels were stained with ethidium bromide. The amplified fragments were visualized with UV transilluminator (Alpha Innotech, USA). The 123 bp DNA ladder (Sigma, USA) was used as a DNA size marker.

Data analysis

Clonal relatedness of the *Escherichia coli* was estimated by ERIC-PCR and RAPD-PCR from image of gel photographs using Gel Compar II image analysis software (Applied Math, Kortjik, Belgium) with the band matching coefficient of Dice (Sahilah *et al*., 2000) The position of the markers run in both ERIC and RAPD were used to normalize the sample data by correcting lane-to-lane and gel-to gel variation. Isolates were clustered using average linkage (UPGMA, unweighted group pair method with arithemetic averages) and displayed in dendrogram form.

Results

Detection of shiga toxin gene

Preliminary study to detect the presence of shiga toxin 1 (*stx*1) gene was conducted by a pair of primer as describe by Fagan *et al*., (1999). Of 43 *E. coli* isolates, 9 isolates of beef meet samples (ECB21, ECB23, ECB24, ECB25, ECB26, ECB27, ECB28, ECB29, and ECB31) were positive to *stx*1, indicated by formation of one band with its molecular weight of 614 bp (Figure 1). While, None of *E. coli* egg isolates were positive towards *sxt*1 (data not shown).

Enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR)

The ERIC-PCR fingerprinting of *E. coli* revealed 39 distinct patterns namely E1 to E39 (Table 1 and Figure 2-5). Two isolates were identical in ERIC-PCR patterns were *E. coli* strain ECB5 and ECB18 which shows ERIC-PCR patterns of E15; and *E. coli* strain ECB11 and ECB16 were identical in ERIC-PCR patterns of E21. The possible number of ERIC-PCR pattern was estimated on the basis of changes in one or more clear bands or band sizes (Figure 2-5). In this work, ERIC-PCR produced complex fingerprints and analysis data using GelCompar II produced fingerprints which could group all the *E. coli* isolates into 6 clusters and 10 single isolates at 80% similarity (Figure 6).

Random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR)

Two primers were chosen to analyse the whole set of 43 *E. coli* isolates. A total of 10-mer of different oligonucleotide primers (Gen1-Gen10) were screening for RAPD-PCR analysis of a subset of 2 isolates to detect polymorphism within *E. coli*. The possible number of RAPD-profiles was estimated on the basis of changes in one or more clear bands or band sizes as tabulated in Table 1. Thirty four and 36 of RAPD-PCR profiles were apparent from primer Gen8 (P1-P32) and Gen9 (Q1-Q34), respectively. However, no band produced with *E. coli* strain ECE3 (Table 1) with both primers (Gen8 and Gen9). While *E. coli* strain ECE8 was untypable with only primer Gen8. Both isolates (ECE3 and ECE8) were not typable in repeated experiment.

Table 1. DNA profiling among eggs and beef meats isolates of *Escherichia coli* using PCR-based techniques

| Strain | $Stx1$ | ERIC-PCR | RAPD-PCR profiles with primers | | Genome types |
			Gen 8	Gen 9	
ECE1	-	E1	P1	Q1	1
ECE2	-	E2	P2	Q1	2
ECE3	-	E3	UT	UT	3
ECE4	-	E4	P2	Q2	4
ECE5	-	E5	P3	Q2	5
ECE6	-	ND	P4	Q3	6
ECE7	-	E6	P5	Q4	7
ECE8	-	E7	UT	Q5	8
ECE9	-	E8	P6	Q5	9
ECE10	-	E9	P6	Q6	10
ECE11	-	E10	P7	Q7	11
ECB1	-	E11	P8	Q8	12
ECB2	-	E12	P9	Q9	13
ECB3	-	E13	P10	Q10	14
ECB4	-	E14	P11	Q11	15
ECB5	-	E15	P12	Q12	16
ECB6	-	E16	P13	Q13	17
ECB7	-	E17	P14	Q14	18
ECB8	-	E18	P15	Q15	19
ECB9	-	E19	P16	Q16	20
ECB10	-	E20	P17	Q17	21
ECB11	-	E21	P18	Q18	22
ECB12	-	E22	P19	Q19	23
ECB13	-	E23	P20	Q19	24
ECB14	-	E24	P21	Q20	25
ECB15	-	E25	P22	Q21	26
ECB16	-	E21	P23	Q22	27
ECB17	-	E26	P24	Q23	28
ECB18	-	E15	P25	Q24	29
ECB19	-	ND	P26	Q25	30
ECB20	-	E27	P27	Q26	31
ECB21	+	E28	P28	Q27	32
ECB22	-	E29	P29	Q28	33
ECB23	+	E30	P29	Q29	34
ECB24	+	E31	P30	Q30	35
ECB25	+	E32	P31	Q31	36
ECB26	+	E33	P31	Q30	37
ECB27	+	E34	P31	Q30	38
ECB28	+	E35	P32	Q32	39
ECB29	+	E36	P32	Q33	40
ECB30	-	E37	P29	Q34	41
ECB31	+	E38	P33	Q35	42
ECB32	-	E39	P34	Q36	43

Negative for *sxt*1; + Positive for *sxt*1; ND-Not determined; UT- could not be classified according to type
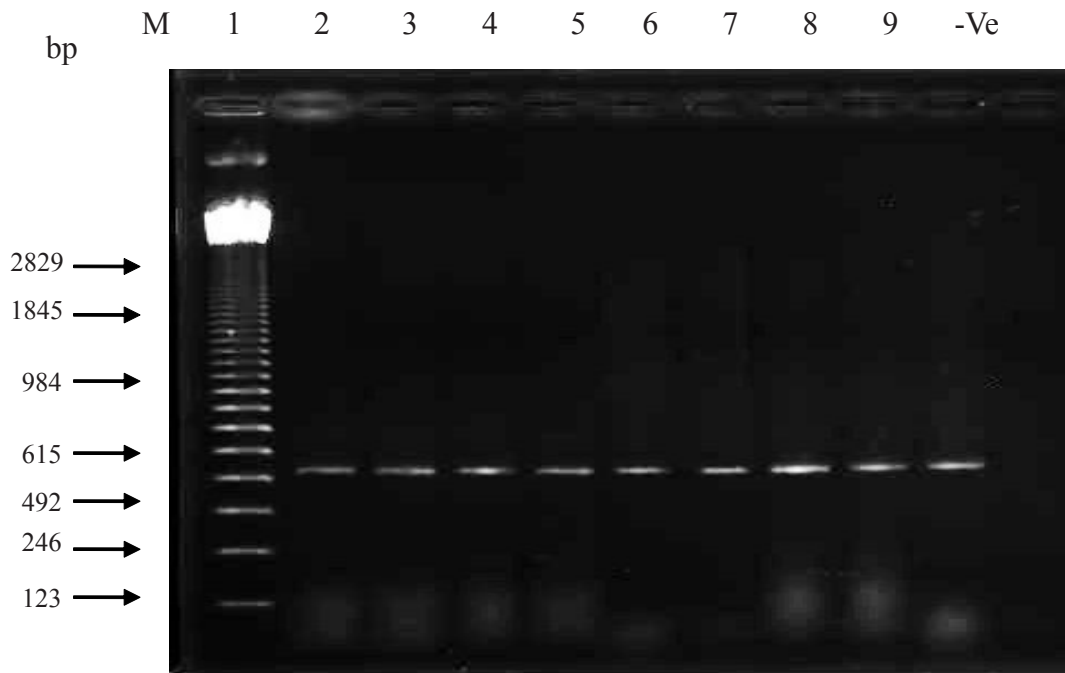
Figure 1. Detection of shiga toxin 1 (*stx*1) gene of *Escherichia coli* isolates by polymerase chain reaction (PCR) electrophoresed on 1.5 % agarose gel. M, Molecular weight sizes (base pairs, bp) are indicated by numbers on the left; lane 1-9: ECB21, ECB23, ECB24, ECB25, ECB26, ECB27, ECB28, ECB29, and ECB31; Lane 11: Negative control.
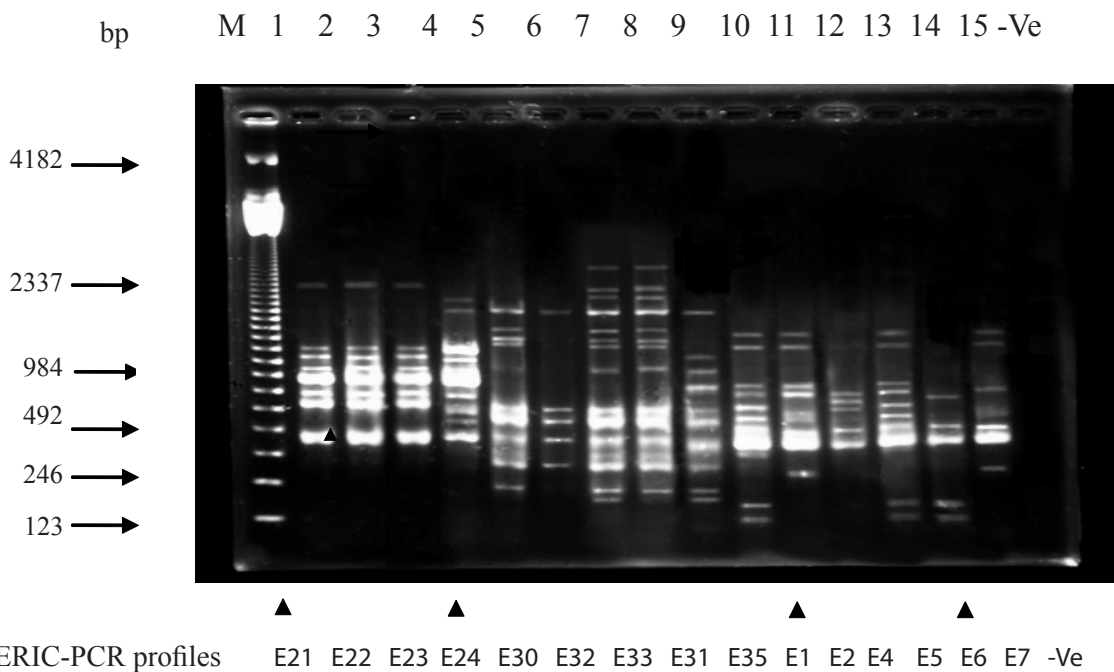
Figure 2. ERIC-PCR profiles of *Escherichia coli* electrophoresed on 1.5% agarose gel. M, Molecular weight sizes (base pairs, bp) are indicated by numbers on the left; lane 1-15: ECB12, ECB13, ECB14, ECB15, ECB22, ECB24, ECB25, ECB23, ECB27, ECE1, ECE2, ECE4, ECE5, ECE7 and ECE8; Lane 16: Negative control.
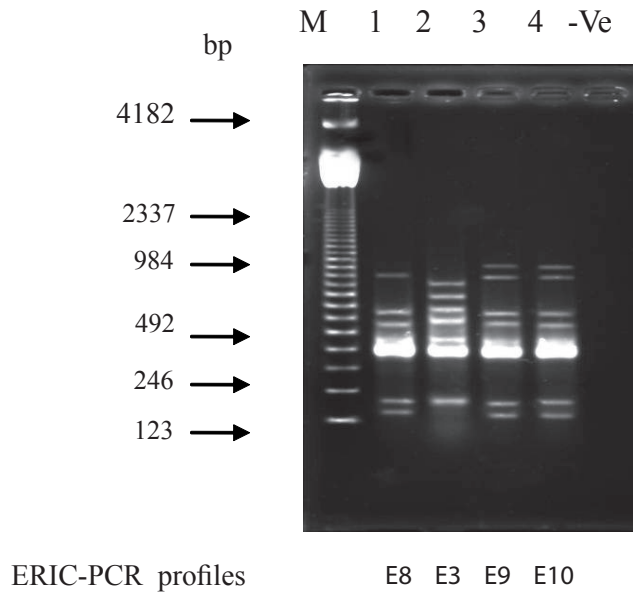
Figure 3. ERIC-PCR profiles of *Escherichia coli* electrophoresed on 1.5% agarose gel. M, Molecular weight sizes (base pairs, bp) are indicated by numbers on the left; lane 1-4: ECE9, ECE3, ECE10, and ECE11; Lane 5: Negative control.
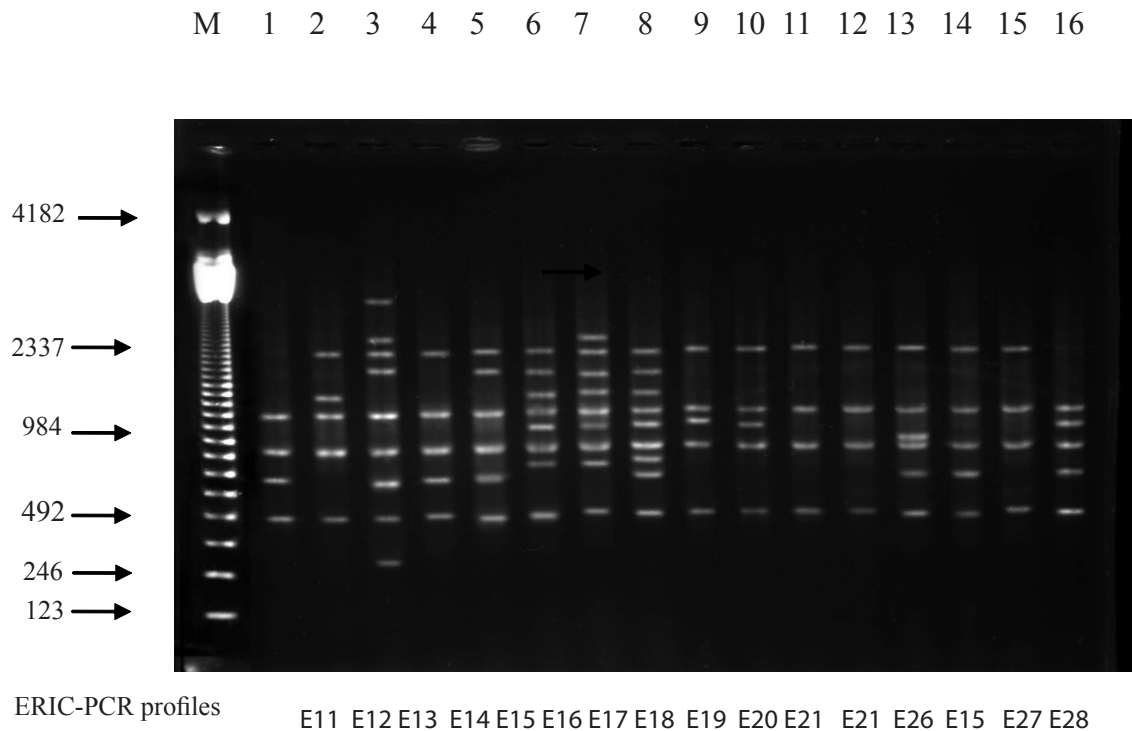
Figure 4. ERIC-PCR profiles of *Escherichia coli* electrophoresed on 1.5% agarose gel. M, Molecular weight sizes (base pairs, bp) are indicated by numbers on the left; lane 1-16: ECB1, ECB2, ECB3, ECB4, ECB5, ECB6, ECB7, ECB8, ECB9, ECB10, ECB11, ECB16, ECB17, ECB18, ECB20 and ECB21.
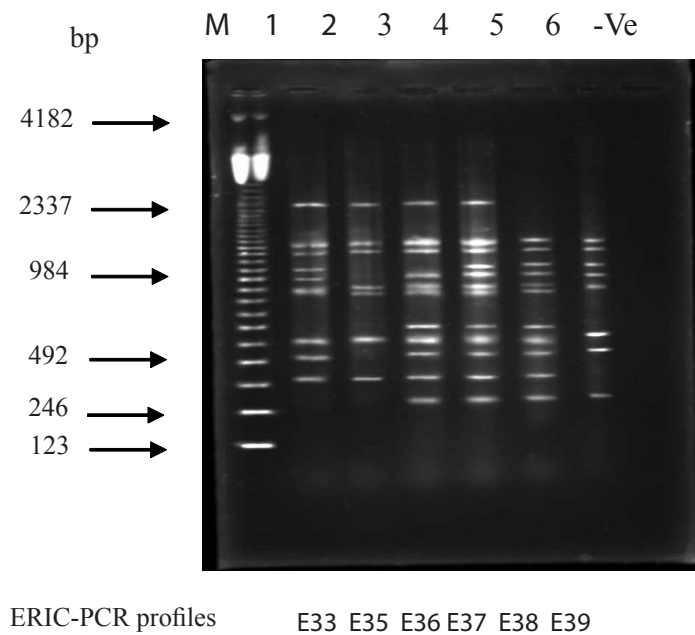
Figure 5. ERIC-PCR profiles of *Escherichia coli* electrophoresed on 1.5% agarose gel. M, Molecular weight sizes (base pairs, bp) are indicated by numbers on the left; lane 1-6: ECB26, ECB28, ECB29, ECB30, ECB31 and ECE32; Lane 7: Negative control.

The dendrograms of primer Gen8 and Gen9 were constructed as shown in Figures 7 and 8. Primer Gen8 produced complex fingerprint that could discriminate the *E. coli* isolates into 10 clusters and 15 single isolates at a similarity level of 80% (Figure 7). Whereas, primer Gen9 produced 12 clusters and 14 single isolates at a similarity level of 80% (Figure 8).

Discussion

Escherichia coli is an opportunist bacteria pathogen and most isolates of *E. coli* are harmless an as a part of the normal intestinal microflora of man, mammal and birds (Sahilah, 1997). However, the *E. coli* associated with shiga toxin (*stx*1 and *stx*2) gene can cause serious disease in humans by possess at least one of the toxin gene (Griffin, 1995; Paton and Paton, 2002; Wang *et al*, 2002). The detection of shiga toxins is very useful for the identification of enterohemorrhagic *E. coli* (EHEC) and Non-EHEC isolates were negative for both *stx1* and *stx*2. *Although, large outbreaks of E. coli shiga toxin-producing (STEC)* or EHEC infection in humans have not been reported in Malaysia the data obtained here show that animal source may act as reservoir of both type of *E. coli.* This is an early phase of an epidemic due to clones of these bacteria that may play an important role as source of human infection.

In this work, 9 (9/31) of *E. coli* beef isolates were positive towards shiga toxin 1 (sxt1) indicated by the presence of bands with of 614 bp in molecular weight (Fagan *et al.*, 1999). Our results are in general agreement and consistent with our previous study (Sahilah *et al.,* 2010) who reported the presence of E. coli O157:H7 in local retail beef marketed in Malaysia concomitant with the presence of shiga toxin gene, *stx*1 and *stx*2. However, in the presence work, the 9 *E. coli* beef isolates may either probably in the group of STEC or EHEC isolates. Futher characterizations on the 9 *E. coli* beef meat isolates are under way in our laboratory to determine their group. It has been reported that majority human infections of STEC and EHEC disease is correlated with the consumption of undercooked ground beef, unpasteurized milk and cheese (Griffin, 1995; Pruimboom-Brees *et al.,* 2000; Rey *et al.*, 2006; Caro and Garcia-Armesto, 2007)**.** Our results are in contrast with those Doyle and Schoeni (1987) who reported the presence of *E. coli* O157:H7 in poultry. None of *E. coli* egg isolates were positive toward shiga toxin which may probably indicate the absent of STEC and EHEC in eggs. Despite, the detection of *stx*1 was detected from imported beef meat samples, limited number of isolates examined from local *E. coli* isolates made it difficult for us to draw any conclusion on the possibility of STEC or EHEC locally.
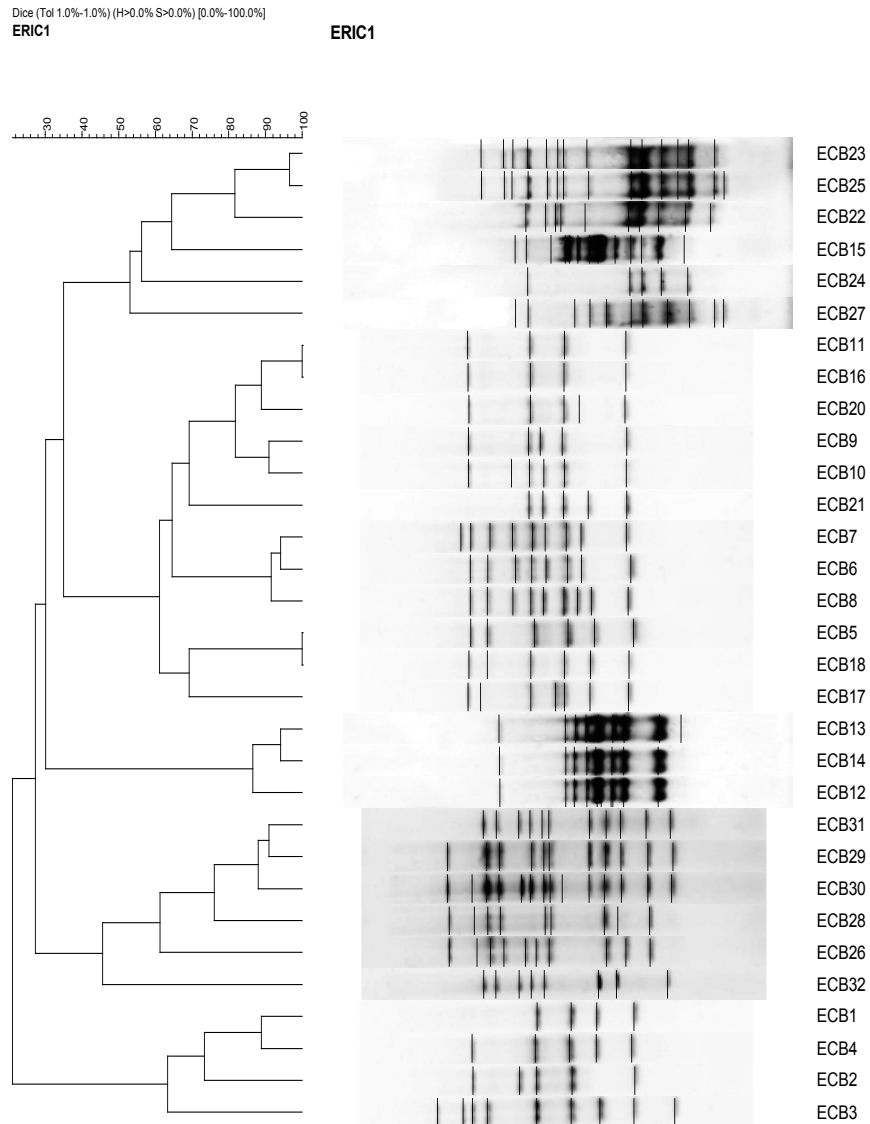
Figure 6. Dendogram showing the percentages of similarity between typable *Escherichia coli* among eggs and beef meats strains generated from random enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) fingerprinting with the band matching coefficient of Dice and the UPGMA clustering method.
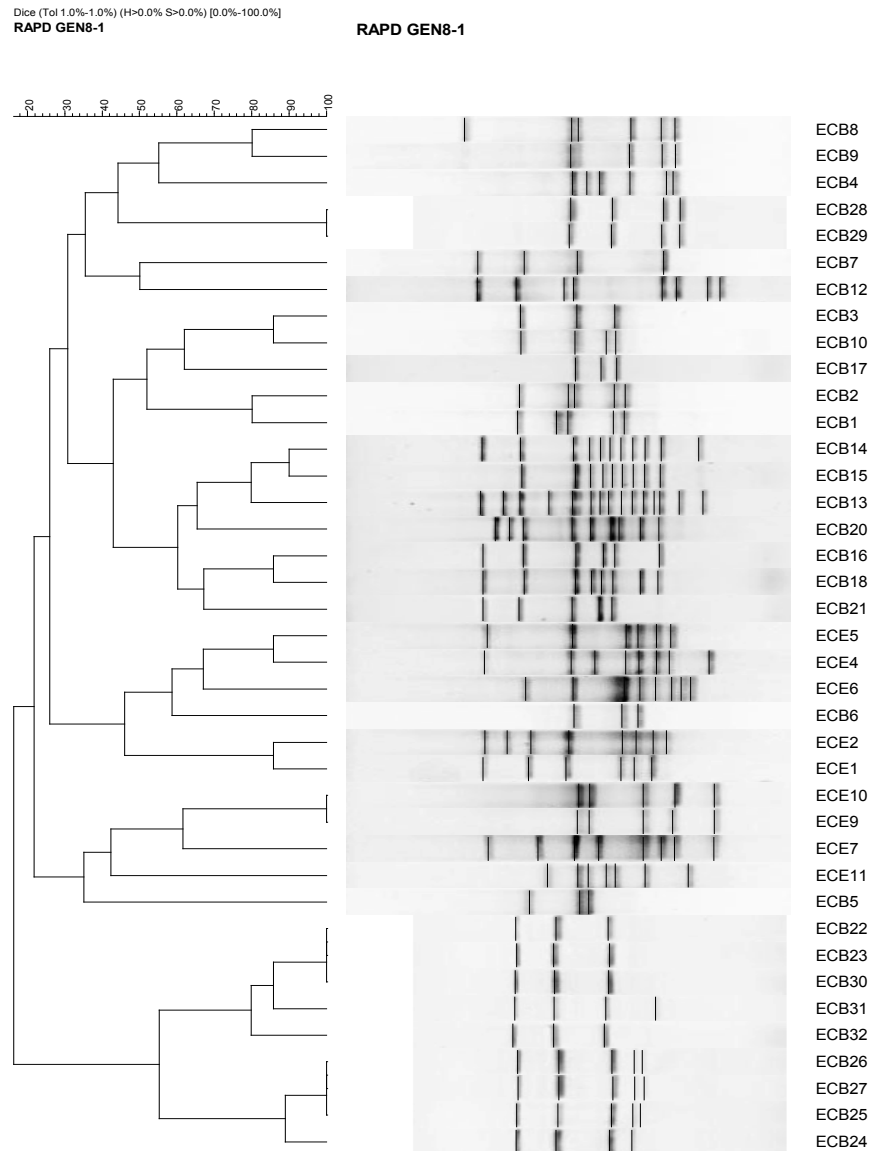
Dice (Tol 1.0%-1.0%) (H>0.0% S>0.0%) [0.0%-100.0%]
RAPD GEN8-1

RAPD GEN8-1

Figure 7. Dendogram showing the percentages of similarity between typable
Escherichia coli among eggs and beef meats strains generated from random amplified polymorphic DNA-PCR (RAPD-PCR) fingerprinting (Gen8) with the band matching coefficient of Dice and the UPGMA clustering method.
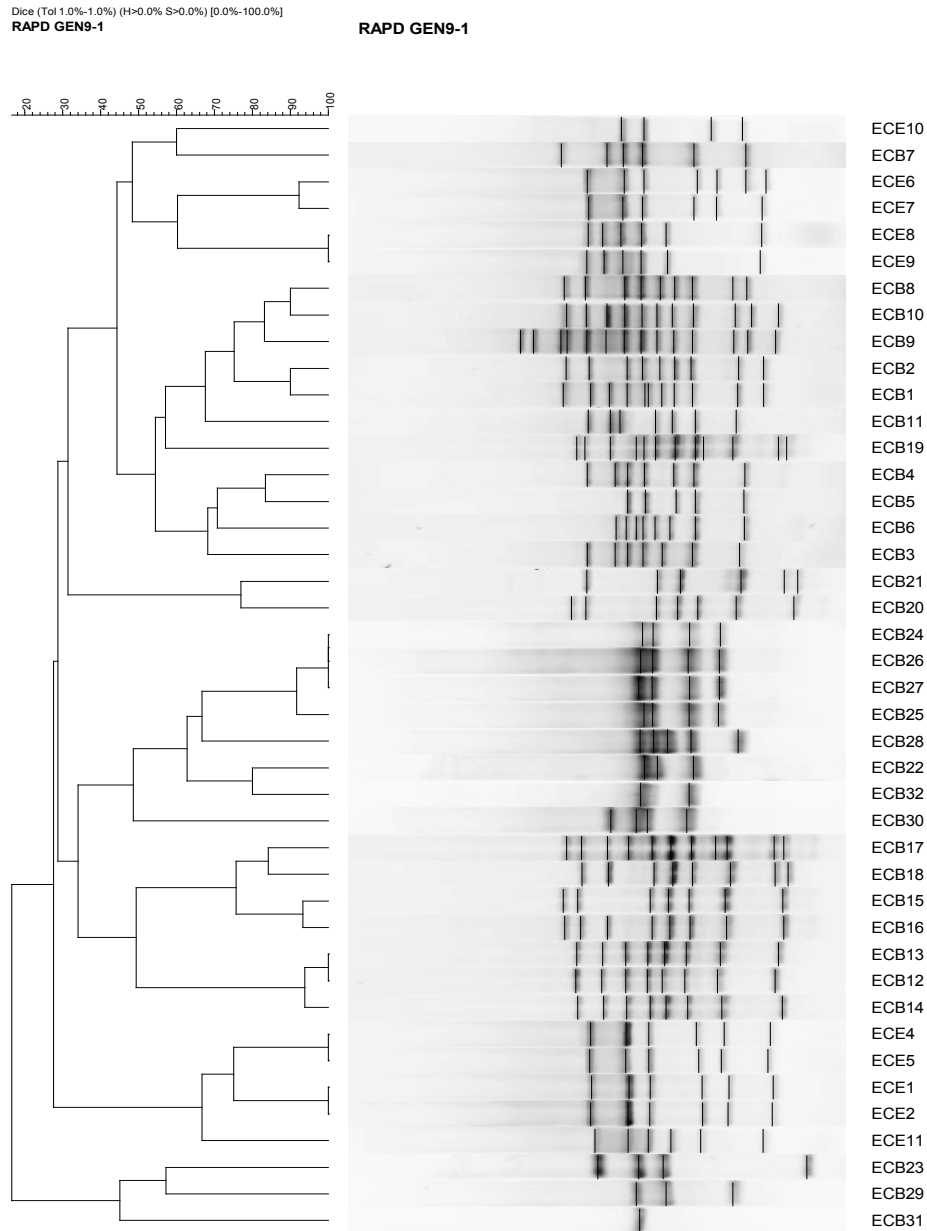
Figure 8. Dendogram showing the percentages of similarity between typable *Escherichia coli* among eggs and beef meats strains generated from random amplified polymorphic DNA-PCR (RAPD-PCR) fingerprinting (Gen9) with the band matching coefficient of Dice and the UPGMA clustering method.

The application of DNA-based techniques is way to examining the *E. coli* that may help to elucidate the epidemiology of STEC and EHEC isolates. *P*recise determination of clones of *E. coli* is an important part in the investigation of human epidemics of outbreaks of STEC or EHEC. The techniques used in this study indicated ERIC-PCR and RADP-PCR typing methods have almost similar capacity to differentiate amongst 43 isolates of *E. coli* from eggs and beef meat samples. ERIC-PCR profiles demonstrated a genetic variety among *E. coli* eggs and beef meat isolates. All isolates were typable using ERIC primers with 2 isolates (ECE11 and ECB32) were not tested. Despite, ECE11 and ECB32 have not examined their ERIC-PCR profiles, both isolates were differentiated using RADP-PCR typing method (Table 1). ERIC-PCR produced 39 DNA patterns and clustered into 6 clusters and 10 single isolates at 80% similarity level (Table 1, Figure 2-6). Only, two *E. coli* isolates were sharing their ERIC-PCR patterns which *E. coli* strain ECB11 and ECB16 showed E21 ERIC-PCR pattern. While, *E. coli* strain ECB5 and ECB18 showed E15 ERIC-PCR patterns (Table 1). In the present work, ERIC-PCR typing method shows a high degree of discriminative power and demonstrated in many previous studies to be useful for strain differentiation of *E. coli* (Dell-Costa *et al.,* 1998; Da Silveira *et al.,* 2002; Wieczorek *et al*., 2004).

In RAPD-PCR typing methods, two primers were used which were Gen8 and Gen9. Using primer Gen 8 and Gen9, RAPD-PCR produced 34 and 36 DNA patterns, respectively. Data clustered using average linkage (UPGMA, unweighted group pair method with arithemetic averages) in dendrogram form, showed primer Gen8 produced 10 clusters and 12 single isolates at a similarity level of 80% (Figure 7). Strain ECE3 and ECE8 were not typable using primer Gen8. This could be interpreted as the loss of specific sites for primer binding in the chromosomal DNA of those isolates. Whereas, primer Gen9 produced 10 clusters and 15 single isolates at a similarity level of 80% (Figure 8). Only one strain was not typable using primer Gen9, which is *E. coli* strain ECE3. Comparing with ERIC-PCR, RAPD-PCR was shown to be less discriminatory than ERIC-PCR. However, it is evident that ERIC-PCR and RAPD-PCR can be complement to each other, for example *E. coli* isolates which were untypable by RAPD-PCR were resolved by ERIC-PCR or vice-versa (Sahilah *et al.,* 2000).

The combination of the ERIC-PCR results with two RAPD-PCR primers, increased the number of different Genome types (Table 1) to 43. The results showed here, ERIC-PCR and RAPD-PCR primers enhance the polymorphism detection, for instance

E. coli isolates ECB26 and ECB27 were similar in patterns using RAPD-PCR primers of Gen8 and Gen9, respectively. But they were differentiated with ERIC-PCR typing method. This was in general agreement with those Rath *et al.,* (1995) and Son *et al.,* (1998b) that isolates with identical RAPD patterns generated by a few primers could be different when it was examined with more primers or primer combination. However, when we tried to look at a specific pattern or clusters to be associated with the origin of *E. coli* isolates using ERIC-PCR and RAPD-PCR techniques, we did not find any specific patterns or clusters to be associated with local eggs and imported beef meat samples. Neither strain from local nor distant localities the results showed a wide heterogeneity within *E. coli* isolates. All *E. coli* beef meat isolates which were positive for shiga toxin 1 (stx1) gene were also not able to fit with any specific patterns or clusters using ERIC-PCR and RAPD-PCR typing methods. They also exhibited high level of genetic variation.

Conclusion

In conclusion our data show the presence of a wide heterogeneity within egg and beef meat isolates of *E. coli*. Despite, the *E. coli* isolates examined were from two distantly geographical locations, we could not correlate between source of samples and location to genetic patterns relatedness. However, it is an evident ERIC-PCR and RAPD-PCR typing methods have shown to be useful for intraspecies discrimination.

Acknowledgements

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