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Analysis of *Escherichia coli* O157:H7 Isolates from Beef by RAPD-PCR and Plasmid Profiles

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Abstract: A total of 70 isolates of *Escherichia coli* O157:H7 isolated from beef samples were examined with respect to plasmid profiles and random amplified polymorphic DNA (RAPD) patterns. All isolates carried the 90 kb pO157 plasmid alone or in combination with other smaller plasmids. Using Gen1-50-02 (5′-CAATGCGTCT-3′), Gen1-50-09 (5′-AGAGGCGATG-3′) and Gen1-50-10 (5′-CCATTTACGC-3′) as primers, respectively, we obtained DNA polymorphisms which allowed us to discriminate the *E. coli* O157:H7 isolates into one, six and five RAPD patterns; providing bands ranging in size from 0.25 to 4.0 kb. Our results demonstrate that both plasmid profiling and RAPD-PCR fingerprinting methods are suitable tools for a fast and reliable molecular typing of *E. coli* O157:H7. The RAPD-PCR method is more sensitive with respect to the individualization of isolates and that RAPD-PCR assay could be a valuable technique for epidemiological studies.

Key words: *Escherichia coli* O157:H7, beef, plasmid, RAPD-PCR

Introduction

Interest in the clinical and ecological significance of enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7 has increased tremendously during the past decade as this organism has been recognized as agent of haemorrhagic colitis, which can progress to the hemolytic-uremic syndrome or thrombotic thrombocytopenic purpura (Griffin and Tauxe, 1991). The important determinants of disease include the production of a Shiga-like toxin (SLT) or Verotoxin (VT), the ability to cause attaching-and-effacing lesions, and the possession of a large plasmids encoding adhesins ans hemolysis (Tesh and O'Brien, 1992; Griffin, 1995). The majority of infections in humans are associated with the consumption of contaminated and improperly cooked beef. In addition, unpasteurized milk, faeces-contaminated vegetables, water, apple cider and other more novel foods have become contaminated and were implicated with *E. coli* O157:H7 infections (Karmali, 1989; Griffin and Tauxe, 1991; Swerdlow *et al*., 1992; Besser *et al*., 1993; Feng, 1995; Cody *et al*., 1999). Identification of the source of contamination of foods with *E. coli* O157:H7 is important to the understanding of the epidemiology of human infection and devising strategies for its control. Thus, this study was undertaken to determine the prevalence of *E. coli* O157:H7 from imported frozen beef. The strains were characterized by the determination of their plasmid profiles and randomly amplified polymorphic DNA (RAPD) patterns.

Materials and Methods

Samples, selective agar and immunomagnetic separation reagents: The beef samples (tenderloins) were taken from different retail outlets in Selangor and Kuala Lumpur. CT-SMAC (Sorbitol MacConkey agar) was used for the isolation of *E. coli* O157:H7 following enrichment and Dynabeads coated with antibodies to *E. coli* O157 (Dynal UK Ltd) were used for the immunomagnetic separation technique.

IMS procedure: Forty samples of frozen beef were tested. Twenty-five grams of meat sample were added to 225 ml of modified EC (mEC) broth containing novobiocin (20 mg/ml) (Kyokuto, Japan), in a stomacher bag containing a nylon filter, and homogenized using stomacher. The homogenates were incubated at 37°C without agitation for 6 h. The immunomagnetic separation (IMS) procedure was conducted according to the manufacturer's instructions using 1 ml of the 6 h enrichment culture added to 20 µl of Dynabeads anti-*E. coli* O157:H7. A volume of 50 µl of the resuspended Dynabeads was then inoculated onto CT-SMAC (Zadik *et al*., 1993) and plated to produce discrete colonies after incubation at 37°C for 18-24 h.

Confirmation and characterization of isolates: A maximum of 10 non-sorbitol fermenting (NSF) colonies were randomly selected, and were inoculated onto the surface of fresh CT-SMAC agar. Non-sorbitol-fermenting colonies were presumptively tested for agglutination with a latex test kit (Oxoid DR622) for detection of *E. coli* O157:H7. Latex agglutinating isolates were confirmed biochemically as *E. coli* by API 20E (Biomeriux) and as serogroup O157 by agglutination with *E. coli* O157 latex kit (Oxoid DR620). The isolates were further confirmed as serotype O157:H7 with a primer pair (flicH7R, 5'-GCGCTGTCGAGTTCTATCGAGC-3' and flich7F, 5'-CAACGGTGACTTTATCGCCATTCC-3') specific for the H7 flagellar gene as described elsewhere by Gannon *et al*. (1997).

DNA extraction: Genomic DNA were extracted by the mini-preparation method of Ausubel *et al*. (1987). Extraction of plasmid DNA from *E. coli* O157:H7 isolates, followed by electrophoresis were performed essentially as described by Sambrook *et al*. (1989). The approximate molecular mass of each plasmid was determined by comparison with plasmids of known molecular mass of *E. coli* V517 (Macrina *et al*., 1978).

PCR-based fingerprinting and identification: PCR-based fingerprinting was carried out in a volume of 25 µl containing 20-30 ng of *E. coli* O157:H7 total DNA, 2.5 mM $MgCl_2$, 20 pmol of primer, 1 U of *Taq* DNA polymerase (Promega), 250 µM each of dNTPs (Promega). A thermal cycler (Perkin Elmer 2400) was used for amplification. The program for amplification was 30 cycles at 94°C for 2 min, 36°C for 1 min and 72°C for 2 min. A final elongation step of 72°C for 5 min was included. The PCR assays for specific identification of the isolates by their flagellar H7 genes were performed in 25 µl volumes containing 2.5 µl 10x PCR buffer, 2.5 mM $MgCl_2$, 1 mM each (final conc.) dNTPs, 5 pmol of the primer H7 set, 1 U *Taq* polymerase and 10 µl of DNA samples extracted from boiled cells. Thirty five cycles were performed, each consisting of 1 min at 94°C, 1 min at 65°C and 2 min at 72°C, followed by one cycle consisting of 5 min at 72°C. After PCR, 10-20 µl aliquots of products were electrophoresed in 1.2% agarose gels, followed by ethidium bromide staining and photography under uv light.

Results and Discussion

Of recent years, Malaysia has increasingly obtained its supply of beef meat from other countries and this has prompted this investigation since beef meat infected by strains of *E. coli* O157:H7 potentially may serve as reservoirs for these bacteria and aid in their dissemination. Of the 40 samples examined, 70 strains of *E. coli* O157:H7 were isolated from 19 (47.5%) of

Table 1: Plasmid profiles and RAPD patterns among the *Escherichia coli* O157:H7 isolates used in this study.

Strain	Plasmid size (kb)	RAPD patterns obtained using primer		
		Gen1-50-02	Gen1-50-09	Gen1-50-10
1, 3, 4, 37, 38, 39, 40, 41, 42, 43, 44, 45, 56, 47	90 (1)[a]	a	a	a
2	90 (1)	a	a	c
5	90 (1)	a	e	a
9	90 (1)	a	c	a
13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 50, 51, 62, 63, 64, 65, 66, 67, 68, 69, 70	90, 3.7, 2.9, 2.6 (2)	a	a	a
12	90, 3.7, 2.9, 2.6 (2)	a	c	a
30, 31, 32, 33	90, 3.7, 2.9, 2.6 (2)	a	b	a
34	90, 3.7, 2.9, 2.5 (2)	a	d	b
48, 49, 52, 53, 54, 55	90, 3.7, 2.9, 2.6 (2)	a	f	a
57, 58, 59, 60, 61	90, 3.7, 2.9, 2.6 (2)	a	a	e
6, 7, 8, 10, 11	90, 60, 6.9, 5.6 (3)	a	c	a
56	90, 60, 6.9, 5.6 (3)	a	f	d

a Number in parenthesis indicates plasmid profiles pattern

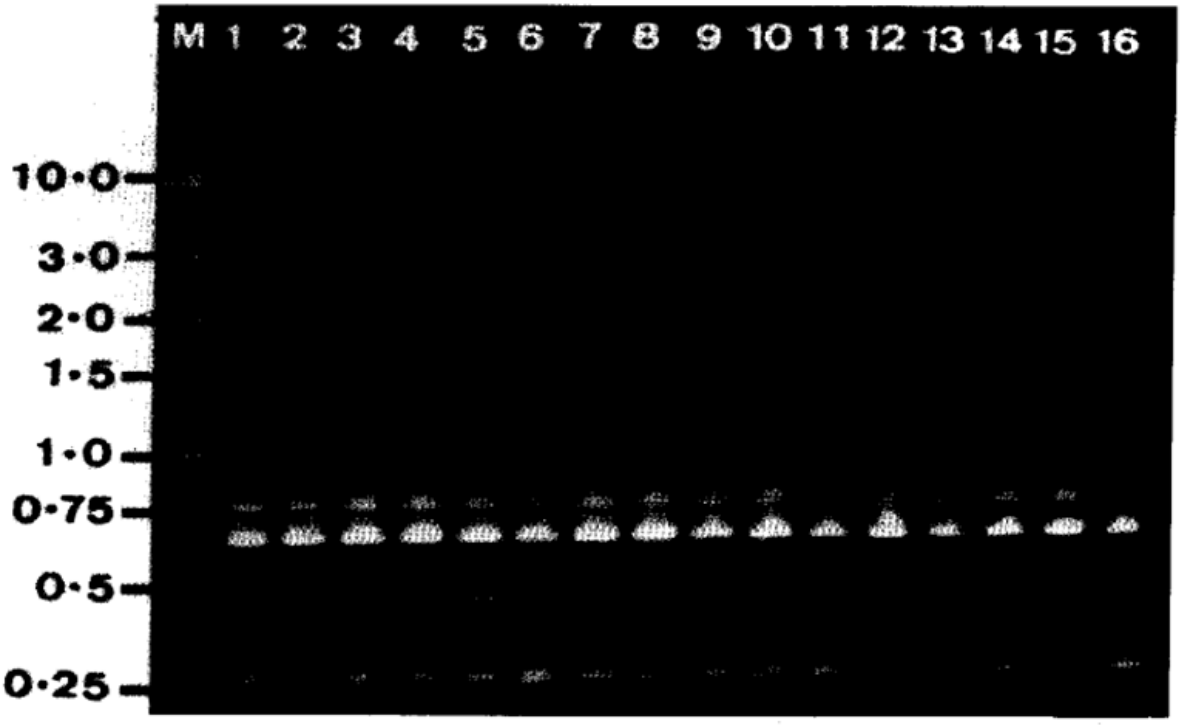

Fig. 1: Representative of the single type of RAPD-PCR patterns obtained with primer Gen1-50-02 electrophoresed on 1.2% agarose gel. Lane M, molecular weight sizes (kb) are indicated by numbers on the left

the beef samples. We reported earlier that imported beef retailed in Malaysia are reservoir for *E. coli* O157:H7 (Son *et al*., 1998). This study expand on that observation and showed that imported beef meat continues to be a source of *E. coli* O157:H7 as the present study showed an increased (47.5 versus 45% in our earlier study) in the isolation rate of this human pathogen. The increased detection of *E. coli* O157:H7 from beef does not dispose the idea that beef harbour the organism; it only reinforce the observation that this pathogen is now common at the moment in Malaysia, thus indicating the need for increased attention to microbiological safety implications associated with their consumption.

Plasmid profiles of the 70 *E. coli* O157:H7 isolates are shown in Table 1, with molecular mass ranging from 2.6 to 90.0 kb. In this study a typical predominant plasmid, the 90 kb pO157 plasmid was detected alone or in combination with other smaller plasmid(s) among the *E. coli* O157:H7 isolates, which resemble those plasmids found in *E. coli* O157:H7 in other countries (Johnson *et al.*, 1983; Karch *et al.*, 1987; Toth *et al.*, 1990; Schmidt *et al.*, 1994; Mechie *et al.*, 1997). The profiles identified may be helpful serotype-specific reference patterns for detecting and tracking individual strain with possible variations in plasmid content that are acquiring new epidemiological importance in Malaysia.

Because of the increasing importance and incidence of infections caused by *E. coli* O157:H7 worldwide, laboratories studies to track the origin and to fingerprint the organisms are interesting and important. The genetic relatedness among the *E. coli* O157:H7 strains was clarified by performing RAPD tests on total DNA preparation from all strains. Ten primers were screened with

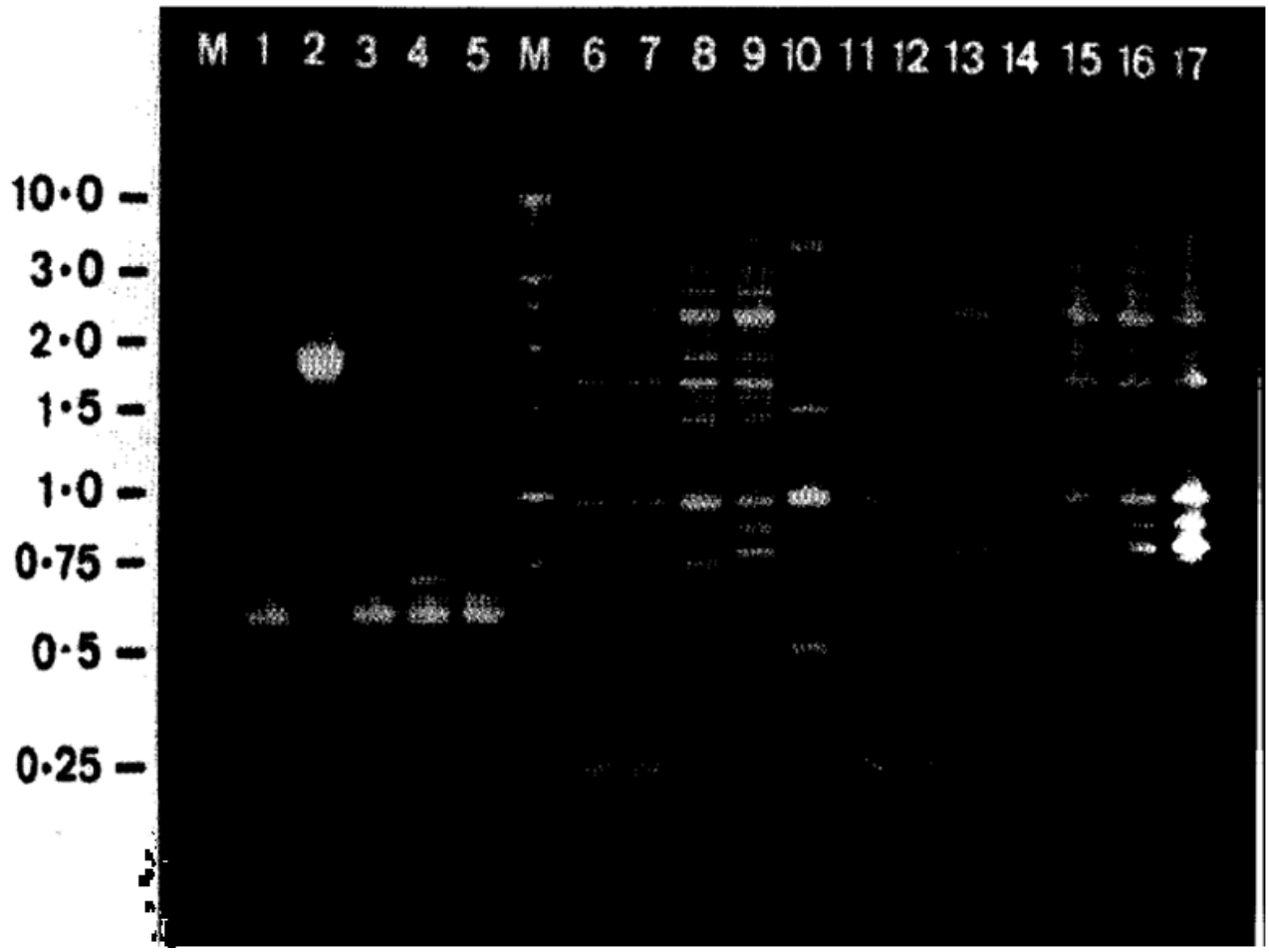

Fig. 2: Representative of the RAPD-PCR patterns obtained with primer Gen1-50-10 (Lanes: 1 [a], 2 [b], 3 [c], 4 [d] and 5 [e] and Gen1-50-09 (Lanes: 6, 7, 11 and 12 [a], 8 [b], 9 [c], 10 [d[, 13 [e], 15, 16 and 17 [f]) electrophoresed on 1.2% agarose gel. Lane M, molecular weight sizes (kb) are indicated by numbers on the left

a subset of fice isolates to detect polymorphic within *E. coli* O157:H7. Three primers (Gen1-50-02: 5′-CAATGCGTCT-3′, Gen1-50-09: 5′-AGAAGCGATG-3′ and Gen1-50-10: 5′-CCATTTACGC-3′; Genosys Biotechnologies Inc.) were studies further because they showed DNA polymorphism and were used further to analyse the whole set of 70 *E. coli* O157:H7 isolates. Representatives results of the RAPD patterns obtained using three suitable primers to detect informative arrays of PCR products are shown in Figure 1 and 2. The possible number of RAPD patterns were estimated on the basis of changes in one or more clear bands or band sizes. A single pattern was apparent from primer Gen1-50-02, whereas six and five RAPD patterns were constantly observed from primers Gen1-50-09 and Gen1-50-10. The number of RAPD bands produced for a given primer ranged from 1 to 12, with molecular sizes ranging from 0.25 to 4.0 kb. For primers Gen1-50-09 and Gen1-50-10, several strains of *E. coli* O157:H7 had indistinguishable RAPD profiles, suggesting that these strains are either duplicates or were closely related (Table 1).

In the present study, plasmid analysis allow us to recognized the presence of only three clone among the *E. coli* O157:H7 isolates as opposed to the multiple clones obtained from the RAPD results (Table 1). Although plasmid profiling was found to be less discriminatory than RAPD for this group of *E. coli* O157:H7 isolates, it provided a measure of genetic distance separating the isolates into three groups and demonstrate the value of using more than one typing technique in epidemiological investigation. The genetic diversity within this group of isolates, as determined by RAPD also demonstrated that although these isolates were isolated from a single source (raw beef), there was almost as much genetic diversity among these isolates. Because *E. coli* O157:H7 is an enteric pathogen, and meat contaminated with this pathogen have been reported as sources of disease outbreaks, surveillance of contamination in food samples in shelf stock and of retail meat is important to reduce potential health risk.

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