PREVALENCE AND MOLECULAR CHARACTERISATION OF *ESCHERICHIA COLI* O157: H7 AND NON-O157

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Introduction

Most *E. coli* strains are harmless commensals in the human guts. However, within this species, there are pathogenic strains that are associated with intestinal infection. These strains produced toxin, which causes diarrhoea in humans. Enterotoxigenic (ETEC) and enterohemorrhagic (EHEC) are among the most frequent enteric pathogen. ETEC strains produce toxin known as ST and LT, whereas EHEC strains produce toxin known as shiga-like toxin (SLT-I) and SLT-II, because of their similarity to the toxins produced by *Shigella dysenteriae* (Karmali, 1989). *E. coli* O157:H7 causes a spectrum of illness ranging from haemorrhagic colitis to hemolytic-uraemic syndrome and thrombocytopenic purpura. The majority of infections are associated with raw meat products, untreated and pasteurised milk and water, and more novel foods have become contaminated and implicated as a source of infection (Feng, 1995). Thus the food industry and public health microbiologists therefore need to screen high risk foods for *E. coli* O157: H7. The objectives of the present study were to isolate and characterise *E. coli* O157:H7 and non-O157 from clinical, environment and food sources by their plasmid profiles and antibiotic susceptibility, as well as to determine the genetic transfer of their plasmid and antibiotic resistance phenotypes. They were further typed by random amplified polymorphic DNA (RAPD) and pulsed field gel electrophoresis (PFGE).

Materials and Methods

E. coli strains isolated from clinical, environmental and food sources were identified by standard biochemical tests, and were tested for presence of the H7 antigen by using antiserum as described by the manufacturer. Isolates were screened for resistance to selected antibiotics by the disc diffusion technique. Plasmid DNA isolation were performed by the alkaline lysis method. Molecular typing of the *E. coli* strains by PFGE and RAPD techniques were performed as described previously (Son et al. 1998a). Detection of the presence of virulence factors (ST, SLT-I and SLT-II) were conducted by radioactively labelled DNA probe or by biotinylated DNA probe in hybridisation assay. Conjugation studies were conducted to determine the transferability of their plasmid and resistance phenotypes as described previously (Son et al. 1997).

Results and Discussion

A total of 586 strains of *E. coli* were isolated from beef (274), raw milk (64), duck intestines (64), poultry (76), aquatic (25), animals (30) and clinical sources (33). *E. coli* O157:H7 strains were isolated from beef samples (77 of 274) and clinical sources (3 of 33), but not from the other sources examined. The antimicrobial susceptibility analysis revealed that all the *E. coli* strains tested demonstrated resistance to one or more of the antimicrobial agents tested. Though resistance is not considered a virulence factor, the multiple resistance phenotypes of the *E. coli* strains may assist in the establishment and persistence of the organism in the host. The multiple antibiotic resistance index (MAR) of the *E. coli* strains ranged from 0.1 to 1.0, and are considered to have originated from high risk sources of contamination like humans, commercial poultry farms, swine and dairy cattle where antibiotics are often used. It is a well accepted notion that when plasmids are present in an organism, some selective advantage is operative even though plasmids, under normal circumstances, are not essential for the viability of the host. Determination of the plasmid profiles among the *E. coli* strains revealed that plasmids of 1.4 to >60 megadalton (MDa) were detected. Selected strains of *E. coli* O157:H7 and non-O157 were used for genetic analysis to perform matings with *E. coli* K12 as recipient to screen for transfer of antibiotic resistance. *Enbloc* transfer of the donor's resistance phenotypes and the concomitant transfer of plasmids ranging in sizes from 1.8 to 60 MDa were observed. In DNA hybridization assay, using radioactively labeled and biotinylated DNA probes, both clinical and beef isolates of *E. coli* O157:H7 and non-O157 were positive for virulence factors (SLT-I, SLT-II and ST, respectively) (Son et al. 1997). Twelve strains of *E. coli* O157:H7 isolated from beef were characterized by RAPD and PFGE analysis. The results obtained showed that the 12 strains were genetically distinct. An important outcome of this study is the first report on the isolation of *E. coli* O157:H7 from beef meat marketed in Malaysia (Son et al. 1998b).

Conclusions

E. coli O157:H7 and non-O157 strains possessing important virulence traits were shown to be distributed at a considerable frequency. The differences in antibiogram, plasmid profiles, RAPD and PFGE among the *E. coli* strains examined suggest that the strains may have originated from diverse sources.

References

Feng, P. 1995. *Escherichia coli* O157:H7: novel vehicles of infection and emergence of phenotypic variants. *Emerging Infectious Diseases.* 1: 47-52.

Karmali, M.A. 1989. Infection by Vero cytotoxin-producing *Escherichia coli. Clinical Microbiology Review.* 2: 15-38.

Son, R., Rusul, G., Karim, M.I.A. and Yusoff, K. 1997. Detection of enterotoxigenic *Escherichia coli* associated with diarrhoea by biotinylated heat-stable (ST) DNA probe. *Malaysian Applied Biology.* 26: 19-23.

Son, R., Zainuri, A., Rusul, G., Zaiton, H., Sahilah, A.M., Morigaki, T. and Nishibuchi, M. 1998a. Molecular typing of *Escherichia coli* O157:H7 by pulsed field gel electrophoresis. *Malaysian Applied Biology.* 27: 135-138.

Son, R., Sahilah, A.M., Rusul, G., Raha, A.R., Rozila, A. and Samuel. L. 1998b. Beef as a source multi-resistant *Escherichia coli* O157 harbouring transferable plasmid and resistance phenotype. *Journal of Bioscience.* 9: 20-27.

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