ENTEROPATHOGENIC *ESCHERICHIA COLI* IN RAW AND COOKED FOOD

A Norazah[1], I Rahizan[1], T Zainuldin[1], MY Rohani[1] and AGM Kamel[2]

[1]Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia; [2]National University of Malaysia, Jalan Raja Muda Abdul Aziz, 50300, Kuala Lumpur, Malaysia

Abstract. A total of 402 *Escherichia coli* isolates were obtained from a variety of food samples and screened for enteropathogenic *E. coli* (EPEC). Screening was carried out using 15 specific monovalent antisera from Murex Diagnostic Limited. A total of 19 *E. coli* isolates were serotyped as EPEC. The EPEC strains were shown to belong to 8 serotypes. Eight out of 19 EPEC strains belonged to serotype 018C:K77 (B21). Seventeen out of 19 of the EPEC strains were isolated from cooked food. The presence of *E. coli* in cooked food is an indicator of fecal contamination and a sign of unhygienic food handling. The presence of EPEC in food could be a potential source of food-borne outbreak. Hygiene training for every food-handler is a necessity.

INTRODUCTION

Enteropathogenic *Escherichia coli* (EPEC) has been recognised as a common cause of watery diarrhea in children (Moyenuddin *et al*, 1989; Sethi and Khuffash, 1989). A study conducted in Malaysia (Jegathesan *et al*, 1975) showed that 9% of the diarrheal cases in children under 10 years of age in Malaysia were due to EPEC. Although rare, enteropathogenic *E. coli* has also been implicated as causing gastroenteritis in adults (Schroeder *et al*, 1968).

The contamination of food by enteric pathogens is considered a particularly important cause of diarrheal diseases, most notably in developing countries. Various food items including water have been traced as sources of EPEC gastroenteritis. EPEC were also isolated from raw cheese and other milk products (Abbar and Kaddar, 1991). Hospital food was shown to contain EPEC and might have been the source for nosocomial diarrhea (Das *et al*, 1996). Cold pork, meat pies, contaminated water supply and other raw and cooked ready-to-eat food were implicated in several EPEC outbreaks (Doyle and Padhye, 1989).

While there have been extensive studies on pathogens such as *Salmonella* species in food, there is far less information on diarrheagenic *Escherichia coli* in food. This study was undertaken to determine the isolation of EPEC in raw and cooked food in Malaysia.

MATERIALS AND METHODS

Escherichia coli isolated during bacteriological examination of raw and cooked food conducted in government food and microbiology laboratories were collected and sent to the Bacteriology Division, Institute for Medical Research. The laboratories involved were the Selangor Food Quality Control Laboratory, Microbiology Laboratory of Chemistry Department, Veterinary Diagnostic Laboratory Petaling Jaya, Terengganu Food Quality Control laboratory, Perlis Food Quality Control Laboratory, Mak Mandin Food Quality Control Laboratory and Johor Food Quality Control Laboratory.

In these laboratories, the *Escherichia coli* isolates were subcultured onto nutrient agar slants and despatched to IMR where the isolates were then subcultured onto MacConkey agar. Biochemical tests were subsequently conducted to confirm the identity of *E. coli.*

Confirmed *E. coli* isolates were first serotyped with polyvalent antisera and then with 15 specific monovalent antisera for serotypes namely 018C:K77 (B21), 026:K60 (B6)), 044:K74 (L), 055:K59 (B5), 086:K61 (B7), 0111:K58 (B4), 0112:K66 (B11), 0114:K90 (B), 0119:K69 (B14), 0124:K72 (B17), 0125:K70 (B15), 0126:K71 (B16), 0127:K63 (B8), 0128:K67 (B12) and 0142:K86 (B). The slide agglutination method was conducted for detection of K antigen on the surface of the organism and the tube agglutination method was conducted for confirmation of 0 (heat stable antigen) group. The

methods were carried out following the manufacturer's recommendations.

RESULTS

A variety of food was screened for *Escherichia coli* by the government laboratories. The food was grouped into (a) cooked food which consist of Malaysian cakes (kueh), noodles, rice and cereals, vegetables, seafood, meat, sauce and gravy, (b) raw food consisting of raw vegetables, seafood and meat, (c) drinks namely soft drinks, milk and water, and (d) type of food not mentioned (Table 1).

A total of 402 *E. coli* isolates were serotyped for EPEC. Out of these isolates, 19 were shown to be EPEC. The frequency of enteropathogenic *E. coli* serotypes and the type of food from which the *E. coli* was isolated is shown in Table 2. When serotypes with the 15 specific monovalent antisera, the EPEC isolated were shown to belong to 8 serotypes. The majority of the isolates (8 out of 19) were of serotype 018C:K77 (B21) while other serotypes were encountered less frequently.

Table 1

Type and frequency of food from which *Escherichia coli* was isolated.

Type of food	Frequency of food in which *E. coli* was isolated
Cooked food:	
Malaysian cakes (kueh)	46
Noodles	19
Rice and cereals	20
Vegetables	11
Seafood	9
Meat	16
Sauce and gravy	28
Raw food:	
Vegetables and fruits	26
Seafood	6
Meat	11
Drinks:	
Soft drinks	39
Milk	35
Plain water	9
Not mentioned	127
Total	402

Except for on EPEC isolated from soft drinks and one from unknown food source, all the other EPEC were isolated from cooked food. Four out of the 8 serotypes were isolated from cooked food. Four out of the 8 serotypes were isolated from rice and cereal based food. There is no predilection of any EPEC serotypes to the type of food.

DISCUSSION

Food poisoning is a major public health concern worldwide. Mass food production, catering and wide distribution of food would undoubtedly increase the incidence of food poisoning especially if there is improper or unhygienic food handling or peparation. The food itself provides sufficient nutrients and with adequate moisture, warmth and time lapse between preparation and consumption of food, multiplication of the pathogen will inevitably take place.

Table 2

Serotype of EPEC isolated and source of food.

EPEC serotype/ type of food	No. of isolates
018C:K77 (B21)	
Noodles	3
Malaysian cakes (kueh)	4
Soft drinks	1
055:K59 (B5)	
Cooked vegetables	1
Rice and cereal	1
026:K60 (B6)	
Rice and cereal	2
086:K61 (B7)	
Rice and cereal	1
Cooked meat	1
0125:K70 (B15)	
Noodles	1
Rice and cereal	1
0128:K67 (B12)	
Not mentioned	1
0124:K72 (B17)	
Malaysian cakes (kueh)	1
0142:K86 (B)	
Noodles	1

The dose of *E. coli* isolated per gram of food was not determined in this study but these contaminated foods were at a potential to cause food poisoning if optimal growth conditions are available. The enteropathogenic *E. coli* like other pathogens may be present initially in food at harmless level, but if conditions favoring its growth is present, high doses may be obtainable causing the people who consume the food to succumb to food poisoning. In general, the dose of 10^6-10^7 organisms/gram is normally required for *E. coli* to cause infection.

In a study conducted by Jegathesan *et al* (1975), the commonest EPEC serotype isolated from pediatric diarrheal cases was 086:B7 (or 086:K61) followed by 0119:B14 (or 0119:K69). The commonest serotype of EPEC isolated from food is unknown in Malaysia. In this study the commonest serotype isolated was 018C:K77 (or 018C:B21). This serotype was not sought out in the previous study as the antisera were not yet available. Serotype 086:K61 (or 086:B7) was isolated from 2 types of cooked food while serotype 0119:K69 was not isolated in any of the food screened. However, no correlation can be made between the serotypes isolated from food and diarrheal cases because of the many variables involved including host factors, food hygiene, geographical, climatic, environmental and socio-economic factors.

In this study it was noted that most of the EPEC were isolated from cooked food. Contamination may occur during the handling of the cooked food. Even though food originating from warm-blooded animals may be contaminated with *E. coli*, contamination from human sources were found to be more common for food involved in outbreak disease (Olsvik *et al*, 1991). As *Escherichia coli* form part of human intestinal flora, its presence in food may also be a sign of unhygienic food handling and can serve as an indicator of fecal contamination. The presence of EPEC in food could be a potential source of food-borne outbreak.

Hygiene training for every food-handler is a necessity, be it for small retail business or large food production business. Each food business should have all staff trained in food hygiene, but the question arises on who to take the responsibility of educating the food handlers. Reinforcement of food safety legislation and act is one of the way to control food and premises involved in the chain from producer to consumer and heavy penalties should be given to those who do not reach the prescribed standard.

ACKNOWLEDGEMENTS

We would like to thank the Director of Institute for Medical Research for his permission to publish this article and to the food technologists of the laboratories involved in this study. This work was supported by the IRPA grant number 94-33.

REFERENCES

Abbar F, Kaddar HK. Bacteriological studies on Iraqi milk products. *J Appl Bacteriol* 1991; 71 : 497-500.

Das AS, Mazumder DN, Pal D, Chattopadhyay UK. A study of nosocomial diarrhoea in Calcutta. *Indian J Gastroenterol* 1996; 15 : 12-3.

Doyle MP, Padhye VV. *Escherichia coli*. In: Doyle MP, ed. Foodborne Bacterial Pathogens. Marcel Dekker 1989: 236-81.

Jegathesan M, Bhagwan Singh R, Kanagayaghy M, Lim ES. Enteropathogenic *Escherichia coli* diarrhoea in Malaysian children. *Southeast Asian J Trop Med Public Health* 1975; 6 : 61-7.

Moyenuddin M, Wachsmuth IK, Moseley SL, Bopp CA, Blake PA. Serotype, antimicrobial resistance and adherence properties of *Escherichia coli* strains associated with outbreaks of diarrhoeal illness in children in the United States. *J Clin Microbiol* 1989; 27 : 2234-9.

Olsvik O, Wasteson Y, Lund A, Hornes E. Pathogenic *Escherichia coli* found in food. *Int J Food Microbiol* 1991; 12 : 103-13.

Schroeder SA, Caldwell JA, Vernon TM, White PC, Granger SI, Bennett JV. A waterborne outbreak of gastroenteritis in adults associated with *Escherichia coli*. *Lancet* 1968; 1 : 737-40.

Sethi SK, Khuffash F. Bacterial and viral causes of acute diarrhoea in children in Kuwait. *J Diarr Dis Res* 1989; 7 : 85-8.