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Characterization of *Enterobacter cloacae* Isolated from Street Foods

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Abstract: A total of 78 samples comprising different types of street foods, sold in different locations in Malaysia, were examined for the presence of *Enterobacter cloacae*. *E. cloacae* contamination was recorded in 9% of the samples examined. Tests for susceptibility to 12 different antibiotics showed that all were resistant to six or more antibiotics, but susceptible to chloramphenicol and gentamicin. Plasmids of four different sizes were detected from the three plasmid positive isolates. RAPD analysis using four primers yielded completely different banding patterns for all *E. cloacae* studied. In Malaysia, no published information on street foods in the epidemiological investigation of *E. cloacae* related disease is available. However, their occurrences have provided compelling evidence that the risk of disease transmission caused by *E. cloacae* through street foods is moderate.

Keywords: Street foods, *E. cloacae*, plasmid, antibiotic resistance, RAPD, PCR

INTRODUCTION

Street foods have been defined by FAO as ready-to-eat foods and beverages prepared and/or sold by vendors especially in streets and other public places (Umoh and Odoba, 1999; Hanashiro *et al.*, 2005). The ubiquitous presence of street foods in most Asian countries is one of the fascinating aspects of urban social life. However, street foods have been reported to be contaminated by pathogens and have also been implicated in foodborne epidemics, particularly in developing countries (King *et al.*, 2000; Kubheka, *et al.*, 2001; Azanza and Ortega, 2004).

Enterobacter spp. are not primary human pathogens, but *E. cloacae* have been implicated in a broad range of clinical syndromes. The literature is replete with descriptions of bacteremia, meningitis, bloodstream infection,

sepsis, urinary tract infection, septicaemia, wound infection, infections of skin and soft tissues, central nervous system and gastrointestinal tract (Lambert-Zechovsky *et al.*, 1992; Sanders and Sanders, 1997; Dijk *et al.*, 2002; Kaminska *et al.*, 2002; Liu *et al.*, 2003; Liu *et al.*, 2004).

To the best of our knowledge, there have been no reports of outbreaks of foodborne disease caused by *E. cloacae* in Malaysia. However, assessing the prevalence of this species in street foods sold in Malaysia is important since these products are often consumed by children, students and other individuals who are forced by circumstances to eat away from their homes. Antibiotic resistance, the occurrence of plasmids, and the molecular fingerprint by Random Amplified Polymorphic DNA (RAPD) assay were used for phenotype and genotype characterization.

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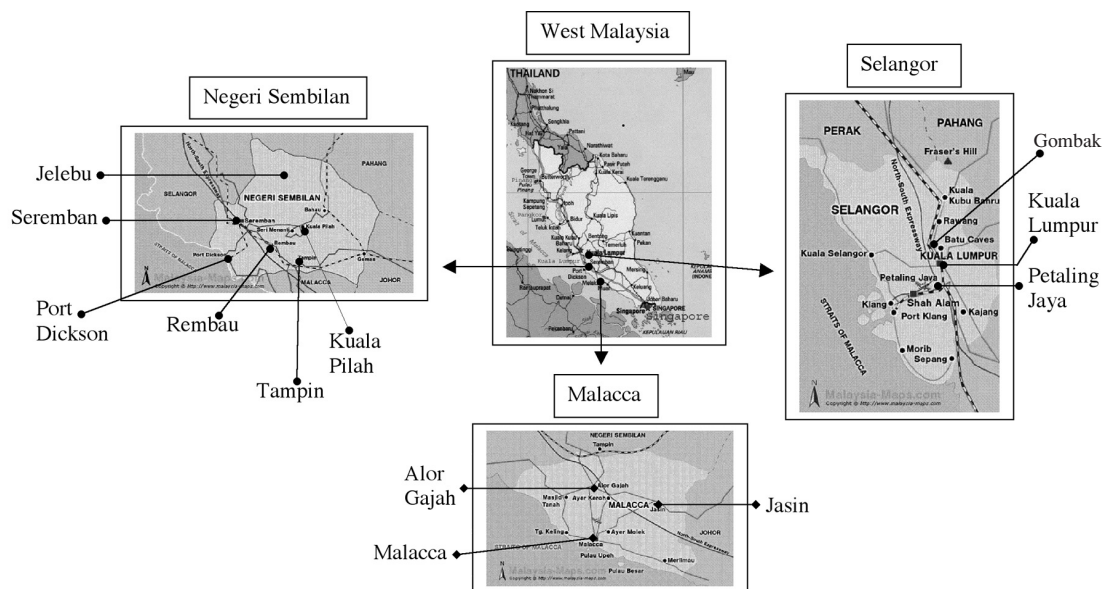


Figure 1: Sample locations

MATERIALS AND METHODS

Samples

Seventy-eight street foods, sold in different locations in Malaysia (Figure 1) were sampled during the period of January 2003 to May 2004 and were analyzed for the presence of *E. cloacae*. The types, numbers and the sources of street foods analyzed are shown in Table 1. Bacterial cultures obtained from the samples were isolated and identified using standard biochemical methods (API 20E) as described in the study carried out by Haryani *et al.* (2007).

Antibiotic Susceptibility Testing

Antibiotics susceptibility testing was done by Kirby-Bauer disk diffusion following the definition of National Committee for Clinical Laboratory Standards (NCCLS) for agar diffusion tests (NCCLS, 1997). Eleven antibiotic disks (Oxoid Ltd., Oxoid Limited Wade Road, Basingstoke, Hampshire, RG24 8PW, England) were tested, namely ampicillin,

10 µg; cefuroxime, 30 µg; chloramphenicol, 30 µg; tetracycline, 30 µg; gentamicin, 10 µg; rifampicin, 5 µg; erythromycin, 15 µg; ciprofloxacin, 5 µg; streptomycin, 10 µg; sulphamethoxazole, 25 µg; and trimethoprim, 1.25 µg. The Multiple Antibiotic Resistance (MAR) index of the isolates is defined as a/b where 'a' represents the number of antibiotics to which the particular isolate was resistant, and 'b' represents the number of antibiotics to which the isolate was exposed (Krumperman, 1983).

Plasmid Isolation

Plasmid DNA was extracted using the alkaline extraction method as described by Birnboim and Doly (1979), analysed by gel electrophoresis through 0.8% agarose gel, visualized under UV transilluminator, and photographed and recorded using the Gel Documentation System (Model Gene Genius, GMV20, Syngene). To estimate the molecular weight of plasmids, *Escherichia coli* V517 was used as molecular size markers.

Table 1
Prevalence, antibiotic resistance patterns and plasmid profiles of the seven *E. cloacae* isolates

No.	Type of street foods	Location ^a	No. of positive samples (Source)	Code	Resistance patterns ^b (Antibiotic resistance profile) (MAR index)	plasmid size ^c (plasmid profile)
1	Kuah chutney	Seremban (1)	1 (Seremban)	E1	AmpCxmTeRdESRI (RE1) (0.64)	ND
2	Tauhu sumbat	Kuala Pilah (3)	2 (Kuala Pilah)	E2 E7	AmpRdECipSRIW (RE2) (0.64) AmpRdECipSRI (RE6) (0.54)	ND 3.7 (PE1)
3	Kuih Koci	Petaling Jaya (1)	1 (Petaling Jaya)	E3	AmpTeRdESRI (RE3) (0.54)	3.7, 2.6, 2, 1.8 (PE2)
4	Kuih Lapis	Kuala Lumpur (1)	1 (Kuala Lumpur)	E4	AmpRdESRI (RE4) (0.45)	ND
5	Curry Samosa	Gombak (1)	1 (Gombak)	E5	AmpRdESRI (RE4) (0.45)	ND
6	Surimi lobster	Kuala Pilah (1)	1 (Kuala Pilah)	E6	AmpCxmTeRdECipRIW (RE5) (0.73)	2.6, 2, 1.8 (PE3)
7	Rojak Mee	Seremban (2)	none	NA	*	-
8	Kek Pandan	Seremban (1), Malacca (1), Rembau (1),		-		
9	Mee goreng	Kuala Pilah (2), Petaling Jaya (1)	none	NA	*	-
10	Ikan masak sambal	Kuala Pilah (1), Tampin (1), Rembau (1)	none	NA	*	-
11	Ayam goreng	Malacca (6), Jasin (5), Petaling Jaya (2), Seremban (3), Port Dickson (1)	none	NA	*	-
12	Air sarsi	Kuala Lumpur (1)	none	NA	*	-
13	Nasi lemak	Tampin (2), Kuala Lumpur (2), Seremban (3), Jasin (2), Port Dickson (1), Petaling Jaya (2)	none	NA	*	-
14	Kuih rengas	Kuala Lumpur (1)	none	NA	*	-
15	Nasi goreng	Alor Gajah (4), Petaling Jaya (3), Jebebu (3), Seremban (2), Port Dickson (2)	none	NA	*	-
16	Milo ais	Rembau (1), Tampin (1)	none	NA	*	-
17	Goreng kacang panjang	Jebebu (1)	none	NA	*	-
18	Hati ayam dan pedal	Malacca (1)	none	NA	*	-
19	Spring roll pastry	Gombak (1)	none	NA	*	-

Table 1: Continued

No.	Type of street foods	Location ^a	No. of positive samples (Source)	Code	Resistance patterns ^b (Antibiotic resistance profile) (MAR index)	plasmid size ^c (plasmid profile)
20	Hati ayam	Jelebu (1), Malacca (1)	none	NA	*	-
21	Ikan masak asam	Jelebu (1)	none	NA	*	-
22	Surimi prawn tail	Gombak (1)	none	NA	*	-
23	Popia goreng sambal	Rembau (1), Tampin (1)	none	NA	*	-
24	Kari kambing	Malacca (1),	none	NA	*	-

^aNumber in parenthesis indicates the number of samples examined

^bSymbols for the drugs: Amp (ampicillin); Cxm (cefuroxime); C (chloramphenicol); Te (tetracycline); Cn (gentamicin); Rd (rifampicin); E (erythromycin); Cip (ciprofloxacin); S (streptomycin); RI (sulphamethoxazole); and W (trimethoprim). ^cEstimated sizes of the plasmids are in Mega Dalton (MDa)

ND: Not-Detected

NA: Not-Applicable

*; -: Not available

RAPD-PCR Fingerprinting

Boil cell method was used to extract the genomic DNA. Out of the 11 primers screened, only 4 primers which produced clear patterns were selected (OPAR3, OPAR8, primer 640 and primer 650) for further analysis.

PCR reactions were prepared in a total volume of 25 μ l per tube, containing 20-30 ng of genomic DNA, 1.5 mM MgCl₂, 1x PCR buffer, 0.2 mM dNTPs, 5pmol primer, and 1 U of *Taq*DNA polymerase (Promega, Madison, USA). Amplification was performed in the thermal cycler (Perkin Elmer GeneAmp PCR System 2400) with a temperature program consisting of the initial denaturation at 94°C for 4 min followed by 49 cycles of denaturation at 94°C for 1 min, annealing for 1 min at 34.5°C and DNA extension at 72°C for 1 min. Final elongation was at 72°C for 10 min, followed by cooling to 4°C.

The DNA fragments produced were separated according to their sizes by electrophoresis in a 1.2% agarose gel. The gels were stained in 0.5 μ g ml⁻¹ ethidium bromide, visualized under UV transilluminator, photographed and recorded as a jpeg file (Gel

Documentation System, Model Gene Genius, GMV20, Syngene. RAPDistance Programs (Version 1.04) was used to analyse the binary code of electrophoresed agarose gels and the generated dendrogram.

RESULTS AND DISCUSSION

A total of seven isolates of *Enterobacter cloacae* were detected in six types of street foods obtained from five locations in Malaysia, revealing a prevalence of 100% in kuah chutney, curry samosa, surimi lobster, kuih lapis and kuih koci; and 66.7% in tauhu sumbat (Table 1). Of the seven *E. cloacae* isolated, two isolates were found in Kuala Pilah's foods, and none were recovered in any food from Malacca, Rembau, Tampin, Jasin, Port Dickson, Alor Gajah, and Jelebu.

The rather high prevalence rates found here may be due to a combination of improper handling of raw materials, poor personal hygiene and use of untreated and contaminated water, and inadequate cleaning of both equipments and surfaces like cutting boards, knives, utensils and floors. Although

the reservoir of *E. cloacae* which contaminated the hawker foods in the current study is unknown and no report has been published on any incidence of *E. cloacae* from street foods, especially in Malaysia, their occurrences have indicated that hawker foods can serve as a potential source of *E. cloacae* infection.

Enterobacter spp. are the sixth most common cause of nosocomial infection and antibiotic-resistant strains are observed with increasing frequency (Peters *et al.*, 2000). Based on European recommendations for antimicrobial resistance surveillance, an *E. cloacae* is one of the few bacteria which has to be monitored in healthcare facilities (Cornaglia *et al.*, 2004). Antibiotic susceptibility testing results showed that all of *E. cloacae* studied were resistant to ampicillin, erythromycin, rifampicin, and sulfamethoxazole. They demonstrated various degrees of resistance to streptomycin (85.71%), ciprofloxacin and tetracycline (42.86%), trimethoprim and cefuroxime (28.57%); but however they were susceptible to chloramphenicol and gentamicin. Six types of antibiotic resistance profiles were produced with MAR index ranging from 0.45 to 0.73. As there are strains of the same antibiotic resistance profile (RE4) isolated from different foods (kuih lapis and curry samosa) sold in different locations (Kuala Lumpur and Gombak), it is probable that there is a transmission of *E. cloacae* within the state (Selangor).

The plasmids were extracted three times to confirm the plasmid profiles of the *E. cloacae* isolates studied. A small number of strains carrying plasmids were not useful in discriminating among the strains (Al-Haddawi *et al.*, 1999) because from an epidemiological view point, the more plasmids an organism contains, the more specific is the plasmid profile as a marker for a single strain (Lihan *et al.*, 1999). In this study, plasmid profiling is of limited epidemiological value since plasmids were only detected in three out of the seven *E. cloacae* isolates. It is in contrast with those

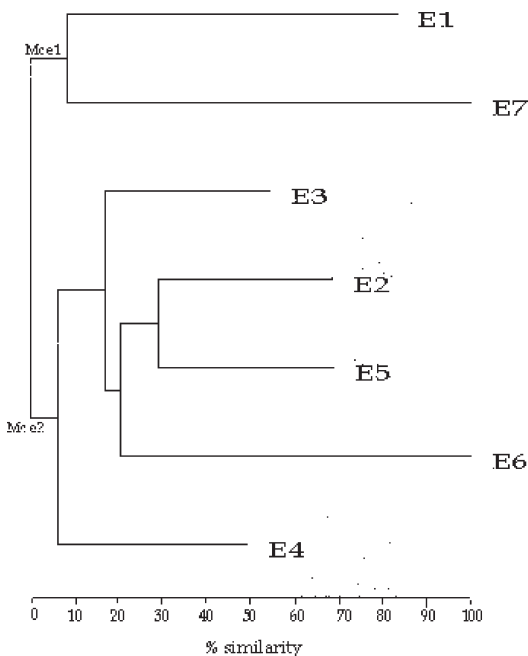


Figure 2: Dendrogram of the *E. cloacae* isolates based on RAPD patterns of the combined results obtained from primers OPAR3, OPAR8, 640 and 650

found in Taiwan where 96% of *E. cloacae* isolates examined carried plasmids (Liu *et al.*, 2004). All three plasmid positive isolates, carrying low molecular weight plasmids, were separated into 3 patterns (designated PE1 to PE3) although E6 and E7 were obtained from the same location.

Antibiotic susceptibility testing showed that *E. cloacae* isolates were resistant to more than one antibiotic with high MAR index. Generally, though the resistance is not considered to be plasmid dependent, some examples of plasmid encoded resistances have been described (Parasakthi *et al.*, 1999; Araque *et al.*, 2000; Bertrand *et al.*, 2003; Munday *et al.*, 2004). However, no correlations can be made between the presence of the plasmids and antibiotic resistances of the *E. cloacae* isolates examined in this study. Rychlik *et al.* (2000), who had detected low molecular weight plasmids among *Salmonella* Enteritidis,

was also unable to conclude any possible role of low molecular weight plasmids in the transmission of antibiotic resistance.

To determine the clonal relatedness of the isolates in this study, RAPD-PCR analysis was done using four primers namely OPAR3 (5'-CCAGGAGAAG-3'), OPAR8 (5'-TGGGGCT-GTC-3'), primer 640 (5'-CGTGGGGCCT-3'), and primer 650 (5'-AGTATGCAGC-3'). To analyse the patterns of the RAPD fragments, dendrograms were constructed using RAPDistance Programs (Version 1.04). Cluster analysis of the RAPD profiles (Figure 2) obtained of *E. cloacae* showed the presence of 2 major clusters (MCe1 and MCe2) by using the combination of four primers used. The genetic diversity observed among the *E. cloacae* isolates emphasized the value of RAPD for detecting the clonalities of this species. Looking through the dendrogram, we observed that isolates from different types of foods from different locations and even from different state (E2 and E5; E1 and E7) were clustering together. This finding indicated the spread of *E. cloacae* in street foods, but the exact mechanism of the distribution could not be determined in this study.

Out of the three methods compared, RAPD proved to be the most effective technique in discriminating the *E. cloacae* isolates as antibiogram and plasmid profiles have limited potential in differentiating them. It is in agreement with conclusions by other researchers' (Hou *et al.*, 1997) that RAPD is a discriminatory and reproducible method for typing *E. cloacae*. Among the four primers used, primer OPAR3, OPAR8, and primer 650 could distinguish the seven strains individually.

The presence of *E. cloacae* in street foods in this study indicates that a potential health risk exists and that cross-contamination to other ready to eat foods sold by the hawkers is possible by this bacterium or other closely related pathogenic members. Some measures should be taken due to the great importance of the street-food trade, such as developing and

enforcing adequate sanitary standards for street-food sales, offering training courses to street-food vendors, and applying the HACCP system as a strategy to prevent contamination.

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