INCIDENCE AND CHARACTERIZATION OF *SALMONELLA* SPECIES IN STREET FOOD AND CLINICAL SAMPLES

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ABSTRACT

The objectives of our study were to investigate the Salmonella *species contamination in various types of ready-to-eat street-vended dishes or drinks, to isolate* Salmonella *spp. from clinical samples and to assess the possible relationship between the serotypes isolated from these two different environments. The isolates were characterized by their antibiotic resistance, plasmid profiles and randomly amplified polymorphic DNA (RAPD) sequences. A total of 24 salmonellae, belonging to seven different serotypes, were isolated from 129 different street-vended foods and drinks and 12 clinical samples (rectal swabs). The encountered serotypes from street foods were* Salmonella *Biafra (*n = *8),* Salmonella *Braenderup (*n = *3) and* Salmonella *Weltevreden (*n = *1), and from clinical samples were* Salmonella *Typhi (*n = *8),* Salmonella *Typhimurium (*n = *2),* Salmonella *Paratyphi A (*n = *1) and* Salmonella *Paratyphi B (*n = *1). The results showed no similarities in the types of* Salmonella *serotypes from street food and clinical samples examined. The* Salmonella *strains were resistant to one or more of the 14 tested antibiotics. Seventeen isolates harbored plasmids, with plasmid sizes ranging from 3.0 to 38.5 MDa. RAPD*

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fingerprinting with primers OPAR3 and OPAR8 produced a combination of 21 fingerprint patterns. The dendrograms generated for the S. *Biafra and* S. *Typhi showed strains of the same serotypes but of very different types of sample and location of sampling clustered together, indicating the possibility of cross contamination during food handling.*

PRACTICAL APPLICATIONS

The identification of pathogenic microorganisms is highly crucial for surveillance, prevention and control of foodborne diseases. It is difficult to properly evaluate the status of salmonellosis in Malaysia because of the lack of detailed epidemiological studies by the veterinary and public health sector. This study was therefore aimed at determining the incidence of *Salmonella* spp. in street food and clinical samples in a limited geographical location in peninsular Malaysia. This study revealed the emergence of *Salmonella* Biafra in street foods in Malaysia, which is, to our best knowledge, the first report of S. Biafra in street foods in Malaysia. There is need for the education and training of street food vendors as well that of educating the public to maintain safe food handling practices. Because of the lack of further information on *Salmonella* isolated from street foods in Malaysia, direct comparisons with this study cannot be made. However, incidence of *Salmonella* is frequently reported in Malaysia (Noorzaleha *et al.* 2003), which shows that the potential hazards of this pathogen should not be underestimated.

INTRODUCTION

Salmonella spp. is one of the major and most common causes of foodborne illness worldwide (Cardinale *et al.* 2005). The incidence of foodborne infections caused by *Salmonella* spp. has increased dramatically during the past few years. Mrema *et al.* (2004) reported that in the U.S.A., salmonellosis is estimated to affect 1.4 million people each year, and 95% of the cases are foodborne. In Japan, salmonellosis became the most frequent health problem as indicated by the number of cases reported and the number of patients affected. Meanwhile, in Malaysia, a few recent outbreaks of salmonellosis have been reported. The *New Straits Times* (Nik and Sharifah 2005) reported that 171 people were infected with salmonellosis, which resulted in two deaths.

In developing countries, street foods in particular have been reported to be contaminated with *Salmonella* spp. and have been implicated in a few outbreaks of foodborne diseases (Mankee *et al.* 2003). To better understand

the spread of infection within outbreaks, different typing tools are being applied to characterize isolates from individual outbreaks. These include traditional typing methods such as serological typing or antibiotic resistance patterns. Currently, these techniques are being supported by molecular genetic techniques such as plasmid profiling or DNA fingerprinting (Rychlik *et al.* 2000).

In Malaysia where ready-to-eat street-vended foods are common, there is no information regarding the incidence of street foods related to *Salmonella* spp. isolation. The aims of this study were to investigate the contamination of various types of ready-to-eat street-vended dishes or drinks, to isolate *Salmonella* spp. from clinical samples and to assess the possible relationship between the serotypes isolated from these two different environmental setups.

MATERIALS AND METHODS

Isolation and Identification

From January to September 2004, 129 samples of street-vended foods and iced drinks were collected from randomly visited locations in Selangor, Federal Territory, Negeri Sembilan and Malacca, while 12 rectal swabs samples were obtained from patients suspected of having human salmonellosis at a hospital in Klang, Selangor. Two hundred grams of each food sample or 250 mL of each iced water sample was collected and immediately transported to the laboratory for analysis within 6 h of the collection. The 12 samples of the rectal swabs from patients were obtained from the forwarding laboratory of the hospital. Food samples (25 g) or iced water samples (25 mL) were homogenized in 225 mL of buffered peptone water (Oxoid, Basingstoke, U.K.), and the rectal swabs were each used to inoculate 225 mL of buffered peptone water. The pre-enrichments were incubated at 37C for 24 h, and 1 mL culture from buffered peptone water was transferred to 10 mL selenite cystine broth (Oxoid). After incubation at 37C for 24 h, 0.1 mL of the selective enrichment broth was spread plated onto xylose lysine desoxycholate agar and bismuth sulfite agar (Oxoid). The plates were incubated at 37C for 24 h, and the presumptive *Salmonella* spp. colonies obtained were identified through biochemical and serological tests.

Antibiotic Resistance

The antimicrobial resistance tests conducted were carried out following the standard disk diffusion method described by Bauer *et al.* (1966). Sensitivity to 14 different antibiotics (Oxoid Ltd., Basingstoke, Hampshire, England) was tested on all isolates. The following concentrations were used: strepto-

mycin, 10 µg/disk (S10); trimethoprim, 1.25 µg/disk (W1.25); sulphamethoxazole, 25 µg/disk (RL25); tetracycline, 30 µg/disk (TE30); cefuroxime, 30 µg/disk (CXM30); ciprofloxacin, 5 µg/disk (CIP5); ampicillin, 10 µg/disk (AMP10); chloramphenicol, 30 µg/disk (C30); gentamicin, 10 µg/disk (CN10); rifampin, 5 µg/disk (RD5); penicillin, 10 µg/disk (P10); nalidixic acid, 30 µg/disk (NA30); norfloxacin, 10 µg/disk (NOR10); erythromycin, 15 µg/disk (E15). Cultures were grown in Luria–Bertani (LB) broth (containing 2% tryptone and 0.5% yeast extract) at 37C for 18–24 h, and were swabbed onto dry Mueller–Hinton agar (Merck, Darmstadt, Germany) plates. The antibiotic disks were dispensed onto each plate and incubated at 37C for 18–24 h. Results were recorded by measuring the inhibition zones as recommended by the National Committee for Clinical Laboratory Standards (1966).

Plasmid Profiling

Plasmid DNA extraction was carried out based on the method described by Sambrook *et al.* (1989) with a minor modification. Cultures were grown in LB broth at 37C for 18–24 h, with vigorous agitation at 250 rpm. One milliliter of the cultures was centrifuged at 10,000 rpm for 1 min. The cell pellets were resuspended in 150 µL of solution I, which contained 50-mM glucose, 25-mM Tris–Cl (pH 8.0) and 10-mM ethylenediamine tetraacetic acid (pH 8.0). The suspensions were mixed by vortexing vigorously and then added with 250 µL of lysing solution (0.2-N natrium hydroxide and 1% sodium dodecyl sulphate at final concentration). The suspensions were inverted several times and then were stored in ice for 5 min. One hundred fifty microliters of solution II (60 mL 5-M potassium acetate, 11.5 mL glacial acetic acid and 28.5 mL distilled water) was added, and the mixtures were inverted several times before being incubated in ice for 5 min and centrifuged at 12,000 rpm for 5 min. The clear supernatants were carefully transferred into new micro centrifuge tubes and were added with 600 µL cold isopropanol. The mixtures were vortexed and were allowed to stand at room temperature for 2 min before being subjected to centrifugation at 12,000 rpm for 5 min. The precipitates were rinsed in 1 mL 70% ethanol and further subjected to centrifugation at 12,000 rpm for 1 min. The clear pellets were left to dry and resuspended with 70 µL sterile distilled water. The extracted plasmids DNA were subjected to agarose gel electrophoresis.

Randomly Amplified Polymorphic DNA (RAPD)-Polymerase Chain Reaction (PCR) and Analysis

Boiling cell method was carried out to prepare the DNA template from the *Salmonella* spp. isolates. Initial primer screening was conducted using

Gold Oligo (Research Biolabs, Singapore) OPAR3 (CCAGGAGAAG), OPAR4 (GTGAATGCGG), OPAR8 (TGGGGCTGTC) and OPAR10 (CCATT TACGC), and based on the number of bands generated and the quantity of reproducible patterns yielded; OPAR3 and OPAR8 were selected to be used in this study. Amplification reactions were performed in 25-µL volumes containing 1.5 µL of total DNA, 2.5 µL 10X buffer (30-mM KCl, 10-mM Tris–HCl pH 8.3), 1.5 µL $MgCl_2$, 0.5 µL 10-mM dNTP, 0.2 µL 0.5-unit *Taq* polymerase (Bioron GmbH, Dt. Steuer-Nr., Germany), 17.8 µL sterile distilled water, and 1 µL of primer. The amplification reaction was carried out using the Gene Amp PCR system 2400 thermocycler (PerkinElmer, Norwalk, CT) with the temperature program consisting of predenaturation at 94C for 4 min at the start of 45 cycles, denaturation at 94C for 1 min, annealing at 35C for 1 min and extension at 72C for 2 min. The cycles were followed with a final elongation step at 72C for 7 min. The PCR products were applied to gel electrophoresis. The banding patterns were scored based on the presence or absence of the bands and the data obtained were recorded and entered in RAPDistance (version 1.4) software (Research School of Biological Sciences, Australian National University, Canberra, Australia) where a dendrogram was produced for further analysis. Clustering was based on the unweighted pair of group average method.

RESULTS

Of the street foods examined in this study, 9.3% (12/129) were positive for *Salmonella* spp. None of the iced water drinks were positive for *Salmonella* spp. However, all the fecal swab samples from patients in a hospital were positive for the isolation of *Salmonella* spp. Table 1 lists all the samples examined from various locations in the states of Selangor, Federal Territory, Malacca and Negeri Sembilan. Table 2 shows the prevalence of different *Salmonella* serotypes in street food and clinical samples, respectively. Among the 12 *Salmonella* isolates from street food samples, *Salmonella* Biafra ($n = 8$) was the predominant serovar, followed by *Salmonella* Braenderup ($n = 3$) and *Salmonella* Weltevreden ($n = 1$). Of the clinical isolates, the most common serovar was *Salmonella* Typhi ($n = 8$), followed by *Salmonella* Typhimurium ($n = 2$), and *Salmonella* Paratyphi A ($n = 1$) and *Salmonella* Paratyphi B ($n = 1$).

Generally, the isolates from the street food samples were resistant to a greater number of antibiotics as indicated in Table 2. All strains of *S.* Biafra were resistant to at least two antibiotics. Meanwhile, the *S.* Braenderup isolates exhibited resistance to the combination of rifampin, sulphamethoxazole, erythromycin, nalidixic acid or gentamicin. The *S.* Weltevreden isolate

TABLE 1.
INCIDENCE OF *SALMONELLA* SPP. IN THE VARIOUS STREET FOOD AND CLINICAL SAMPLES EXAMINED

Samples examined	Type of dish	Location (no. of samples examined)	No. of samples positive for *Salmonella* spp.	*Salmonella* spp. identity (no. of isolates obtained)
Street food samples				
Air sarsi ais	Drinks	Kuala Lumpur (3)	–	BD
Ayam goreng	Chicken dish	Malacca Town, Malacca (6)	–	BD
		Jasin, Malacca (5)	1	*Salmonella* Biafra (1)
		Petaling Jaya, Selangor (2)	–	BD
		Seremban, Negeri Sembilan (3)	–	BD
		Port Dickson, Negeri Sembilan (2)	–	BD
Curry Samosa/Lily	Vegetable dish	Gombak, Selangor (2)	–	BD
Popia goreng sambal	Vegetable dish	Rembau, Negeri Sembilan (3)	–	BD
		Tampin, Negeri Sembilan (3)	–	BD
Goreng kacang panjang	Vegetable dish	Jelebu, Negeri Sembilan (3)	–	BD
Hati ayam	Chicken dish	Jelebu, Negeri Sembilan (2)	–	BD
		Malacca Town, Malacca (2)	–	BD
Ikan masak asam	Fish dish	Jelebu, Negeri Sembilan (2)	–	BD
Kari kambing	Beef dish	Malacca Town, Malacca (2)	–	BD
		Seremban, Negeri Sembilan (3)	–	BD
Kerabu jantung pisang	Vegetable dish	Jasin, Malacca (5)	3	*Salmonella* Braenderup (3)
Kuah chatni	Vegetable dish	Malacca Town, Malacca (2)	–	BD
		Rembau, Negeri Sembilan (2)	–	BD
Kuih lapis	Cake	Kuala Lumpur (3)	–	BD
Kuih koci	Cake	Petaling Jaya, Selangor (3)	–	BD
Kuih rengas	Cake	Kuala Lumpur (3)	–	BD
Sambal ikan	Fish dish	Bahau, Negeri Sembilan (9)	6	*S.* Biafra (5) *Salmonella* Weltevreden (1)
Pedal ayam	Chicken dish	Malacca Town, Malacca (2)	–	BD

Mee goreng	Noodle dish	Malacca Town, Malacca (2)	–	BD
		Rembau, Negeri Sembilan (2)	–	BD
		Kuala Pilah, Negeri Sembilan (2)	–	BD
		Petaling Jaya, Selangor (2)	–	BD
Air milo ais	Drinks	Rembau, Negeri Sembilan (2)	–	BD
		Tampin, Negeri Sembilan (2)	–	BD
Nasi goreng	Rice	Alor Gajah, Malacca (4)	–	BD
		Petaling Jaya, Selangor (3)	–	BD
		Jelebu, Negeri Sembilan (3)	–	BD
		Seremban, Negeri Sembilan (2)	–	BD
		Port Dickson, Negeri Sembilan (2)	–	BD
Nasi lemak	Rice	Tampin, Negeri Sembilan (2)	–	BD
		Port Dickson, Negeri Sembilan (2)	–	BD
		Seremban, Negeri Sembilan (3)	–	BD
		Jasin, Malacca (2)	–	BD
		Petaling Jaya, Selangor (2)	–	BD
		Kuala Lumpur (2)	–	BD
Rojak mee	Noodle dish	Seremban, Negeri Sembilan (2)	–	BD
Sayur campur	Vegetable dish	Bahau, Negeri Sembilan (5)	2	*S*. Biafra (2)
Spring roll pastry	Vegetable dish	Gombak, Selangor (2)	–	BD
Surimi lobster	Prawn dish	Kuala Pilah, Negeri Sembilan (2)	–	BD
Surimi prawn tail	Prawn dish	Gombak, Selangor (2)	–	BD
Tauhu sumbat	Vegetable dish	Kuala Pilah, Negeri Sembilan (3)	–	BD
Clinical samples				
Rectal swab		Klang, Selangor (12)	12	*Salmonella* Typhi (8) *Salmonella* Paratyphi A(1) *Salmonella* Paratyphi B(1) *Salmonella* Typhimurium (2)

Total number of samples analyzed = 129 street food samples, 12 clinical samples.
–, negative for *Salmonella* spp.
BD, below detection.

TABLE 2.
SALMONELLA SPP. ISOLATED FROM STREET FOOD AND CLINICAL SAMPLES, ANTIBIOTIC RESISTANCE PATTERNS, PLASMID SIZES AND RAPD TYPE

Code	Sample	Source	Species name	Resistance (MAR index)	Plasmid sizes (MDa)	RAPD type
SF1	Sambal ikan	Bahau, Negeri Sembilan	*Salmonella* Biafra	RdEP (0.21)	–	1
SF2	Sambal ikan	Bahau, Negeri Sembilan	*S.* Biafra	RdRlNor (0.21)	35.8	2
SF3	Kerabu jantung pisang	Jasin, Malacca	*Salmonella* Braenderup	RdRl (0.14)	6.3	3
SF4	Kerabu jantung pisang	Jasin, Malacca	*S.* Braenderup	RdERlNaCn (0.36)	35.8, 6.3	3
SF5	Sambal ikan	Bahau, Negeri Sembilan	*S.* Biafra	RdERlPS (0.36)	38.5	4
SF6	Kerabu jantung pisang	Jasin, Malacca	*S.* Braenderup	RdERlNa (0.29)	35.8, 33.0	5
SF7	Sambal ikan	Bahau, Negeri Sembilan	*S.* Biafra	RdRl (0.14)	38.5	4
SF8	Sayur campur	Bahau, Negeri Sembilan	*S.* Biafra	RdEP (0.21)	–	6
SF9	Ayam goreng	Malacca Town, Malacca	*S.* Biafra	RdERlNor (0.29)	–	7
SF10	Sambal ikan	Bahau, Negeri Sembilan	*S.* Biafra	RdES (0.21)	35.8	4
SF11	Sayur campur	Bahau, Negeri Sembilan	*S.* Biafra	RdEP (0.21)	33.0	8
SF12	Sambal ikan	Bahau, Negeri Sembilan	*Salmonella* Weltevreden	RdERlPSCTe (0.50)	33.0	9
C1	Rectal swab	Selangor	*Salmonella* Typhi	RdERlPSW (0.43)	6.3	10
C2	Rectal swab	Selangor	*S.* Typhi	RdCip (0.14)	–	11
C3	Rectal swab	Selangor	*Salmonella* Typhimurium	RdERl (0.21)	–	12
C4	Rectal swab	Selangor	*Salmonella* Paratyphi A	RdERlPC (0.36)	3.4	13
C5	Rectal swab	Selangor	*S.* Typhi	RdRlPSAmp (0.36)	3.0	14
C6	Rectal swab	Selangor	*S.* Typhi	RdERlS (0.29)	–	15
C7	Rectal swab	Selangor	*S.* Typhi	RdCAmp (0.21)	–	16
C8	Rectal swab	Selangor	*S.* Typhi	RdRlPSNaW (0.43)	3.4	17
C9	Rectal swab	Selangor	*S.* Typhimurium	RdERlCCn (0.36)	12.5	18
C10	Rectal swab	Selangor	*S.* Typhi	RdE (0.14)	–	19
C11	Rectal swab	Selangor	*S.* Typhi	RdRlS (0.21)	4.0	20
C12	Rectal swab	Selangor	*Salmonella* Paratyphi B	RdE (0.14)	–	21

Total number of samples analyzed = 129 street food samples, 12 clinical samples.
Antibiotic agents: Rd, rifampin; E, erythromycin; Rl, sulphamethoxazole; P, penicillin; S, streptomycin; C, chloramphenicol; Na, nalidixic acid; W, trimethoprim; Amp, ampicillin; Cn, gentamicin; Nor, norfloxacin; Te, tetracycline; Cip, ciprofloxacin; CXM, cefuroxime.
MAR, multiple antibiotic resistance; RAPD, randomly amplified polymorphic DNA.

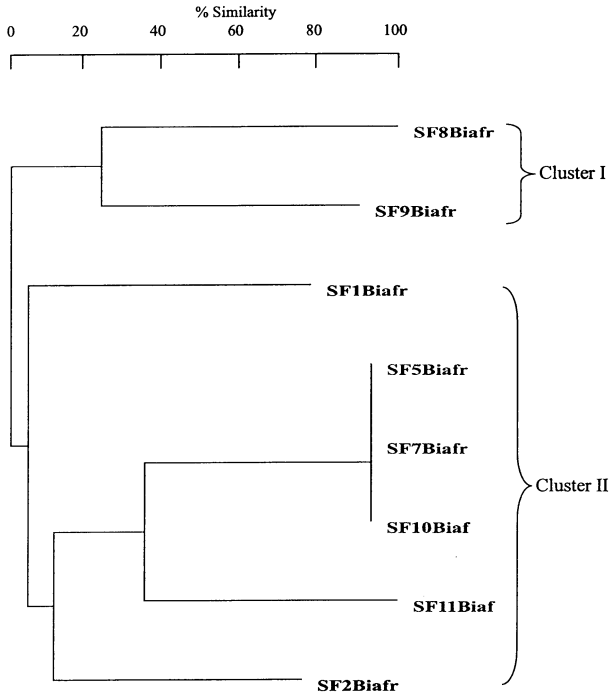

FIG. 1. RANDOMLY AMPLIFIED POLYMORPHIC DNA DENDROGRAM OF *SALMONELLA* BIAFRA FROM STREET FOOD SAMPLES USING PRIMERS OPAR3 AND OPAR8
Total number of isolates analyzed = 8.

obtained from sambal ikan was resistant to rifampin, erythromycin, sulphamethoxazole, penicillin, streptomycin, chloramphenicol and tetracycline. *S.* Typhi isolates had more diverse patterns of antibiotic resistance. The eight isolates of *S.* Typhi isolated from clinical samples exhibited eight profiles of antibiotic resistance, and all the isolates were resistant to at least two antibiotics. Plasmids ranging in size from 3.0 to 38.5 MDa (figures not shown) were detected among the *S.* Biafra, *S.* Braenderup, *S.* Weltevreden, *S.* Typhi, *S.* Typhimurium and *S.* Paratyphi A isolates. However, plasmids were not detected in *S.* Paratyphi B.

In the RAPD-PCR analysis of the isolates, the combination of the results obtained using primers OPAR3 and OPAR8 yielded a total of 21 different RAPD banding patterns (Table 2). The RAPD results were analyzed using RAPDistance software to generate dendrograms for strains of *S.* Biafra and *S.* Typhi (Figs. 1 and 2). The dendrogram of the *S.* Biafra generated two major clusters. In Cluster I, SF8 (sayur campur from Bahau, Negeri Sembilan) and

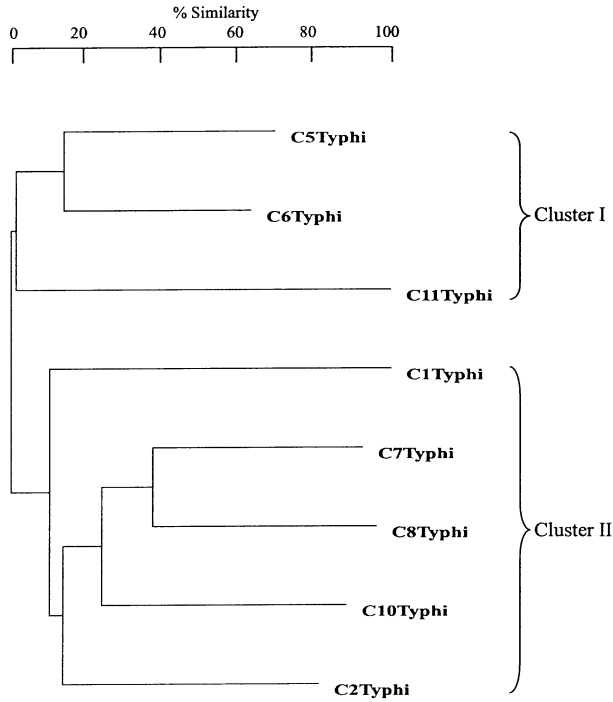

FIG. 2. RANDOMLY AMPLIFIED POLYMORPHIC DNA DENDROGRAM OF *SALMONELLA* TYPHI FROM CLINICAL SAMPLES USING OPAR3 AND OPAR8
Total number of isolates analyzed = 8.

SF9 (ayam goreng from Malacca Town, Malacca) were clustering together. Meanwhile, in Cluster II, SF1 (sambal ikan from Bahau, Negeri Sembilan) was a single isolate. SF5, SF7 and SF10 from sambal ikan in Bahau, Negeri Sembilan were 100% similar, and they formed a larger cluster with SF2 from sambal ikan and SF11 from sayur campur in Bahau, Negeri Sembilan. The RAPD dendrogram of *S.* Typhi from clinical samples (Fig. 2) showed two major clusters in which Cluster I consisted of C5, C6 and C11, and Cluster II consisted of C1, C2, C7, C8 and C10.

DISCUSSION

From the results obtained in this study, different *Salmonella* serotypes were isolated from 12 of the 129 street food samples and all the 12 clinical samples (Table 1). In a study by Cardinale *et al.* (2005), *Salmonella* spp. was

regularly isolated from ready-to-eat poultry dishes of the street foods examined. In previous works carried out by Noorzaleha *et al.*, 18 and 31 different serotypes of *Salmonella* spp. were isolated from poultry and vegetable samples, respectively (Noorzaleha 2003). When compared to other studies, the *Salmonella* serotypes recovered from the street foods examined in this study were different. Generally, the difference in the reported incidences and the serotypes recovered could be associated with the sampling plan procedures, sample type, and distribution of salmonellae in the lot examined and method of detection employed. This assumption can be corroborated by an earlier study by Noorzaleha *et al.* (2003), in which the methods (media, temperature and enrichment broth) used were found to have influence on the types of *Salmonella* serotypes recovered from vegetables and poultry samples examined. Although the more severe disease-causing *S.* Typhi, *S.* Typhimurium, *S.* Paratyphi A and *S.* Paratyphi B serotypes are only isolated from the clinical samples, the nontyphoidal salmonellae (*S.* Biafra, *S.* Weltevreden and *S.* Braenderup) isolated from street foods are also known to produce foodborne salmonellosis in humans in developed and developing countries (Miller *et al.* 2000). The observation in this study in which the clinical samples were positive for *S.* Typhi, *S.* Paratyphi A and *S.* Paratyphi B may be factors associated with the frequency of typhoid and paratyphoid fever in Malaysia.

The prevalence of *Salmonella* spp. was greatest in sambal ikan followed by kerabu jantung pisang and sayur campur. However, the poultry dishes of the street foods had very low prevalence with only one sample positive for *Salmonella* spp. (Table 1). Other studies have reported that *Salmonella* spp. were most prevalent in poultry, especially chicken and eggs (Mare *et al.* 2001; Lim *et al.* 2005). *S.* Weltevreden, *S.* Braenderup, *S.* Typhimurium and *S.* Weltevreden were among the various serotypes detected in poultry, raw milk and clinical sources in Malaysia (Rusul *et al.* 1996; Yassin *et al.* 1997; Noorzaleha *et al.* 2003; Chye *et al.* 2004). The serotypes recovered from street food samples were different from the serotypes in clinical samples, indicating a possible different mode of infection and transmission of the serotypes from different sources. *S.* Biafra is less frequently encountered throughout the world but is isolated in Malaysia. This is the first report of *S.* Biafra in street foods in Malaysia. *S.* Biafra was first isolated from a province in Nigeria in 1968 (Van and Du 1968). According to the websites report of the United Arab Emirates Agriculture Information Centre, *S.* Biafra was isolated from ducks in Sharkia Governorate (United Arab Emirates Agriculture Information Centre 2005).

Greater education and training of street food vendors may decrease the incidence of *Salmonella* spp. detected in street food samples. According to Kubheka *et al.* (2001), lack of hygiene was not a major determinant of the

quality and safety of ready-to-eat street foods, but instead, they reported that short holding times of the prepared foods were instrumental in reducing the growth of the bacterial populations. Street-vended foods have been implicated in outbreaks in many countries (Dawson and Canet 1991; Ries *et al.* 1992; Weber *et al.* 1994). Foodborne illness was estimated to be the cause of 76 million illnesses, 325,000 hospitalizations and 5,000 deaths in the U.S.A. annually (Mead *et al.* 1999; Badrie *et al.* 2005). In Malaysia, 14 deaths were reported in October 1988 in the Malaysian state of Perak and were attributed to the consumption of loh see yun (rice noodles) bought from different vendors (Perdigon 1990). In this study, it was observed that many of the street food vendors targeted for sampling operate from strategic locations at all hours of the day and night. In some cases, it was also observed that some of the vendors have limited infrastructure, with restricted access to drinking water, toilets, water disinfecting methods, refrigeration, ice or hand washing and waste disposal facilities. For example, the washing of hands, utensils and dishes is often done in the same bucket or bowl. In some cases, the raw materials are observed to be of generally poor quality, and foods are stored in temperatures that are not safe for a long period of time. Street food vending plays an important role in the economies of developing countries. As much as 25–30% of the household expenditure is spent on street foods in Kuala Lumpur (Malaysia) and Iloilo in the Philippines, respectively (Barth 1985; Winarno and Allain 1986). Perdigon (1990) reported that in Penang (Malaysia), a hawker could be earning an average annual income of RM18, 864 (U.S.$5,113). With a total of 5,030 hawkers participating in the survey, the estimated gross sales of RM95 million (U.S.$25.7 million) is estimated for one city (Penang alone) in 1 year. Thus, for the whole of Malaysia, the values of the annual sales of the street foods are estimated to be as high as U.S.$2.2 billion (Dawson and Canet 1991).

A total of 14 antimicrobial agents were used in this study. Isolates from the clinical samples gave 12 antibiotic resistance patterns, while isolates from the street food samples produced only 10 antibiotic resistance patterns (Table 2). All the isolates of the various serotypes examined displayed multiple resistances. All of the isolates were found to be resistant to at least two antibiotics, namely, rifampin and erythromycin. This pattern was uniform among the isolates from street food samples and clinical samples even though they were sampled on different locations and occasions, thus indicating the spread of antimicrobial resistance (Bartoloni *et al.* 2005). Antibiotic resistance has been suggested to result from a variety of factors, including escalating use of broad-spectrum antibiotic agents in human medical practice, in animal health and in food production, and also the increasing reservoirs of pathogens among patients who are unable to completely clear infections because of underlying immune disorders. In some countries, insusceptibility rates are

greater because antibiotics are available on an over-the-counter basis and hence tend to be used inappropriately. Furthermore, poor hygiene standards may lead to contamination of food products with strains of resistant pathogens (Current Topics 1995).

In the majority of cases, infection with *S.* Typhi, the causative agent for typhoid fever and, in this study, the serotype with the most number of isolates recovered from clinical samples, is not lethal if effective antibiotic therapy is administered in a timely fashion (Mills-Robertson *et al.* 2002). Chloramphenicol is the "gold standard" agent for the treatment of *S.* Typhi infection (Shanahan *et al.* 1998), but like many pathogens in today's world, chloramphenicol-resistant strains have emerged (Mills-Robertson *et al.* 2002). With the emergence of chloramphenicol-resistant strains, ampicillin and sulphamethoxazole are considered suitable alternatives. In this study, one *S.* Typhi isolate was resistant to chloramphenicol, while two isolates were resistant to ampicillin and six isolates showed resistance to sulphamethoxazole. While issues of antimicrobial use and concerns about antimicrobial resistance have garnered considerable attention in the scientific community and in the general public, little attention is given on the economic impacts of antimicrobial data. The limited information based on reliable data on antibiotic insusceptibility for low-resource countries is usually from studies conducted on pathogens isolated during disease outbreaks or from community- or hospital-acquired infections observed in the few health centers where high-quality laboratories are available (Bartoloni *et al.* 2005).

Plasmid profiles are very useful when plasmids (preferably more than two) are present in most of the isolates examined. In this study, all the *Salmonella* isolates were grouped into specific profiles based on the plasmid sizes. It was observed that the *Salmonella* strains isolated from street food and clinical samples examined in this study are made up of a heterogenous group of isolates exhibiting many different plasmid profiles. There were no plasmid profiles common to all isolates that could be used as clonal marker. There was no correlation between plasmid content and source of isolates, and no correlation could be made on the presence of plasmid with their antibiotic resistance patterns.

From the RAPD dendrograms generated in this study, different strains of the same serotypes could be differentiated. In the RAPD dendrogram of *S.* Biafra from street food samples (Fig. 1) where two major clusters were produced, it was observed that most of the strains tend to cluster together based on the source of samples and location of sampling. SF8 and SF9 in Cluster I were clustered together although from different types of food and locations, indicating the possibility of cross contamination of food probably during food handling. In Cluster II, all the strains were of sambal ikan that were collected from Bahau, Negeri Sembilan, with the exception of SF11,

which was sayur campur. Even though they were of the same type of samples and locations, the strains could be differentiated, in which SF1 and SF2 were of different strains, while SF5, SF7 and SF10 were 100% similar. As for the RAPD dendrogram of *S.* Typhi from clinical samples (Fig. 2), all the strains were found to be different, although they were of the same types of clinical samples collected from the same hospital. The clustering showed evidence of the possibility that the patients might have been infected with this *S.* Typhi from different places and sources.

CONCLUSION

The prevalence of *Salmonella* spp. in this study indicates that the potential risk posed by street foods cannot be ignored. The incidence of salmonellae was not limited to a particular location, as salmonellae were present in various samples and from different locations, indicating the potential problem of spread of *Salmonella* spp.

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