Characterization of *Salmonella* enterica Isolated From Street Food and Clinical Samples in Malaysia

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Abstract: *Salmonella* enterica is one of the major causes of bacterial foodborne infection. The aims of this study were to determine the antibiotic resistance and the genetic diversity of *Salmonella* enterica isolated from street foods and clinical samples and to understand the correlation between the prevalence of serovars and genotypes with their source (street food and clinical samples) and geographic origin (Negeri Sembilan, Malacca and Selangor in Peninsular Malaysia). The enterobacterial repetitive intergenic consensus (ERIC) PCR analysis distinguished the *Salmonella* isolates into 19 ERIC types, with one untypable isolate. Dendrograms were specifically constructed for the *S.* Biafra and *S.* Typhi isolates. Identical or very similar ERIC types among the *S.* Biafra isolates from street food samples indicate transmission of the *S.* Biafra among the street foods, as well as possible cross-contamination of the street foods. In addition, the identical or very similar ERIC types among the *S.* Typhi isolates from human samples examined suggest possible similarity in their source of infection. All the twenty four isolates were resistant to rifampin and none were resistant to cefuroxime. Most isolates displayed multiple resistances. Dendrogram of antibiotic resistances produced six clusters, with similarity levels between 18.8% and 100%. Generally, street food and clinical isolates tend to cluster apart. Dendrogram to cluster the antibiotic groups showed that they could be grouped according to classes based on mode of inhibition. The findings suggest that street food contaminated with drug-resistant *Salmonella* enterica can be an important factor in the continuous emergence of antibiotic resistant *Salmonella* enterica.

Key words: *Salmonella* enterica, street food, clinical sample, antibiotic resistance, clustering analysis, ERIC-PCR

INTRODUCTION

Salmonella is considered to be the cause of the largest number of outbreaks, cases, and fatalities that result from food borne infections among enteric pathogens. Mrema *et al.* (2004) reported that in the United States, salmonellosis is estimated to affect 1.4 million people each year, and 95% of the cases are food borne. In developing countries, street foods in particular have been reported to be contaminated with *Salmonella* and have been implicated in a few outbreaks of food borne diseases (Mankee *et al.,* 2003). Salmonellosis is caused by the two species of *Salmonella*; *Salmonella* enterica and *Salmonella* bongori, and it is an infectious disease of humans and animals.

Antibiotics such as chloramphenicol, ampicillin and sulphamethoxazole are first-line antibiotics used for the treatment of salmonellosis. However, *Salmonella* strains

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which are resistant to these first-line antibiotics have recently emerged worldwide, and is causing great concern. With that increase, the risk to public health has also increased (Current Topics, 1995). It is particularly serious in low-resource countries where bacterial infections remain among the major causes of death, especially in childhood (Bartoloni *et al.,* 2005). Surveillance of antibiotic resistance is a key element for providing updated information on the magnitude and trends in resistance and for planning and monitoring intervention strategies targeted at preserving the therapeutic efficacy of antimicrobial agents.

A variety of DNA-based genotyping methods are now been utilized to delineate epidemiological relationships between various isolates. Rapid typing methods are needed to differentiate *Salmonella* spp. for the identification of sources of contamination in food-processing, and also for epidemiological surveillance and the monitoring of food borne outbreaks (Lim *et al.*, 2005). Enterobacterial repetitive intergenic consensus (ERIC) sequence is a short interspersed repetitive nucleic-acid sequence which was originally found in *Escherichia coli* and *S.* Typhimurium. Recently, this technique has been proven to be useful in epidemiological studies of various isolates. However in Malaysia, to the best of our knowledge there is no information on antibiotic resistance and other characterization of *Salmonella* species isolated from street foods. Thus, this study was carried out to characterize *Salmonella* isolates using antibiotic resistance and ERIC.

MATERIALS AND METHODS

Bacterial Strains Used

The 24 isolates of *Salmonella* examined for antibiotic resistance in this study were isolated from street food and clinical samples (Tunung *et al.*, 2007). 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 with human salmonellosis at a hospital in Klang, Selangor. Food samples (25g) or iced water samples (25 ml) were homogenized in 225 ml of buffered peptone water (Oxoid, Basingstoke, UK) and the rectal swabs were each used to inoculate 225 ml of buffered peptone water. The pre-enrichments were incubated at 37°C for 24 hours, and 1 ml culture from buffered peptone water was transferred to 10 ml selenite cystine broth (Oxoid, Basingstoke, UK). After incubation at 37°C for 24 hours, 0.1 ml of the selective enrichment broth was spread-plated on xylose lysine desoxycholate agar (XLD) and Bismuth Sulfite Agar (BSA) (Oxoid, Basingstoke, UK). The plates were incubated at 37°C for 24 hours and the presumptive *Salmonella* spp. colonies obtained were identified through biochemical and serological tests.

Antibiotic Resistance Test

The 24 isolates of *Salmonella* were grown overnight at 37°C. The antimicrobial resistance tests conducted was carried out following the standard disc diffusion method described by Bauer *et al.* (1966). The antibiotic susceptibility discs used for the tests were purchased from Oxoid Ltd., England. Sensitivity to 14 different antibiotics was tested on all isolates. The following concentrations were used; streptomycin 10 μg/disc (S10), trimethoprim 1.25 μg/disc (W1.25), sulphamethoxazole 25 μg/disc (RL25), tetracycline 30 μg/disc (TE30), cefuroxime 30 μg/disc (CXM30), ciprofloxacin 5 μg/disc (CIP5), ampicillin 10 μg/disc (AMP10), chloramphenicol 30 μg/disc (C30), gentamicin 10 μg/disc (CN10), rifampin 5 μg/disc (RD5), penicillin 10 μg/disc (P10), nalidixic acid 30 μg/disc (NA30), norfloxacin 10 μg/disc (NOR10), erythromycin 15 μg/disc (E15). Results were recorded by measuring the inhibition zones as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2004).

Antibiotic Resistance Clustering Analysis

Associations between the resistance profiles obtained for each isolates were performed using the software package BioNumerics Version 4.5 (Applied Maths, Kortrijk, Belgium) and employing Pearson correlation coefficient and UPGMA for dendrogram construction (Lopes *et al.*, 2005). All the results obtained were coded using '0' for susceptible and intermediate and '1' for resistant phenotypes for each antimicrobial drug.

ERIC-PCR

Cultures were grown in Luria Bertani broth at 37°C overnight. Template preparation was carried out by the boiling method (Baumler *et al.*, 1997). The DNA extracts were amplified using primers ERIC1 (ATGTAAGCTCCTG GGGATTCAC) and ERIC2 (AAGTAAGTGA CTGGGGTGAGCG) (Research Biolabs). Amplification reactions were performed in 25 µl volume containing 2.5 µl 10X reaction buffer, 1.5µl 2 mM MgCl$_2$, 1 mM each of dNTP, 1.0 µl 5 μM each of the forward and reverse primers, 0.5 μl 2.5 units of *Taq* DNA polymerase and 2.0 μl template DNA. The ERIC – PCR amplification was performed using a Perkin Elmer 2400 thermocycler. The amplification reactions were started with pre-denaturation at 94°C for 5 minutes, and 29 cycles of denaturation at 90°C for 30 seconds, annealing at 55°C for 1 minute and extension at 65°C for 4 minutes, followed by a final step of elongation of 65°C for 8 minutes at the end of the cycles. The PCR products were applied to gel electrophoresis and were visualized under UV transilluminator (SynGene Gel Documentation System).

ERIC Analysis

The banding patterns of individual strains were scored based on the presence or absence of the bands, and were analyzed using the RAPDistance Package Software (version 1.04) program. The scoring was made in the form of binary code with the score '1' indicating presence of band and''0' the absence of band. The data obtained were recorded and entered in the software where a dendogram was produced for further analysis. Clustering was based on the unweighted pair of group average method (UPGMA) and was performed with the RAPDistance software.
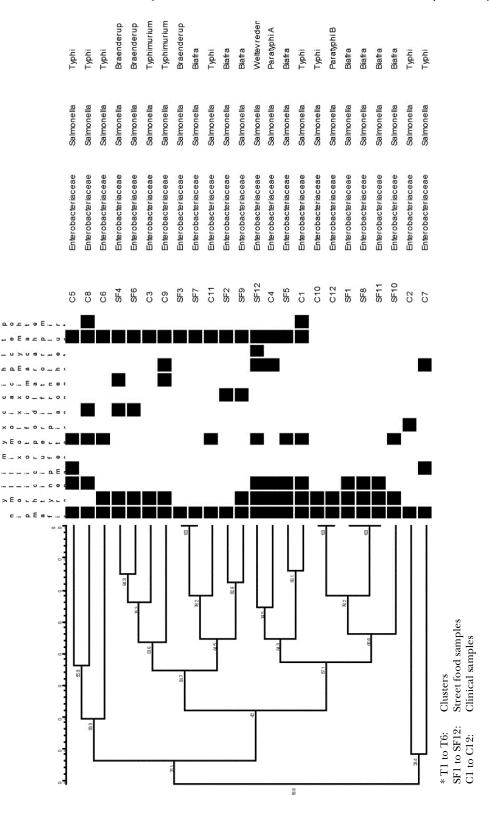
RESULTS

A total of 14 antimicrobial agents were used in this study. As shown in Table 1, isolates from the clinical samples gave 12 antibiotic resistance patterns while isolates from the street foods samples produced only 10 antibiotic resistance patterns. The prevalence of resistance to rifampin was highest (100%) followed by erythromycin and sulpha-methoxazole (66.7% each). All serotypes examined displayed resistance to either of these antibiotics, while some serotypes were resistant to both. It is worthy to note that more of the street foods isolates displayed multiple resistances when compared to the clinical strains. 83.3% (10/12) of isolates from the street foods samples were resistant to three or more antibiotics, compared to only 75% (9/12) of the clinical isolates. None of the isolates were resistant to cefuroxime. Cluster analysis with disk diffusion susceptibility results was performed in order to investigate if resistance to one antibiotic could be correlated with resistance to another and to compare resistance patterns among street food and clinical *Salmonella* isolates.

From the antibiotic resistances dendrogram in Figure 1, six clusters (T1 to T6) corresponding to 24 strains could be defined, with similarity levels ranging from 18.8% to 100%. T1 consisted of C5, C6, and C8, which are all *S.* Typhi of clinical isolates. T2 was generated from SF4 and SF6 (*S.* Braenderup of street food isolates), and C3 and C9 (*S.* Typhimurium of clinical isolates). T3 mainly consists of street food isolates, which are SF2 (*S.* Biafra), SF3 (*S.* Braenderup), SF7 (*S.* Biafra), and SF9 (*S.* Biafra), with only one clinical isolate which is C11 (*S.* Typhi). T4 contained C1 (*S.* Typhi) and C4 (*S.* Paratyphi A) of clinical isolates, and SF5 (*S.* Biafra) and

Table 1: Antibiotic resistance patterns of *Salmonella* isolates from street food and clinical samples

Salmonella serovars	Code	Sample	Type of dish	Location	Antibiotic resistance patterns
S. Biafra	SF1	Sambal ikan	Fish	Bahau, Negeri Sembilan	RdEP
	SF2	Sambal ikan	Fish	Bahau, Negeri Sembilan	RdRlNor
	SF5	Sambal ikan	Fish	Bahau, Negeri Sembilan	RdERIPS
	SF7	Sambal ikan	Fish	Bahau, Negeri Sembilan	RdRl
	SF8	Sayur campur	Vegetable	Bahau, Negeri Sembilan	RdEP
	SF9	Ayam goreng	Chicken	Malacca Town, Malacca	RdERlNor
	SF10	Sambal ikan	Fish	Bahau, Negeri Sembilan	RdES
	SF11	Sayur campur	Vegetable	Bahau, Negeri Sembilan	RdEP
S. Braenderup	SF3	Kerabu jantung pisang	Vegetable	Jasin, Malacca	RdRl
	SF4	Kerabu jantung pisang	Vegetable	Jasin, Malacca	RdERlNaCn
	SF6	Kerabu jantung pisang	Vegetable	Jasin, Malacca	RdERlNa
S. Weltevreden	SF12	Sambal ikan	Fish	Bahau, Negeri Sembilan	RdERIPSCTe street food samples
S. Typhi	C1	Rectal swab		Selangor	RdERIPSW clinical samples
	C2	Rectal swab		Selangor	RdCip
	C5	Rectal swab		Selangor	RdRIPSAmp
	C6	Rectal swab		Selangor	RdERIS
	C7	Rectal swab		Selangor	RdCAmp
	C8	Rectal swab		Selangor	RdRIPSNaW
	C10	Rectal swab		Selangor	RdE
	C11	Rectal swab		Selangor	RdRIS
S. Typhimurium	C3	Rectal swab		Selangor	RdERl
	C9	Rectal swab		Selangor	RdERICCn
S. Paratyphi A	C4	Rectal swab		Selangor	RdERIPC
S. Paratyphi B	C12	Rectal swab		Selangor	RdE

Figure 1: Dendrogram of the resistance patterns of *Salmonella* isolates from street food and clinical samples using Pearson correlation and UPGMA for clustering

* T1 to T6:   Clusters
SF1 to SF12:   Street food samples
C1 to C12:   Clinical samples

* T1 to T3:   Clusters
SF1 to SF12:   Street food samples
C1 to C12:   Clinical samples

Figure 2: Dendrogram based on the numerical analysis of the resistance patterns to 14 antibiotics of 24 *Salmonella* isolates from street food and clinical samples

SF12 (*S*. Weltevreden) of street food isolates. T5 once again consisted of mainly street food isolates, namely SF1, SF8, SF10, and SF11, which are all *S*. Biafra strains, and with only C10 (*S*. Typhi) and C12 (*S*. Paratyphi B) from clinical samples. T6 however consisted of only clinical isolates, which are all *S*. Typhi strains (C2 and C7).

Figure 2 shows clustering of antibiotic agents studied based on the resistance patterns of the *Salmonella* isolates towards the agents. At 10% similarity, three clusters (T1 to T3) could be defined, together with five single isolates. At 24% similarity, T1 consisted of the antibiotics nalidixic acid, gentamicin, and sulphamethoxazole. T2 at 37.7% similarity clustered streptomycin, trimethoprim, and penicillin together. At 13.5% similarity, T3 contained chloramphenicol, tetracycline, and ampicillin. The five single antibiotics are

erythromycin, norfloxacin, rifampin, cefuroxime, and ciprofloxacin.

ERIC-PCR fingerprinting of *Salmonella* isolates from street food samples is shown in Figure 3, in which 11 different banding patterns were constructed. Meanwhile, ERIC fingerprinting of *Salmonella* enterica isolated from clinical samples is shown in Figure 4, and 9 banding patterns were produced. A total of 19 banding patterns were generated using ERIC for the *Salmonella* spp. from both street food and clinical samples. SF12 was untypable using ERIC-PCR.

The ERIC dendrogram of *S*. Biafra from street foods samples (Figure 5) produced 3 major clusters. Cluster I contained a single isolate, SF1 (sambal ikan from Bahau, Negeri Sembilan). Cluster II consisted of SF5 (sambal ikan from Bahau, Negeri Sembilan), SF7 (sambal ikan from Bahau, Negeri Sembilan),
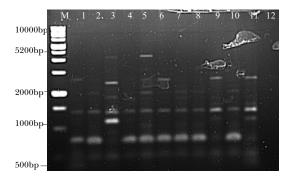
Figure 3: ERIC fingerprints of *Salmonella* isolates from street food samples. Lane M contains 1 kb marker. Lane 1: SF1, Lane 2: SF2, Lane 3: SF3, Lane 4: SF4, Lane 5: SF5, Lane 6: SF6, Lane 7: SF7, Lane 8: SF8, Lane 9: SF9, Lane 10: SF10, Lane 11: SF11, Lane 12: SF12.

Figure 4: ERIC fingerprints of *Salmonella* isolates from clinical samples. Lane M contains 1 kb marker. Lane 13: C1, Lane 14: C2, Lane 15: C3, Lane 16: C4, Lane 17: C5, Lane 18: C6, Lane 19: C7, Lane 20: C8, Lane 21: C9, Lane 22: C10, Lane 23: C11, Lane 24: C12.

SF8 (sayur campur from Bahau, Negeri Sembilan), and SF10 (sambal ikan from Bahau, Negeri Sembilan), in which SF5 were clustered away from SF7, SF8, and SF10. In a smaller cluster, SF8 and SF10 were clustered together away from SF7. Cluster III meanwhile contained SF2 (sambal ikan from Bahau, Negeri Sembilan), SF9 (ayam goreng from Malacca Town, Malacca), and SF11 (sayur campur from Bahau, Negeri Sembilan). In this cluster, SF2 was a single isolate while SF9 and SF11 were in the same minor cluster.

Meanwhile, the ERIC dendrogram of *S.* Typhi from clinical samples (Figure 6) generated 2 major clusters. Cluster I consisted of C1 and C11, and Cluster II contained C2, C5, C6, C7, C8, and C10. In Cluster I, C1 and C11 were well differentiated. In Cluster II, C6 was a single isolate, clustering away from the other strains. In the smaller cluster, C5 was clustered away from C2, C7, C8, and C10 which were 100% similar.

DISCUSSION

The worldwide overuse and misuse of antimicrobials in human medicine, veterinary medicine, agriculture and as prophylactic supplements or growth promoters in the feed
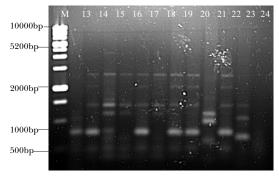
of food animals has created enormous pressure for the selection of antimicrobial resistance among bacterial pathogens (WHO, 2000; Aarestrup *et al.*, 2001). Hence, the increasing prevalence of resistance among microorganisms has become an increasingly important public health problem which has serious implications for treatment and prevention of infectious diseases in both human and animals (European Commission, 1999). This study is the first to report on antimicrobial resistance in *Salmonella* enterica from street food and clinical samples in Malaysia.

In this study, all the encountered serotypes were of *Salmonella* enterica, and to date, *Salmonella* enterica is recognized as one of the most common bacterial causes of foodborne diarrheal illness worldwide (Butaye *et al.*, 2006). From the results, it was found that none of the serovars involved in the clinical samples were encountered in the street food samples. However, the prevalence of *Salmonella* in the street foods indicates their potential as vehicle for human infection. In fact, Arumugaswamy *et al.* (1995) reported the prevalence of *S.* Paratyphi B in street foods in Malaysia, and that *S.* Weltevreden found in street food samples in this study is the third most
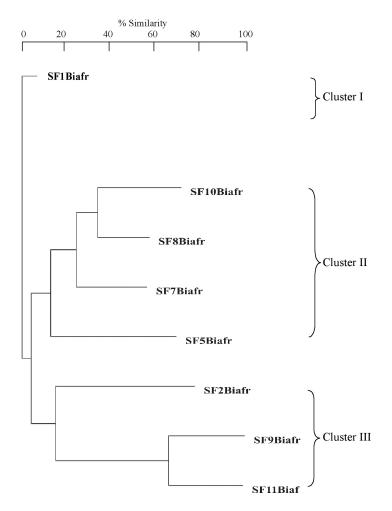
Figure 5: ERIC dendrogram of *S.* Biafra from street food samples

frequently isolated serotype from humans. As stated by Butaye *et al.* (2006), all *Salmonella* serotypes may be regarded as potential human pathogens, although the vast majority of infections are caused by a very limited number of serotypes.

As observed from the antibiotic resistance dendrogram in Figure 1, generally, street food and clinical strains tend to cluster apart. This is shown in clusters T1 and T6, which only consisted of clinical strains (*S.* Typhi), while the remaining clusters are majority street food strains. Isolates in Cluster T1 and T6 when subjected to ERIC characterization, were found to be clustering together in one major

cluster, in which this supports the relatedness of the isolates in antibiotic resistance patterns. It is worthy to note that more of the street foods isolates displayed multiple resistances when compared to the clinical strains.

Approximately 83.3% of isolates from street foods were resistant to three or more antibiotics, while clinical isolates only displayed 75% multiple resistances. Resistance genes can be obtained by bacteria in a number of ways including conjugation, transformation, and transduction (Yan *et al.*, 2003), which is probably why in this study, it was observed that the *Salmonella* serotypes encountered in street food samples acquire resistance to antibiotics
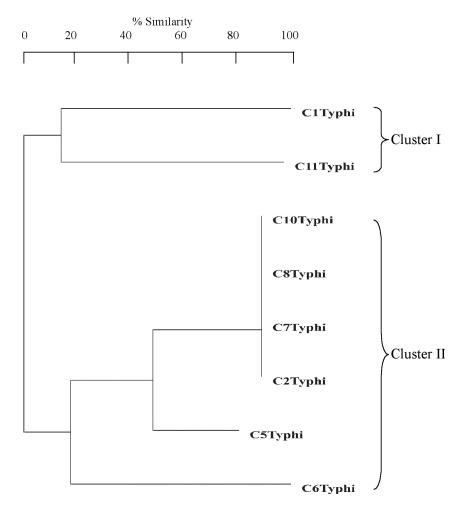
Figure 6: ERIC dendrogram of *S.* Typhi from clinical samples

although they were not among the serotypes usually found in clinical cases. As stated by Yan *et al.* (2003), many resistance mechanisms are acquired through mutation or acquisition of antimicrobial resistance genes.

Antibiotic resistance is on the rise and it has been suggested that it results 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 due to underlying immune disorders. It was suggested that the antibiotic resistance of isolates were

related to the possession of plasmids. Low molecular weight plasmids are of unknown role and function, but on the other hand, high molecular weight plasmids have been reported to affect the phenotype of *S.* Enteritidis, including their virulence, phage type and antibiotic resistance. Some examples of plasmid encoded resistances have been described (Rychlik *et al.*, 2000). It was also suggested that the bacteria owe their antibiotic resistance to chromosomally encoded characteristics, as reported by Threlfall *et al.* (1994) in their study.

In the remaining antibiotic resistance clusters, some clinical strains were clustered

together with street food strains. This was observed in cluster T2 (50% street food, 50% clinical, cluster T3 (80% street food, 20% clinical), cluster T4 (50% street food, 50% clinical), and cluster T5 (67% street food, 33% clinical). This indicates that although antibiotic prescriptions and administration practices are different for the two set-ups, there seems to be no barriers between both environments.

Cluster T3 mainly consisted of street food isolates but of different *Salmonella* serotypes, with only one clinical isolate. SF3 (*S.* Braenderup) and SF7 (*S.* Biafra) were 100% similar and were clustered together with C11 (*S.* Typhi), SF2 and SF9 of *S.* Biafra. They share the same resistance to rifampin and sulphamethoxazole. Even though serotypes, type of sample and location of sampling differed, the 100% similarity between SF3 and SF7 may be due to cross-resistance among different serotypes. The ERIC dendrogram for *S.* Biafra strains in this cluster showed that SF2 and SF9 were closely related, while SF7 was only clustering together in a major cluster, in concordance with their antibiotic resistance profiles.

In T4, the street food and clinical isolates that were clustered together are also of totally different serotypes. Here, SF5 (*S.* Biafra), SF12 (*S.* Weltevreden), C1 (*S.* Typhi), and C4 (*S.* Paratyphi A) showed the most number of resistances, in which almost all the strains were resistant to rifampin, erythromycin, penicillin, streptomycin, and sulphamethoxazole. In this cluster, SF5 and C1 showed the highest percentage of similarity (86.1%) even though of very different source of sample, in which one is of street food and the other is of clinical sample, most probably also due to cross-resistance.

Cluster T5 on the other hand generally consisted *S.* Biafra street food isolates (SF1, SF8, SF10, and SF11), and only two clinical isolates (C10 of *S.* Typhi and C12 of *S.* Paratyphi B). Here, C10 and C12 are 100% similar, sharing resistance to rifampin and erythromycin, while SF1, SF8, and SF11 are also 100% similar, with the same resistance to rifampin, erythromycin, and penicillin. The 100% similarity between the strains may be because they are from the same street food samples. From their ERIC discrimination however, all *S.* Biafra strains in this cluster were clustering away from each other, except for SF8 and SF10.

In Figure 2, the antibiotics can be grouped according to classes based on their mode of inhibition although not so evident: inhibition of cell wall synthesis (A), inhibition of protein synthesis (B), and inhibition of nucleic acid synthesis (C). This is in concordance with the finding reported by Lopes *et al.* (2005) in which they stated that their antimicrobials appeared to be grouped according to their mode of inhibition, despite belonging to different classes of antibiotics. The dendrogram in Figure 2 revealed that the most related compounds were chloramphenicol and tetracycline (46.6% similarity) in T3, which is acceptable as they both share the same mode of inhibition (Group B). Ampicillin was also grouped in this cluster at 13.5% similarity, although of different mode of inhibition (Group A), in which the explanation may be due to cross-resistance. In T1, gentamicin (Class aminoglycoside) of Group B is clustering together with nalidixic acid (Class quinolone) and sulphamethoxazole (Class sulphonamide) of Group C. Surprisingly, T2 constituted streptomycin (Class amino-glycoside, Group B), trimethoprim (Class trimethoprim, Group C), and penicillin (Class β-lactam penicillin, Group A). They are all of very different group of antibiotics, but were clustering together. Resistance to the remaining antibiotics did not seem to be related, with the exception of norfloxacin which is clustered with T1 to T3 at 2.1% similarity, and erythromycin which clustered together at 6.9% similarity. As reported by Sherley *et al.* (2000), co-resistance to more than one antibiotic belonging to the same class may be expected due to shared resistance mechanisms, while correlation between

unrelated antibiotics may reflect a shared broad resistance mechanism.

When using ERIC-PCR to discriminate the *Salmonella* isolates, a total of 19 different banding patterns were constructed (Figure 3 and 4), showing the sensitivity of this method. SF12 was untypable when subjected to ERIC, possibly due to the loss or absence of the specific sites for primer binding in the chromosomal DNA. A combination of typing methods would be beneficial. The ERIC dendrogram of *S.* Biafra from the street foods samples (Figure 5) showed that SF1 (sambal ikan from Bahau, Negeri Sembilan) was clustered further away from the other strains of the same type of sample and location. The other *S.* Biafra strains were observed to be clustering together (SF5, SF7, SF8, SF10), in concordance with the location of sample. Also SF9 (ayam goreng from Malacca Town, Malacca) and SF11 (sayur campur from Bahau, Negeri Sembilan) were clustered together although of very different type of sample and location of sampling. The ERIC dendrogram of *S.* Typhi from clinical samples (Figure 6) showed that the clinical strains of *S.* Typhi were different as well, although from the same location of sampling, which indicated the possibility of the patients getting the infections at very different places and sources. Even though most of the strains were well differentiated, C2, C7, C8, and C10 were shown to be 100% similar. These analyses showed a high heterogeneity among the *Salmonella* strains. They were able to be differentiated even though identified as the same serotypes, indicating the sensitivity of ERIC method in differentiating the varying and unique strains.

The possible emergence of resistance among salmonellae, isolated from humans or food products, therefore justifies continued surveillance. This applies to the *Salmonella* enterica in this study, given their capacity for dissemination and the inefficiency of protective measures for control that might be implemented locally. The potentials of other antimicrobial agents including broad-spectrum antibiotics for treatments of salmonellosis should be investigated (Shanahan *et al.,* 1998). Unfortunately, the expense of these antibiotics precludes their use in developing countries. As all *Salmonella* isolates in this study were susceptible to cefuroxime, there is a potential for its usage to treat salmonelllosis in future, if the costs become less prohibitive. However further extensive studies need to be done for validation.

CONCLUSION

This study has highlighted that there is a high percentage of antibiotic resistance in *Salmonella* enterica isolated from street food and clinical samples. Though the emergence of resistant *Salmonella* spp. is suggested to be due to the over usage of antibiotics in human and veterinary medicine, agriculture and animals food, the incidence of drug-resistant *Salmonella* enterica in street food and clinical samples may contribute to the incidences of resistant *Salmonella* enterica in Malaysia. ERIC proved to be a sensitive, stable, reproducible, rapid and low cost typing method for improved understanding of *Salmonella* enterica characteristics. In order to control this pathogen, continued surveillance and research on their antimicrobial resistance patterns and epidemiology are needed.

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REFERENCES

Aarestrup, F. M., Seyfarth, A. M., Emborg, H. N., Pedersen, K., Hendriksen, R. S. and Bager, F. 2001. Effect of abolishment of the use of antimicrobial agents for growth promotion on occurrence of antimicrobial resistance in decal enterococci from food animals in Denmark. Antimicrobial Agents Chemotherapy, 45: 2054-2059.

Arumugaswamy, R. K., Rusul, G., Abdul Hamid, S. N. and Cheah, C. T. 1995. Prevalence of *Salmonella* in raw and cooked foods in Malaysia. Food Microbiology, 12: 3-8.

Bartoloni, A., Benedetti, M., Pallecchi, L., Larsson, M., Mantella, A., Strohmeyer, M., Bartalesi, F., Fernandez, C., Guzman, E., Vallejos, Y., Villagran, A. L., Guerra, H., Gotuzzo, E., Paradisi, F., Falkenberg, T., Rossolini, G. M. and Kronvall, G. 2005. Evaluation of a rapid screening method for detection of antimicrobial resistance in the commensal microbiota of the gut. Transactions of the Royal Society of Tropical Medicine and Hygiene, 100: 119-125.

Bauer, A. W., Kirby, W. M. M. and Sherris, J. C. 1966. Antibiotic susceptibility testing by a standardized single disc. American Journal of Clinical Pathology, 45: 493-496.

Baumler, A. J., Tsolis, R. M. and Heffron, F. 1997. Fimbrial adhesins of *Salmonella typhimurium.* Role in bacterial interactions with epithelial cells. Advances in Experimental Medical Biology, 412: 149-158.

Butaye, P., Michael, G. B., Schwarz, S., Barrett, T. J., Brisabois, A. and White, D. G.   2006. Forum on antimicrobial resistance: The clonal spread of multidrug-   resistant non-typhi *Salmonella* serotypes. Microbes and Infection, 8: 1891-1897.

Current Topics. 1995. FDA seeking advice on antibiotic resistance surveillance. American Society for Microbiology News, 61(2).

European Commission (EC). 1999. Opinion of the Scientific Steering Committee on Antimicrobial Resistance. Directorate-General XXIV, Consumer Policy and Consumer Health Protection Directorate B-Scientific Health Opinions Unit B3-Management and Scientific Committees II, pp. 7-8.

Lim, H., Lee, K. H., Hong, C. H., Bahk, G. J. and Choi, W. S. 2005. Comparison of four molecular typing methods for the differentiation of *Salmonella* spp. International Journal of Food Microbiology, 105: 411-418.

Lopes, M. F. S., Ribeiro, T., Abrantes, M., Marques, J. J. F., Tenreiro, R. and Crespo, M. T. B. 2005. Antimicrobial Resistance Profiles of Dairy and Clinical Isolates and Type Strains of Enterococci. International Journal of Food Microbiology, 103: 191-198.

Mankee, A., Ali, S., Chin, A., Indalsingh, R. Khan, R., Mohammed, F., Rahman, R., Sooknanan, S., Tota-Maharaj, R., Simeon, D. and Adesiyun, A. A. 2003. Bacteriological quality of "doubles" sold by street vendors in Trinidad and the attitudes, knowledge and perceptions of the public about its consumption and health risk. Food Microbiology, 20: 631-639.

Mrema, N., Mpuchane, S. and Gashe, B. A. 2004. Prevalence of *Salmonella* in raw minced meat, raw fresh sausages and raw burger patties from retail outlets in Gaborone, Botswana. Food Control, 17: 207-212.

National Committee for Clinical Laboratory Standards. 2004. Performance standards for antimicrobial susceptibility testing, 5th International Supplement Document M100-SF. National Committee for Clinical Laboratory Standards. Villanova, P. A.

Rychlik, I., Svestkova, A. and Karpiskova, R. 2000. Subdivision of *Salmonella enterica* serovar *enteritidis* phage types PT14b and PT21 by plasmid profiling. Veterinary Microbiology, 74: 217-225.

Shanahan, P. M. A., Jesudason, M. V., Thomson, C. J. and Amyes, S. G. B. 1998. Molecular analysis of and identification of antibiotic resistance genes in clinical isolates of *Salmonella typhi* from India. Journal of Clinical Microbiology, 35: 1595-1600.

Sherley, M., Gordon, D. M. and Collignon, P. J. 2000. Variations in antibiotic resistance profile in Enterobacteriaceae isolated from wild Australian mammals. Environmental Microbiology, 2(6): 620-631.

Threlfall, E. J., Frost, J. A., Ward, L. R. and Rowe, B. 1994. Epidemic in cattle and humans of *Salmonella typhimurium* DT104 with chromosomally integrated multiple drug resistance. Veterinary Record, 134: 577-578.

Tunung, R., Chai, L. C., Usha, M. R., Lesley, M. B., Cheah, Y. K., Patrick, G. B., Farinazleen, M. G., Fatimah, A. B., Pradeep, K. M. and Son, R. 2007. Incidence and characterization of *Salmonella* species in street food and clinical samples. Journal of Food Safety (In Press).

World Health Organization. 2000. WHO global principles for the containing of antimicrobial resistance in animals intended for food. WHO Department of Communicable Disease Surveillance and Response, Geneva, Switzerland, 5-9 June 2000. Report of a WHO Consultation. pp. 1-7.

Yan, S. S., Pendrak, M. L., Abela-Ridder, B., Punderson, J. W., Fedorko, D. P., and Foley, S. L. 2003. An overview of *Salmonella* typing: Public health perspectives. Clinical and Applied Immunology Reviews, 4: 189-204.