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Full Length Research Paper

Isolation and molecular sub typing of Salmonella Enterica from chicken, beef and street foods in Malaysia

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This study was conducted to determine and identify *Salmonella* isolated from chicken meat, beef and street food (poultry and beef products) and the genetic diversity of *Salmonella* by pulsed-field gel electrophoresis (PFGE). Eighty-eight non-repeat *Salmonella* isolates from 300 food samples were isolated and characterized using conventional culture, biochemical and serological methods and confirmation of *Salmonella* was determined by polymerase chain reaction (PCR) using specific primers. Among the 88 *Salmonella* isolates, 11 serovars [Corvallis (n = 33), Typhimurium (n = 18), Hadar (n = 7), Enteritidis (n = 5), *Weltevreden* (n = 5), Agona (n = 5), Newport (n = 4), Albany (n = 3), Istanbul (n = 2), Emek (n = 1) and Wandsworth (n = 1)] were identified. All the 88 *Salmonella* isolates were typable by PFGE with 61 distinct pulsed-field profiles. The cluster analysis indicates that 61 different pulsotypes were separated into groups of identical serovars based on their PFGE profiles. The isolation of various serovars of *Salmonella* from different sources indicates the presence and distribution of *Salmonella* in raw meats and street foods. Knowledge of variability of subtypes of different serovars and sources of *Salmonella* may provide valuable added information for research, risk management, and public health strategies.

Key words: *Salmonella enterica*, food, polymerase chain reaction (PCR), pulsed-field gel electrophoresis (PFGE).

INTRODUCTION

The genus *Salmonella*, a member of the family Enterobacteriaceae, is an important foodborne pathogen. It is composed of bacteria related to each other both phenotypically and genotypically. Taxonomically, there are two species in the genus *Salmonella*: *S. enterica* (six subspecies) and *S. bongori* (one subspecies). Members of the seven subspecies can be serotyped into one of more than 2500 different serovars based on somatic (O) and flagellar (H) antigens (Popoff, 2001).

Non-typhoidal *Salmonella* remains the leading cause of bacterial food-borne infections and continue to be a major problem, in terms of both morbidity and economic costs. Although, *Salmonella* serovars Typhimurium and Enteritidis are the most common cause of human salmonellosis worldwide (Archambault et al., 2006; Herikastad et al., 2002), other *Salmonella* serovars associated with food poisoning cases are becoming important in recent years. In Malaysia, S. Enteritidis (28.1%), S. Weltevreden (25.7%), S. Corvallis (10.3%) and S. Typhimurium (6.7%) were the most common non-typhoidal *Salmonella* reported for the period 2003 - 2005 (National Public Health Laboratory, 2005). In some regions, S. Weltevreden, S. Anatum and S. Derby have been reported to be of greater importance (Bangtrakulnonth et al., 2004).

Transmission of *Salmonella* to humans typically occurs by ingesting meat, dairy products, and other foods

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contaminated by animal feces or by cross-contamination from foods contaminated with *Salmonella*. Hanning et al. (2009) reported that in the U.S.A., most cases of salmonellosis originate from the consumption of contaminated meat and poultry products. In meat production, the leading source of contamination of carcasses by *Salmonella* is the evisceration step at the slaughter-house (Bouchrif et al., 2009). The examination of food to detect *Salmonella* is routinely carried out for food safety and food-borne disease surveillance. Conventional typing methods based on phenotypic characteristics such as, biotyping, serotyping and phage typing, have been widely used (Yan et al., 2004). These methods are less discriminative. In contrast, molecular approaches such as pulsed-field gel electrophoresis (PFGE), amplified fragment length polymorphism (AFLP), and multi-locus variable tandem repeats (MLVA) offer higher discrimination (Kotetishvili et al., 2002; Yan et al., 2004). PFGE has been widely regarded as the "gold standard" for *Salmonella* subtyping (Swaminathan et al., 2001).

In the past few years, the incidence of enteric fever has decreased in Malaysia. However, outbreaks and gastroenteritis due to non-typhoidal *Salmonella* remains the leading cause of bacterial foodborne infection. Knowledge about the occurrence and epidemiology of different *Salmonella* in Malaysia may assist in the recognition and tracing of new emerging pathogen. The purpose of this study was to determine and identify *Salmonella* isolated from chicken meat, beef and street food (poultry and beef products) and the genetic diversity of *Salmonella* by PFGE.

MATERIALS AND METHODS

Samples and bacterial isolates

In this study, 200 samples of raw beef and chicken meat and 100 samples of street cooked food were examined during 2006 - 2009. The raw beef and chicken meats were purchased from hyper markets and retail markets in Kuala Lumpur and its vicinity, and street foods (poultry and beef products) were purchased from different localities in Kuala Lumpur. Street foods are defined as cooked foods, which are eaten at the point of sale. The procedures for isolation of *Salmonella* were carried out according to the techniques recommended by the International Organization for Standardization (ISO 6579, 2002). Briefly, 25 g of samples were mixed with 225 ml buffered peptone water (BPW) and incubated at 37°C for 24 h. An aliquot of 0.1 ml was inoculated in 9.9 ml Rappaport-Vasiliadis (RV) broth and incubated at 42°C for another 24 h. A loopful of enriched broth was streaked on Xylose-lysine desoxycholate (XLD) and Hektoen enteric (HE) (Oxoid, Hampshire, England) agar plates and incubated at 37°C for 24 h.

All presumptive *Salmonella* colonies (red colonies with black centers on XLD or blue-gray colonies with black center on HE) were confirmed by some biochemical tests as recommended by the guidelines of the ISO 6579 (2002). These biochemical tests include the Lysine decarboxylase (LIA), glucose (TSI), motility test (SIM), oxidase, indole, Methyl Red-Voges Proskauer (MRVP), Simmons citrate, and urease reactions.

LIA agar was used to determine the ability of bacteria to ferment sucrose. In this test, presumptive colonies have an alkaline (purple) slants and alkaline butts. TSI agar was used to determine the ability of bacteria to ferment glucose and/ or lactose and their ability to produce hydrogen sulfide or other gases. Presumptive colonies have alkaline (red) slants and acid (yellow) butts, with or without H_2S production (blackened agar). For urease test, the production of ammonia from urea was shown by a change in the phenol red indicator from yellow to pink. *Salmonella* species are typically urease-negative. Oxidase test was used to determine the presence of an enzyme cytochrome oxidase, which catalyses the oxidation of reduced cytochrome by molecular oxygen. *Salmonella* species are oxidase–negative. In Simmons citrate agar, the use of citrate as a sole carbon source was indicated by the production of ammonia and a change in the color of the medium from green to blue (presumptive cultures). Testing for indol production is important in the identification of Enterobacteria. Indol production was tested by Kovac's reagent and a positive test was indicated by red colored compound. Methyl Red (MR) test detects the production of sufficient acid during fermentation of glucose by bacteria and sustained the maintenance of pH below 4.5 (positive: red color, negative: yellow color). Motility was indicated by turbidity extending out from the line of stab inoculation. Non-motile organisms grew only in the inoculated area.

Further confirmation of the presumptive *Salmonella* isolates was carried out with a commercial bacterial identification kit such as the Analytical Profile Index (API) system. The API is a miniaturized panel of biochemical tests compiled for identification of groups of closely related bacteria. For *Salmonella* identification, the API-20E was used according to the manufacturer's instruction (BioMerieux, France). After incubation in a humidity chamber for 18 - 24 h at 37°C, the color reactions were read (according to manufacturer's protocol). Positive test results were scored as a seven-digit number (profile). Identity of the bacterium was then derived from the database accessed from the apiweb™ software (bioMérieux, France) and the identity of the cultures were recorded as a percentage of similarity.

PCR primers, DNA amplification and detection of *Salmonella*

PCR targeting the *hilA* (F: 5´-CGGAACGTTATTTGCGCCATGCTGAGGTAG -3´, R: 5´-GCATGGATCCCCGCCGGCGAGATTGTG-3´) and *ompC* (F:5´-ATCGCTGACTTATGCAATCG-3´, R:5´-CGGGTTGCGTTATAGGTCTG-3´) genes of *Salmonella* previously reported by Pathmanathan et al. (2003) and Kwang et al. (1996), respectively, were used to confirm the identity of the presumptive *Salmonella*. Crude DNA was prepared by a suspension of a loopful of well isolated colonies in 50 µl distilled water, boiled at 99°C for 5 min and immediately cooled in ice for 10 min. The cell lysate was centrifuged at 10,000 x g for 2 min and about 3 µl (~10 ng) of supernatant was used as DNA template for PCR. The amplification for *hilA* was carried out using a total volume of 25 µl containing 25 pmol of each primer, 50 µM of each dNTP, 1.5 mM $MgCl_2$, 1.5 U *Taq* DNA polymerase, 1X PCR buffer. A negative control containing the same reaction mixture except the DNA template was included in every experiment. The PCR conditions consisted of an initial denaturation at 94°C for 5 min, which was followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 65°C for 1 min and extension at 72°C for 1 min, with a final extension at 72°C for 10 min. A 5 µl aliquot of each PCR product was electrophoresed on a 1.5% agarose gel for 1.5 h at 100 V, stained for 10 min in ethidium bromide (0.5 $\mu g ml^{-1}$), visualized and photographed under UV illumination. For *ompC*, the PCR conditions consisted of an initial denaturation of 2 min at 95°C; 30 cycles of 1 min at 95°C, 1 min at 58°C, and 2 min at 72°C; and a final elongation step of 5 min at 72°C.

To differentiate Salmonella Typhimurium (including phage type

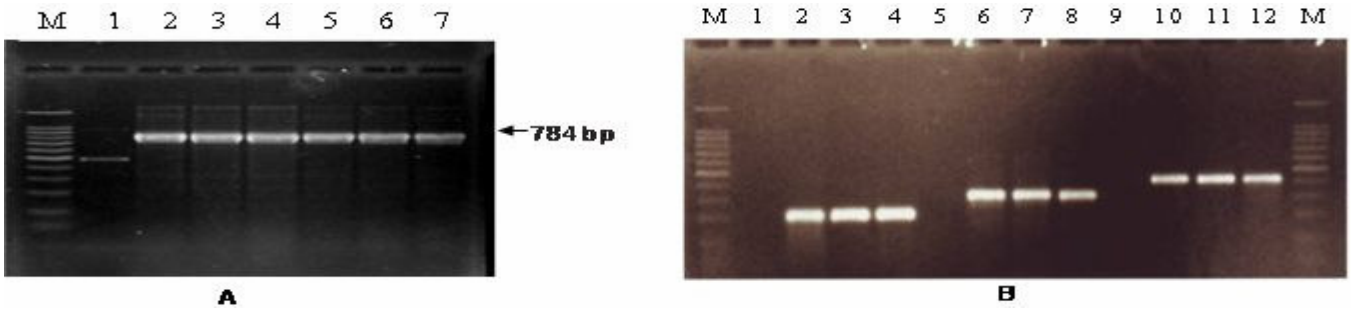

Figure 1. Representative gels of showing the amplification of the *hilA, ompC*, ENT and STM in *Salmonella* isolates. A: Lane M; 100 bp marker, Lane 1; non-*Salmonella*, Lanes 2-7; *Salmonella* (*hilA*;784 bp). B: M.100 bp DNA marker; lanes 1, 5, 9: negative; Lanes 2-4: *Salmonella* genus-specific sequence (*ompC*; 204 bp), Lanes 6-8; serovar Enteritidis-specific sequence (ENT; 304 bp) and Lanes 10-12; serovar Typhimurium-specific sequence (STM; 401 bp).

DT104) and *Salmonella enteritidis*, a multiplex PCR assay incorporating primers specific for *S. enteritidis* (ENTF:5´-TGTGTTTTATCTGATGCAAGAGG-3´, ENTR:5´-TGAACTACGTTCGTTCTTCTGG-3´ (304 bp), *S.* Typhimurium (STMF: 5´-TTGTTCACTTTTTACCCCTGAA-3´, STMR: 5´CCCTGACAGCCGTTAGATATT-3´ (401 bp) and phage type DT104 (104F: 5´-ATGCGTTTGGTCTCACAGCC -3´, 104R: 5´-GCTGAGGCCACGGATATTTA-3´ (102 bp) as previously described was applied (Olsen et al., 1995; Pritchett et al., 2000; Agron et al., 2001). These primers were selected because *S.* Enteriditis and *S.* Typhimurium are the most common non-typhoidal *Salmonella* serovars in Malaysia. PCRs were performed in a final volume of 25 µl. The optimized PCR mixture consisted of 1.5 mM $MgCl_2$, 200 µM each of the four deoxynucleoside triphosphates, 1 U of *Taq* polymerase, 60 pmol of each primer per sample and 5 µl of DNA template. The PCR protocol consisted of the following steps: An initial denaturation step of 2 min at 95°C; 30 cycles of 1 min at 95°C, 1 min at 58°C, and 2 min at 72°C; and a final elongation step of 5 min at 72°C. PCR amplicons (*hilA*, *ompC,* ENT, STM and DT104) were purified and submitted to a commercial company (First Base, Malaysia) for DNA sequencing to validate the identity.

Pulsed-field gel electrophoresis (PFGE)

Genomic DNA was prepared and embedded in agarose plugs as previously described (Thong et al., 2002). Slices of the DNA plugs were digested with 10 U of *Xba*I (Promega, Madison, Wis, USA) restriction enzyme overnight at 37°C and electrophoresed on a CHEF MAPPER (Bio-Rad Laboratories, CA, USA) for 25 h at 6 Vcm^{-1}, 210º, with an initial pulse time of 2.2 s and final a pulse of 63.8 s at 14°C. *Xba*I restricted-*Salmonella* Braenderup H9812 served as a control and DNA size marker. The DNA fragments on 1% w/v agarose gel and stained with ethidium bromide, destained and photographed under UV illumination (Gel Doc™ XR Bio Rad, CA, USA). DNA fragment patterns were visually assessed and distinct profiles were assigned an arbitrary pattern. Analysis of the restricted fragments was carried out using the BioNumerics Software (Applied Maths, Kortrijk, Belgium). A dendrogram based on the Dice coefficient was generated using the unweighted pair group with arithmetic mean (UPGMA) algorithm at 1% position tolerance.

Serotyping

All of the *Salmonella* serovars were identified according to the Kauffman-White scheme (Popoff, 2001) by the *Salmonella* Reference Laboratory at the Institute for Medical Research (IMR) in Kuala Lumpur. According to Kauffman-White scheme, isolates were serotyped based on the agglutination tests on somatic O and phase 1 and phase 2 flagella antigens with antisera.

RESULTS

Isolation and identification of *Salmonella*

Out of 300 food samples, 88 non-repeat *Salmonella* isolates (66 from beef and chicken meat and 22 from street food) were identified by standard biochemical tests. All the isolates showed the expected reactions according to the ISO 6579 (ISO, 2002) guidelines: Lysine decarboxylase positive, Glucose positive, Simmons citrate positive, Urease reaction negative, Methyl red positive, Indole negative, H_2S positive (TSI and LIA), Motility test positive and Oxidase test negative. All 88 biochemically-tested *Salmonella* isolates were further identified by API20E kit and the identities/similarities were from 90 - 95%.

PCR confirmation of the *Salmonella* isolates gave the expected amplicons size of 784 bp for the *hil*A gene (Figure 1A) and 204 bp for *omp*C gene (Figure 1B). DNA sequence analysis of the amplicons showed 90 - 100% homology with the respective genes in GenBank Database. These results showed that both *hil*A and *omp*C genes were present in all *Salmonella* isolates tested.

Among 88 *Salmonella* isolates, 20.5% (n = 18) and 5.5% (n = 5) were positive for Typhimurium-specific sequence (401 bp) and Enteritidis-specific sequence (304 bp), respectively (Figure 1B). There was a concordance in the PCR assay and traditional serotyping in the identification of S. Typhimurium and S. Enteritidis. Only one of the 18 S. Typhimurium (isolated from chicken meat) showed the specific band for phage type DT104. The other serovars identified according to the conventional serotyping results were Salmonella Corvallis 38.5% (n = 33), S. Hadar 8% (n = 7), S. Weltevreden 5.5% (n = 5), S. Agona 5.5% (n = 5), S. Newport

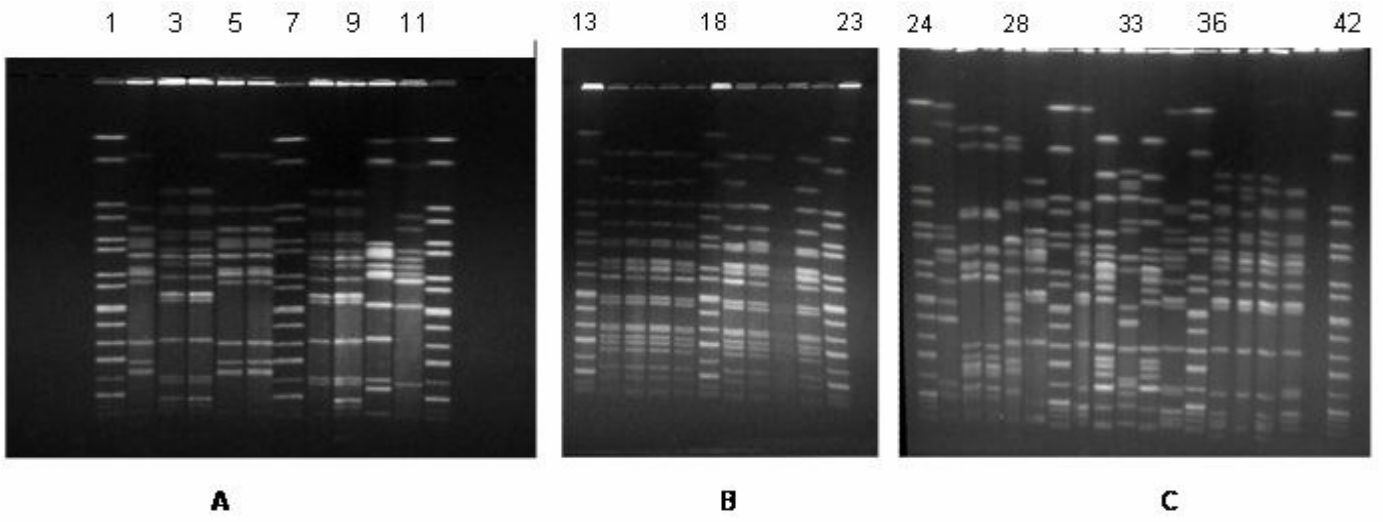

Figure 2. Representative PFGE-*Xba*I profiles of *Salmonella* isolates from raw meats and street foods. A: Lanes 1, 7 and 12: DNA marker (*S.* Braenderup H9812). Lanes 2, 5 and 6: S. Agona. Lanes 3, 4, 8 and 9: S. Corvallis. Lane 10: *S.* Enteritidis. Lane 11*:* S. Albany. B: Lanes 13, 18 and 23: DNA marker (*S.*Braenderup H9812). Lanes 14, 15-17, 19-20 and 22: *S.* Hadar. C: Lane 24, 30, 36 and 42: DNA marker (S. Braenderup H9812). Lane 25: S. Newport. Lane 26 and 27: S. Typhimurium. Lane 28: S. Wandsworth. Lanes 29, 31, 35, 37-39 and 40: S. Corvallis. Lanes 32 and 34: S. Istanbul.

4.5% (n = 4), S. Albany 3% (n = 3), S. Istanbul 2% (n = 2), S. Emek 1% (n = 1) and S. Wandsworth 1% (n = 1).

Macrorestriction analysis by PFGE

All the 88 *Salmonella* isolates were typable by PFGE into 61 distinct pulsed-field profiles (pulsotypes). Pulsotypes consisted of 12 to 19 *Xbal*-restricted fragments with sizes ranging from 33.4 - 1135.0 kb (Figure 2). PFGE was able to distinguish isolates from different serovars as no two serovars shared identical profiles. Reproducible profiles were obtained when PFGE was repeated once.

Cluster analysis of the 61 pulsotypes clustered the 88 isolates into various groups with members of the same serovar being clustered in the same group (Table 1; Figure 3). Among 11 serovars, S. Corvallis (n = 33), S. Typhimurium (n = 18) and S. Hadar (n = 7) were the predominant groups. Out of 33 S. Corvallis isolates, 24 pulsotypes (*F* = 0.69 - 1.0) were observed in various food types (Figure 3). Except for two isolates (SC03607 from raw meat and SC05007 from street food) with indistinguishable profile, all the other S. Corvallis isolates from raw meat and cooked food were genetically different. Among 18 S. Typhimurium isolates, 12 pulsotypes (*F* = 0.8 to 1.0) were detected from various food types. This serovar was more common in raw meats (15/18) and they were closely related (differed in less than three bands). The PFGE profiles of S. Typhimurium isolates recovered from raw meat and cooked street foods (STM05207, STM05607 and STM04704) were different (Table 1; Figure 3). *S.* Hadar (n = 7) recovered from different street foods (chicken curry, beef murtabak, beef lymph, fried chicken, chicken murtabak and chicken liver) purchased from the same locality were indistinguishable (Table 1, Figure 3). These results indicate that cross-contamination could be the main factor for dissemination this serovar in street cooked foods. *S.* Agona was subtyped into four pulsotypes, which were closely related. There was a low genetic diversity among the *S.* Enteritidis as the five isolates were subtyped into three pulsotypes, each with less than 2 bands difference. Members of *S.* Albany, S. Weltevreden, *S.* Istanbul, S. Wandsworth, *S.* Newport and *S.* Emek had unique profiles (Table 1, Figure 3).

DISCUSSION

S. Enterica are common food-borne bacterial pathogens and are most prevalent in chicken, eggs and beef (Lim et al., 2005; Mare et al., 2001; Ray et al., 2007). In this study, *Salmonella* obtained from raw meats and food sources were subtyped by serotyping and pulsed-field gel electrophoresis (PFGE). The present study highlights the considerably high prevalence of *Salmonella* with 27.2 and 72.7% in beef and chicken meats, respectively. Based on the results obtained, there seemed to be a difference in the types of *Salmonella* serovars from the different sources: Certain serovars of *Salmonella* such as S. Enteritidis, S. Weltevreden, S. Albany, S. Agona, and S. Emek were exclusively recovered from retail meats, while S. Hadar, S. Istanbul and S. Wandsworth were recovered from street foods only. Other serovars, S. Typhimurium, *S.* Corvallis, and S. Newport were recovered from both

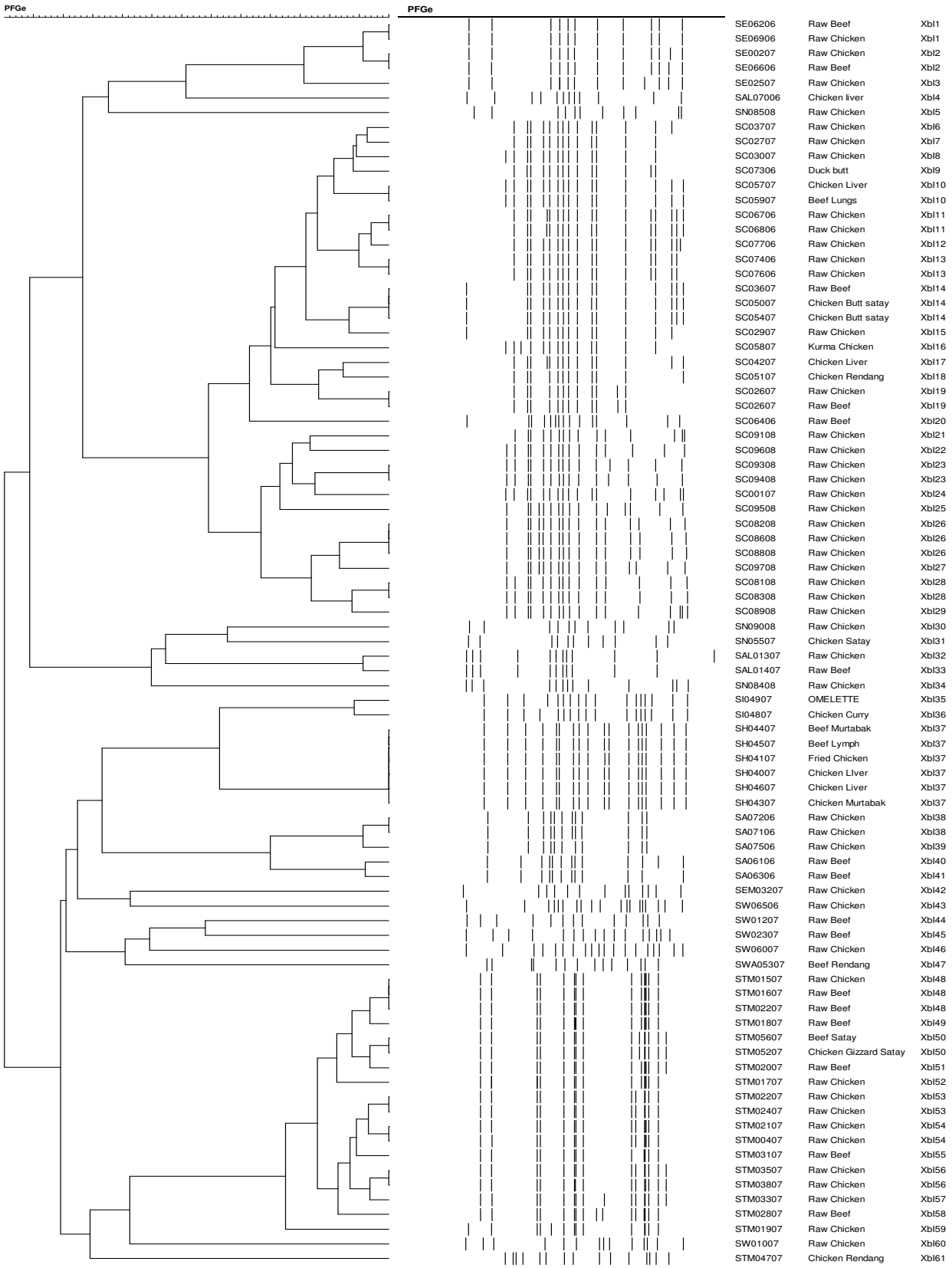

Figure 3. Dendrogram of cluster analysis of *Salmonella* isolates recovered from raw meats and street food. SE = S. Enteritidis, SC = S. Corvallis, SA = S. Agona, SAL = S. Albany, STM = S. Typhimurium, SI = *S.* Istanbul, SW = S. Welterveden, SN = S. Newport, SH = S. Hadar, SEM = S. Emek, SWA = S. Wandsworth.

Table 1. PFGE patterns of *Salmonella* serovars by source, date and locations.

Sample type (No.)	Serovars	Date of sampling	Location of sampling	PFGE patterns
Raw beef (n = 6) Raw chicken (n = 9) [c]Chicken gizzard satay (n = 2) [c]Beef satay (n = 1)	Typhimurium	Mar, Apr 07 Apr 07 Aug, Sep 07 Oct 07	A, B, C, D, E, F	XbI 48-XbI 61
Raw chicken (n = 2) Raw beef (n = 2) Raw chicken (n = 1)	Enteritidis	Jan, Apr 07 Oct, Nov 06 Oct 06	B, G, H	XbI 1 XbI 2 XbI 3
Raw chicken (n = 9) Raw beef (n = 3) [c]Chicken butt satay (n = 2) [c]Chicken rendang (n = 1) [c]Fried chicken liver (n = 2) [c]Kurma chicken (n = 1) Raw chicken (n = 2) Duck butt (n = 1) Beef lungs (n = 1) Raw chicken (n = 11) Raw chicken (n = 2)	Corvallis	Jan, Apr 07 Apr 07 Sep, Dec 07 Sep 07 Dec 07 Dec 07 Aug 06 Sep 06 Dec 07 Dec 08 Sep 08	A, B, G, H, I, J, K, L, M, N, O, P, Q	XbI 6 – XbI 29
Raw chicken (n = 3) Raw beef (n = 2)	Weltevreden	Mar, Dec 07 Apr 07	B, H, M	XbI 43-XbI 46, XbI 60
Raw chicken (n = 1) Raw beef (n = 1) Raw chicken (n = 1)	Albany	Apr 07 Apr 07 Sep 06	B, M	XbI 4, XbI 32, XbI 33
Raw chicken (n = 1) [c]Chicken liver (n = 3) [c]Fried chicken (n = 1) [c]Chicken murtabak (n = 1) [c]Beef murtabak (n = 1) [c]Beef lymph (n = 1)	Emek Hadar	Apr 07 Aug 07 Sep 07 Sep 07 Sep 07 Sep 07	B R, S, T, U	XbI 42 XbI 37
[c]Chicken curry (n = 1) [c]Omelette (n = 1) [c]Beef rendang (n = 1)	Istanbul Wandsworth	Aug 07 Aug 07 Sep 07	V, W X	XbI35, XbI36 XbI47
[c]Chicken satay(n = 1) Raw chicken (n = 1) Raw chicken (n = 2)	Newport	Sep 07 Aug 08 Oct 08	I, F	XbI 5,XbI 30, XbI31,XbI 34

Table 1. Contd.

Raw chicken (n = 1) Raw chicken (n = 1) Raw chicken (n = 1) Raw beef (n = 2)	Agona	Oct 06 Jun 06 Sep 06 Jul 06 Aug 06	M, Y	XbI 38-XbI 41

[c]: Cooked food. A: Hypermarket Putrajaya; B: Night market Seri Kembangan C: Hypermarket Cheras; D: Night market, USJ5; E: Perlis F: Night market, Kerinchi; G: Hypermarket Seri Kembangan; H: SS2, Petaling Jaya; I: Wet market Jinjang KL; J: Wet market Segambut KL; K: Bangsar, KL; L: Jalan Gasing, Petaling Jaya; M: Hawker center Kerinchi; N: Night market Puchong Jaya; O: Night market Putra Heights; P: Night market, Subang Jaya; Q: Hawker center ; Kerinchi; R: USJ14, Subang Jaya; S: Jalan222, Petaling Jaya; T: SS15, Subang Jaya; U: Section 14, Petaling Jaya V: Wet market Puchong W: Hawker center, Shah Alam; X: Night market, SS18, Subang Jaya; Y: Seri Sentosa; KL.

sources.

Among street food samples, chicken meat harbored the highest variety of *Salmonella* serovars. These results are in line with the study's by Bangtrakulnonth et al. (2004) in Thailand, who reported at least 10 different serovars in chicken meat. Previous studies in Malaysia showed that S. Weltevreden was the most frequent serovar isolated from poultry, fish, vegetables and milk (Tunung et al., 2006; Noorzaleha et al., 2003; Thong et al., 2002). However, in this study, the isolation rate of *S. Weltevreden* was low (4.5%) against the other serovars such as S. Corvallis and S. Typhimurium.

S. Typhimurium is a common cause of salmonellosis among humans and animals in many countries (Bouchrif et al., 2009; Chen et al., 2004; White et al., 2001). S. Typhimurium phage type DT104 is of particular public health concern as it is a multiple antimicrobial resistant strain to ampicillin, tetracycline, chloramphenicol, sulfamethoxazole and streptomycin (Bouchrif et al., 2009). Douadi et al. (2010) reported the occurrence of S. Typhimurium DT104 in human and animal hosts. In this study, for the first time S. Typhimurium DT104 was isolated from raw chicken meat in Malaysia. This finding indicates that cross-contamination is an important factor for the dissemination of this phage type in meat products.

PCR is a cost-effective and faster method for the confirmation of *Salmonella* and is useful to complement the conventional culture method. In this study, confirmation of presumptive *Salmonella* was based the presence of *hilA* gene and *ompC.* Both of these targets have been shown to be very specific for *S. enterica* (Pathmanathan et al., 2003). The use of the specific primers for S. Enterititidis and S. Typhimurium provided a rapid identification for these two common *Salmonella* serovars.

Currently, pulsed-field gel electrophoresis (PFGE) is the primary typing method for *Salmonella* outbreak investigations and is suitable for examining the epidemiologically related strains and also for the identification and characterization of *Salmonella* within serovars (Foley et al., 2006; Swaminathan et al., 2001). PFGE is more discriminatory than serotyping for subtyping *Salmonella* strains and would be useful for differentiating *Salmonella* serovars (Harbottle et al., 2006). Analysis of PFGE revealed that all serovars were separated into individual clusters. These finding were very similar to those described by Liebana et al. (2001) and Gaul et al. (2007), in that the cluster analysis grouped strains of similar serovars together. Gaul et al. (2007) reported 56% similarity among 14 different *Salmonella* serovars based on PFGE. In this study, there was a 65% similarity among the 11 *Salmonella* serovars. Also, all serovars differed in their levels of heterogeneity. S. Corvallis isolates were highly diverse while all the seven isolates of S. Hadar were identical with one pulsotype (Figure 3).

The incidence of *Salmonella* was not limited to a particular location, as *Salmonella* were present in various samples and in different locations, indicating the potential problem of the spread of *Salmonella.* Raw meats, particularly minced meats have a very high total count of microorganisms and *Salmonella* are likely to be present in large numbers. Contamination of equipment, hands of workers can spread *Salmonella* to uncontaminated carcasses, which can occur during processing, transport, storage and preparation for consumption. Knowledge of how *Salmonella* disseminates through the food chain is important in understanding how food animals and food-processing procedures contribute to food contamination and subsequent human infections. The lack of food hygiene also needs to be addressed and it is necessary to pay more attention to reduce or eliminate the risk from pathogenic bacteria originating from food and particularly from street food.

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