RESEARH NOTE

MOLECULAR SUBTYPING OF *SALMONELLA ENTERICA* SEROVAR TSHIONGWE RECENTLY ISOLATED IN MALAYSIA DURING 2001-2002

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Abstract. Pulsed field gel electrophoresis (PFGE) and antimicrobial susceptibility analysis were undertaken on twenty-three strains of *Salmonella enterica* serovar Tshiongwe, an unusual serovar, which recently emerged in Malaysia. Antimicrobial susceptibility analysis showed that all the strains were sensitive to ampicilin, chloramphenicol, cotrimoxazole, and kanamycin. Twenty (87%) and 8 (3.5%) strains had resistance to tetracycline and streptomycin respectively. PFGE analysis subtyped 23 strains into 10 profiles (Dice coefficient of similarity, F = 0.7-1.0). The predominant profile, X1 was found in both clinical and environmental isolates and was widely distributed in different parts of Malaysia during the study period. In addition, isolates recovered from food, a hand-towel, apron and the surface of a table-top in one particular location had unique, indistinguishable profiles (X4/4a) and identical antibiograms. Similarly, isolates from cooked meat and a chopping board had PFGE profiles similar to some human isolates. These probably indicated cross-contamination and poor hygiene in food practices, hence contributing to Salmonellosis. Factors causing the emergence of this rare *Salmonella* serovar being responsible for food poisoning episodes during the study period remained unclear. The study reiterated the usefulness and versatility of PFGE in the molecular subtyping of this rare *Salmonella* serovar in Malaysia.

Salmonella infections continued to be an important public health problem in Malaysia. Surveillance programs for the prevention of foodborne diseases have been given high priority by the authorities. The common serotypes associated with gastroenteritis are *S*. Enteritidis, *S*. Typhimurium and *S*.Weltevreden (Yasin *et al*, 1995, Lee *et al*, 1998). Occasionally, rare *Salmonella* serotypes do occur. Between August, 2001 till August, 2002, there was an unusual increase in the number of *Salmonella enterica* serovar Tshiongwe based on the laboratory surveillance at the Salmonella Reference Center, Institute of Medical Research, Malaysia. *S*. Tshiongwe, of serogroup C_3 with an antigenic formula of 6,8:e,n,z_{15} (Ewing, 1986) has never been reported in Malaysia, hence the paucity of data on this rare *Salmonella* serovar. Isolation of *S*. Tshiongwe has been reported in some European countries like Latvia and Moldova (WHO Surveillance report: http//www.bfr.bund.de/internet/7threport/CRS.Iva/MDA) and Poland (Hoszowsky *et al*, 2000). Hence, the study was undertaken in response to an increase in the numbers of *S*. Tshiongwe received in the serotyping laboratory of the Institute for Medical Research, Malaysia to better understand the spread and emergence of this unusual serovar. We determined the genotypes as assessed by pulsed field gel electrophoresis (PFGE) and the antimicrobial susceptibility patterns. In addition, isolates from the available environmental and food specimens were also characterized to determine the association between the clinical and environmental strains.

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PFGE was the method of choice as it is a very reproducible, highly discriminatory subtyping method capable of identifying the transmission source of bacterial pathogens (Tenover *et al*, 1995; Thong *et al*, 2002).

A total of 23 strains (17 humans, 4 environmental, 2 food isolates) were analyzed by antimicrobial susceptibility test and pulsed field gel electrophoresis. All the clinical strains were from patients in various Government hospitals located in different parts of Peninsular Malaysia while the environmental strains (food, apron, handtowel, table top) were from the Public Food Quality Control Laboratory. Antimicrobial susceptibility testing was performed by the disc diffusion method according to National Committee for Clinical Laboratory Standards guideline (NCCLS, 2000). The antimicrobial agents tested include ampicilin, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, streptomycin, and kanamycin. Genomic DNA for PFGE analysis was prepared by the method previously described (Thong and Pang, 1996). A slice of DNA plug was digested with 20 units of *Xba*I at 37ºC for 2 hours. PFGE-*Xba*I was performed using a CHEF DRII /DRIII apparatus (Bio-Rad Laboratories, Hercules, CA) at 6V/cm with a pulse ramped times of 2.2 seconds to 63.8 seconds for 22 hours. The preparation of DNA from the strains was repeated, digested and electrophoresed on at least three occasions to assess the reproducibility and stability of PFGE of the method. Gel analysis was as described previously (Thong *et al*, 2002). PFGE profiles were assigned arbitrary designation and analyzed by defining a similarity (Dice) coefficient, $F= 2n_{xy}/(n_x + n_y)$ where n_x = number of fragments for isolate x, n_y = number of fragments for isolate y.

All the isolates were sensitive to the ampicilin, chloramphenicol, kanamycin, cotrimoxazole. Among the 23 isolates tested, 20 and 8 had resistance to tetracycline and streptomycin respectively. High resistance rates to these two antibiotics was also noted in other *Salmonela* spp and *Shigella* spp in Malaysia (unpublished data). In a study by Hoszowksi and Waysl (2001), a *S.*Tshiongwe strain isolated from sewage sludge had resistance to streptomycin and sulphonamide compounds.

Ten different *Xba*I-PFGE profiles (pulsotypes) consisting 14-16 bands were observed with F values of 0.71 to 0.93 (Table 1, Fig 1). The interpretation of the pulsotypes followed the recommendation of Tenover *et al* (1995) where pro-

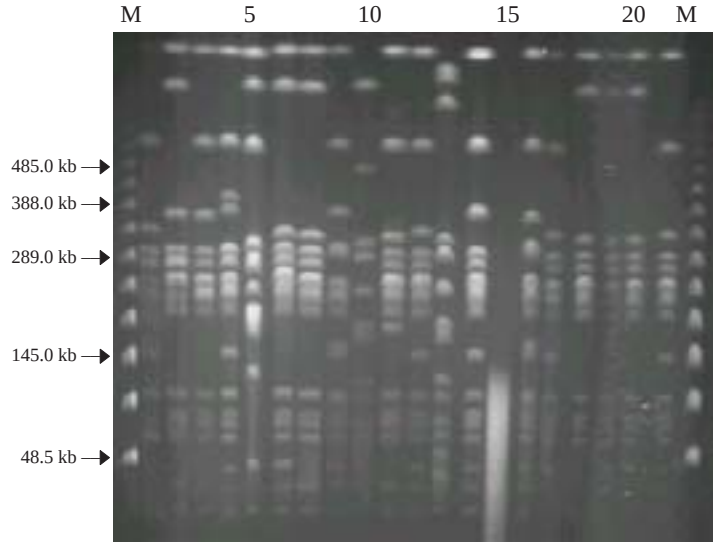

Fig 1–Representative PFGE-*Xba*I profiles of clinical and environmental strains of *S.* Tshiongwe. Lanes 1,22 M=PFGE Lambda DNA concatemer standard; lanes 2-21 : profiles X2b, X3a, X3, X1b, NST, X4, X2, X1, NST, X2, X2a, NST, X1a, DNA degraded, X1, X2a, X4a, X4, X4 and X2a (NST= not *S.* Tshiongwe).

Table 1
PFGE-*Xba*I profiles and antibiograms of Malaysian *Salmonella* Tshiongwe.

Lab No.	Origin /date of islolation	Specimen	Antibiogram profiles[a] A,C, SXT, K,S,T	PFGE
STSW1	NB /2001	Stool	S S S S S S	X2b
STSW2	NB/2001	Stool	S S S S S S	X3a
STSW3	NB /2001	Stool	S S S S S S	X1a
STSW4	NB /2001	Food	S S S S S S	X3
STSW5/24	Penang/2002	Stool	S S S S S R	X1
STSW6	Klang/2002	Stool	S S S S R R	X1
STSW7	KL/2002	Stool	S S S S R R	X1
STSW8	Ipoh/2002	Stool	S S S S S R	X2
STSW9	Alor S./2002	Stool	S S S S S R	X1b
STSW13	KL/25.1.02	Stool	S S S S S R	X1
STSW14	KL/4.2.02	Stool	S S S S R R	X1
STSW16	Ipoh/4.2.2.02	Stool	S S S S S R	X2
STSW17	Seremban/4.4.02	Stool	S S S S S R	X2a
STSW19	Penang/10.5.02	Stool	S S S S S R	X1
STSW20	HKL/16.5.02	Stool	S S S S S R	X1
STSW21	A. Setar/27.3.02	Stool	S S S S S R	X1a
STSW22	Penang/3.4.02	Stool	S S S S S R	X1
STSW25	Klang/15.8.02	Stool	S S S S S R	X2a
STSW26	Kelantan/5.7.02	Food	S S S S R R	X4a
STSW27	Kelantan/5.7.02	Table top	S S S S R R	X4
STSW28	Kelantan/5.7.02	Apron	S S S S R R	X4
STSW11	Kelantan/5.7.02	Hand towel	S S S S R R	X4
STSW12	Kelantan/5.7.02	Chopping board	S S S S R R	X2
STSW29	Perlis/5.7.02	Cooked meat	S S S S S R	X2a

Order of antimicrobial agents: ampicillin (A), chloramphenicol (C), cotrimoxazole (SxT), kanamycin (K), streptomycin (S), and tetracycline (T).

files of less than three bands are closely related and are designated with small letters alphabets to indicate subtypes. The 17 clinical isolates were represented by seven pulsotypes with the most common profile, X1 (8/25 or 32%) (Table 1). This particular pulsotype was represented by strains isolated from different parts of Malaysia, an indication of the wide distribution of this clone within the study period.

Pulsotypes X4 and X4a were unique for isolates from food, apron, a hand-towel and from the surface of a table where the food was prepared (Table 1, Fig 1). This indicated cross-contamination and poor hygiene in food preparation. The first food isolate obtained in 2001 had a unique X3 profile and was very closely related to another human isolate (pulsotype X3a). Similarly, profile X2 was shared by *S.* Tshiongwe strains isolated from cooked meat (in Perlis) and humans (in Seremban and Klang) (Table 1), also indicating the wide distribution and adaptability of this particular strain during the study period.

The association of the different PFGE profiles was indicated by the cluster analysis based on the matrix of similarities. At 85% similarity, two clusters were formed (Fig 2). The first cluster consisted of five pulsotypes (F= 0.8-0.9) represented by clinical and environmental/food isolates. The second cluster also encompassed five profiles, inclusive of the most common pulsotype, X1 (F =0.7-0.9) (Fig 2).

In this study, we report the emergence of a relatively rare serovar, *Salmonella* Tshiongwe isolated from both clinical and environmental speci-

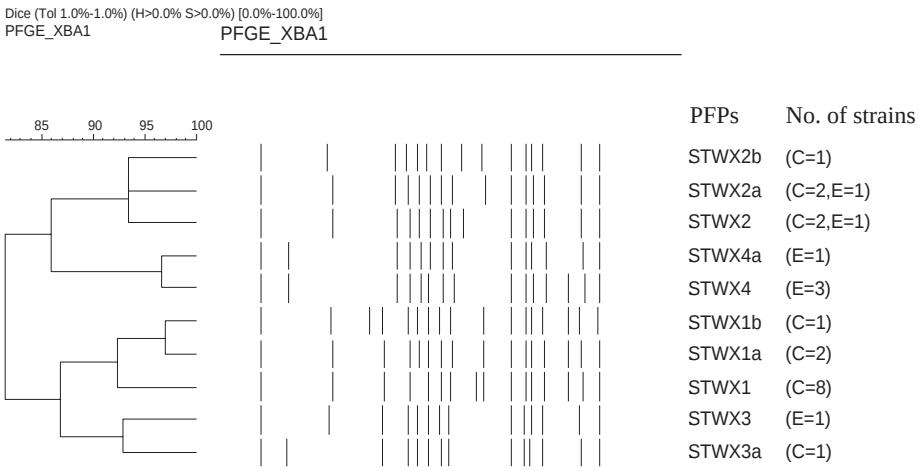

Fig 2–Dendrogram showing cluster analysis of PFGE-*Xba*1 patterns from 24 strains of *Salmonella enterica* serovar Tshiongwe generated with the UPGMA method based on the matrix of F values. The different PFPs and number of strains tested are indicated (C=clinical, E= environmental).

mens in Malaysia. This serovar was first reported in late August, 2001 and continued to surface until October, 2002. It was not clear how this serovar emerged among the prevalent ones like *S.* Enteritidis, *S.* Typhimurium and *S.* Weltevreden. One possible explanation could be through the importation of contaminated meat which was subsequently distributed for sale in various parts of the country. The sporadic cases of food poisoning due to *S.* Tshiongw*e* was probably caused by a few closely related clones (F= 0.7-1.00). The free movement of the population, rapid transportation as well as the availability of common contaminating foods probably helped to further disseminate this strain of *S.* Tshiongwe in the country. The initial clinical isolate obtained in 2001 had profile X1a which was also found in an isolate obtained in 2002. Similarly, other profiles, X2b, X3, X3a found in 2001 isolates were very closely related to the ones observed in 2002 (in both clusters). In addition, the persistence of this serovar could be attributed to its ability to survive in the environment (Wray and Wray, 2000). The habitat of *Salmonella* spp seemed to be limited to the digestive tract of humans and animals. Thus, the presence of *Salmonella* in other habitats such as water, food or other natural environment can be explained by fecal contamination. For example, Salmonella contamination of raw vegetables was due to untreated wastewater (Ait Melloul *et al*, 2001) and the presence of *S.* Tshiongwe in the sewage and veterinary environment (Hoszowksi and Wasyl, 2001).

Overall, PFGE was a useful and discriminative technique to differentiate *S.* Tshiongwe. The work was carried out in a 'blind fashion' that is without prior knowledge of the strains. We initially analyzed 26 strains (labeled as *S.* Tshiongwe) but eliminated three strains as their profiles were very different (more than 10 bands difference) from the dominant profiles (Fig 1, lanes 6,10,13). Subsequent reconfirmation of these three strains by serotyping showed they were indeed of a different *Salmonella* serotype.

In conclusion, the study reiterates the usefulness of PFGE in the molecular epidemiology of *Salmonella i*nfection in Malaysia. The recent increase in food poisoning caused by *S.* Tshiongwe was caused by a few clones circulating in the population. The wide distribution of this particular clone (s) could be associated with a centralized food supply contaminated with *S.*Tshiongwe for urban population. However, the lack of environmental and food specimen hampered further investigation. The source of this *Salmonella* serotype in humans hence remained unknown although it is most probably propagated

through contaminated foods and poor food hygiene practices. The ubiquity of *Salmonella* means that any food, if not handled properly and protected from contamination, can cause infection.

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