GENOTYPIC AND PHENOTYPIC DIFFERENTIATION OF *SALMONELLA ENTERICA* SEROVAR PARATYPHI B IN MALAYSIA

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Abstract. *Salmonella enterica* serovar Paratyphi B is known to cause either paratyphoid fever or gastroenteritis. Differentiation of *Salmonella* ser. Paratyphi B into biotype Java (*d*-tartrate fermenting, dT$^+$) and biotype Paratyphi B (*d*-tartrate non-fermenting, dT$^-$) is important for *Salmonella* epidemiology. This study applied a PCR approach to differentiate the two biotypes to augment the conventional biochemical method and to determine the antibiograms and genomic diversity of Malaysian *S.* Paratyphi B. Among 100 strains tested (clinical, 86; non-humans, 14), only two clinical strains were confirmed as biotype Paratyphi B as indicated by both lead acetate test and PCR. Antibiotic resistance rates were as follows: streptomycin 18%, sulphonamides 13%, ampicillin 10%, chloramphenicol 4%, tetracycline 3%, cefotaxime 2%, cefpodoxime 2%, ceftazidime 2%, gentamicin 1% and trimethoprim 1%. None showed resistance towards amoxicillin-clavulanic acid, ceftiofur, ciprofloxacin, nalidixic acid and trimethoprim-sulphamethoxazole. Seven strains showed multidrug resistance towards 3 or more classes of antimicrobial agents. REP-PCR and PFGE generated 32 and 76 different profiles, respectively. PFGE (D = 0.99) was more discriminative than REP-PCR (D = 0.93) and antimicrobial susceptibility test (D = 0.48) in subtyping the strains. Strains isolated 18 years apart (1982 - 2008) from different localities in Malaysia were clonally related as demonstrated by REP-PCR and PFGE, indicating that these strains were stable and widely distributed. In some clusters, strains isolated from different sources (clinical, food and animal) were grouped together. Thus, biotype Java was the most common biotype of *Salmonella* ser. Paratyphi B in Malaysia. The PCR approach is highly recommended due to its simplicity, specificity and ease of operation. The level of antimicrobial resistance among *Salmonella* ser. Paratyphi B remained relatively low in Malaysia but the emergence of resistance to cephalosporins is a cause for concern.

Keywords: *Salmonella* Paratyphi B, lead acetate test, antimicrobial resistance, REP-PCR, PFGE

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INTRODUCTION

Non-typhoidal *Salmonella* are among the primary causes of food-borne disease in humans. Symptoms due to *Salmonella enterica* serovar Paratyphi B (O:H formula

O1,4,5,12:Hb:1,2) infection is paratyphoid fever or gastroenteritis (Miko *et al*, 2002). It is usually acquired via ingestion of contaminated water or food. *Salmonella* ser. Paratyphi B can be further differentiated into biotypes Java and Paratyphi B based on *d*-tartrate fermentation (Popoff, 2001). Rapid identification of *d*-tartrate fermenting (dT$^+$) and non-fermenting (dT$^-$) strains is important for *Salmonella* surveillance as biotype Java (dT$^+$) is a less virulent strain and is commonly associated with gastroenteritis (Chart, 2003) whereas biotype Paratyphi B (dT$^-$) is often associated with paratyphoid fever (Malorny *et al*, 2003).

Although lead acetate test is the standard protocol to discriminate biotypes Java and Paratyphi B, this method is time consuming and sometimes gives unreliable results (Malorny *et al*, 2003). Previously, we only used the conventional lead acetate test to biotype our *Salmonella* ser. Paratyphi B but it took almost 7 days for confirmation (Goh *et al,* 2003). Thus a more rapid assay is needed. To overcome the deficiency of this biochemical test, Malorny *et al* (2003) proposed a PCR-based test to differentiate dT$^+$ and dT$^-$ *S.* Paratyphi B strains based on the single nucleotide difference in ORF STM3356 gene which codes for a putative cation transporter. In dT$^-$ strains, the AUG translation start codon is altered, resulting in the nonfunctional cation transporter. Hence, the new PCR assay described by Malorny *et al* (2003) was applied to evaluate its usefulness in biotyping *Salmonella* ser. Paratyphi B in Malaysia.

Surveillance of antimicrobial resistance among *Salmonella* strains is important in view of the increasing reports of human infections caused by multiple antimicrobial resistant *Salmonella* (Parry and Threlfall, 2008). Multiple resistance to ampicillin, chloramphenicol and tri-methoprim/sulphamethoxazole has been reported from many countries (Meunier *et al*, 2002; Miko *et al*, 2002; Weil *et al,* 2005). Of greater concern is the growing resistance of *Salmonella* to extended-spectrum cephalosporins and fluoroquinolones which are likely to lead to therapeutic problems (Threlfall, 2002; Parry and Threlfall, 2008). Molecular subtyping to determine the genetic relatedness of *Salmonella* has been shown to be useful for surveillance of salmonellosis (Thong *et al*, 1995). The tools for molecular subtyping include PCR-based typing, pulsed field gel electrophoresis (PFGE), ribotyping, multilocus sequence typing and variable number tandem repeat analysis. For *Salmonella* subtyping, PFGE is still the most common method due to its reproducibility, discriminatory ability, typeability and availability of standardized protocols.

The objectives of this study were to apply a PCR approach to differentiate biotype Java (dT$^+$) and biotype Paratyphi B (dT$^-$) to augment the conventional biochemical method and to determine the antimicrobial susceptibility and genomic diversity of *Salmonella* ser. Paratyphi B in Malaysia.

MATERIALS AND METHODS

Bacterial strains

One hundred *Salmonella* ser. Paratyphi B strains from various sources (humans, animals, food, well-water) were retrieved from the culture collection of the Laboratory of Biomedical Science and Molecular Microbiology, Institute of Graduate Studies, University of Malaya, Malaysia. These strains were previously identified and confirmed by standard microbiology methods. The purity of the strains was checked by using selective media, XLD and BSA agar.

Differentiation of biotypes of *S.* Paratyphi B by lead acetate test

Lead acetate test (*d*-tartrate fermentation) to differentiate *Salmonella* ser. Paratyphi B into biotype Java (dT$^+$) and biotype Paratyphi B (dT$^-$) was carried out as previously described (Goh *et al*, 2003; Malorny *et al*, 2003). In brief, a loopful of cells was inoculated into tubes containing potassium sodium tartrate tetrahydrate in 1% peptone water and 0.0023% bromothymol blue and incubated at 37°C for 6 days. Then, 0.8 ml of saturated aqueous lead acetate solution was added. Positive *d*-tartrate utilization is indicated by precipitation after 1 hour of lead acetate addition. As controls, potassium sodium tartrate tetrahydrate was replaced by distilled water (negative control) and glucose (positive control).

Differentiation of *Salmonella* ser. Paratyphi B biotypes by PCR

DNA was prepared by boiling bacterial cell suspension. A multiplex PCR for the discrimination of dT$^+$ and dT$^-$ *Salmonella* ser. Paratyphi B strains contained 0.4 M primers 166 (5'-GTA AGG GTA ATG GGT TCC-3') and 167 (5'-CAC ATT ATT CGC TCA ATG GAG-3') (Malorny *et al*, 2003), 0.2 M primers ST11 and ST15, 200 M dNTP, 1 x PCR buffer (Promega Madison, WI), 2.5 mM MgCl$_2$, 1 U of *Taq* polymerase (Promega) and a 5 l (approximately 100 ng) DNA solution. The additional primer set ST11/ST15, a *Salmonella* genus-specific primer was included to increase the reliability of the PCR assay (Aoba *et al*, 1993). The PCR thermocycling consisted of initial denaturation at 95°C for 1 minute followed by three cycles of 95°C for 30 seconds, 61°C for 30 seconds, and 72°C for 30 seconds; 27 cycles of 95°C for 30 seconds, 58°C for 30 seconds; 72°C for 30 seconds and a final heating of 72°C for

4 minutes. PCR amplicons were resolved on a 1.5 % (w/v) agarose gel and visualized by staining in ethidium bromide (0.5 g/ml) and destaining in distilled water.

Antimicrobial susceptibility test

All *Salmonella* ser. Paratyphi B strains were tested for susceptibility to 15 antimicrobial agents: ampicillin (10 g), amoxicillin/clavulanic acid (30 g), cefotaxime (30 g), cefpodoxime (10 g), ceftazidime (30 g), ceftiofur (30 g), chloramphenicol (30 g), ciprofloxacin (5 g), gentamicin (10 g), nalidixic acid (30 g), streptomycin (10 g), sulphonamides (300 g), trimethoprim (5 g), trimethoprim/ sulphamethoxazole (23.75 g/1.25 g), and tetracycline (30 g) by a modified Kirby-Bauer disk diffusion method on freshly prepared Mueller Hinton agar (Oxoid, Cambridge, UK). *Escherichia coli* ATCC 25922 was used as control (CLSI, 2006).

Repetitive extragenic palindromic (REP)-PCR

REP-PCR was carried out following the method previously described by Gallardo *et al* (1999). In short, REP-PCR solution (25 l) contained 0.5 M primer REP (5'-GCG CCG ICA TGC GGC ATT-3'), 50 M dNTP, 1 x PCR buffer (Promega), 2.5 mM MgCl$_2$, 1 U of *Taq* polymerase (Promega), and a 5 l aliquot (approximately 100 ng) DNA solution. PCR thermocycling consisted of 2 cycles of 94°C for 5 minutes, 33°C for 5 minutes, 68°C for 5 minutes and 30 cycles of 94°C for 5 minutes, 45°C for 1 minute, 68°C for 2 minutes and a final heating of 68°C for 16 minutes. PCR amplicons were resolved on a 1.5 % (w/v) agarose gel, and electrophoresis was performed in 0.5 x TBE buffer at 100 V for 5 hours. Bands were visualized after staining in ethidium bromide (0.5 g/ml) and destaining in distilled water. A 1 kb DNA ladder and 100 bp DNA ladder (Promega)

were used as DNA size standards.

Pulsed-field gel electrophoresis (PFGE)

PFGE was performed according to Thong *et al* (2003). In brief, equal volumes of standardized cell suspension (turbidity: 0.48 - 0.52) were mixed with 1% Seakem Gold Agarose (Cambrex Bio Science, Rockland, ME) and allowed to set in mold. The gel plugs were lysed with cell lysis buffer containing 1 mg/ml proteinase K at 54ºC for 2 hours followed by washing with sterile de-ionized water and TE buffer. A 2 mm slice of the gel plug was digested with 10 U of *Xba*I (Promega) and PFGE performed using CHEF Mapper (Bio-Rad Laboratories, Richmond, CA) in 1% agarose in 0.5 x TBE buffer at 6 V/cm with a pulse ramped times of 2.16 seconds to 63.8 seconds for 24 hours. A *Salmonella* Braenderup strain (H9812) was used as DNA size marker.

Data analysis

DNA profiles (in TIFF files) were analyzed using BioNumerics software (Applied Math, Kortrijk, Belgium). Quantitative difference between two strains was scored by Dice coefficient of similarity (*F* value) for band matching with 1.0% position tolerance, as previously described (Thong *et al*, 1995) and a hierarchic unweighted pair group method for arithmetic averages (UPGMA) was used to generate a dendrogram for cluster analysis. Discriminatory power (D) was calculated using Simpson's index of diversity (Hunter and Gaston, 1988).

RESULTS

Bacterial strains

A total of 100 *Salmonella* ser. Paratyphi B strains isolated from 1982 to 2008 were tested. These strains included 86 clinical and 14 non-human isolates. The clinical strains originated from different parts of Malaysia and were cultured from stools (*n* = 64), blood (*n* = 6) and unknown sources (*n* = 16). Non-human strains were derived from food (*n* = 9), animals (*n* = 2) and environment well water (*n* = 2). The numbers of *Salmonella* ser. Paratyphi B isolated in different years were: 1982 (*n* = 4), 1983 (*n* = 8), 1995 (*n* = 2), 1998 (*n* = 1), 1999 (*n* = 4), 2000 (*n* = 7), 2001 (*n* = 6), 2002 (*n* = 21), 2003 (*n* = 11), 2004 (*n* = 10), 2005 (*n* = 13), 2006 (*n* = 1), 2007 (*n* = 6), and 2008 (*n* = 5).

Differentiation of Java (dT$^+$) and Paratyphi B (dT$^-$) biotypes by lead acetate test and PCR

Among 100 strains, only two, SPB 45 and SPB 117 were non-tartrate fermenting (dT$^-$, biotype Paratyphi B) as indicated by blue coloration of the media and fluffy fine precipitate with lead acetate. The remaining 98 strains were tartrate fermenters (dT$^+$, biotype Java) as indicated by yellow coloration of the media and small volume of precipitate with lead acetate addition. Similarly, PCR results showed that strains SPB 45 and SPB 117 were dT$^-$ as they lacked the ORF STM3356 gene (290 bp) (Fig 1). The other 98 strains were dT$^+$ as they harbored both 429 bp and 290 bp amplicons (Fig 1). Consistent results were obtained when PCR were repeated twice. Both the Paratyphi B dT$^-$ biotype strains were from patients from a tertiary University Hospital isolated in 1982 and 1983. Based on clinical records, these two patients presented gastroenteritis and fever.

Antibiograms

Among the 100 strains, 28 had resistance to at least one antimicrobial agent and 12 antibiograms were observed (Table 1). All the resistant strains were from humans. The resistance rates are as follows: streptomycin 18%, sulphonamides 13%,
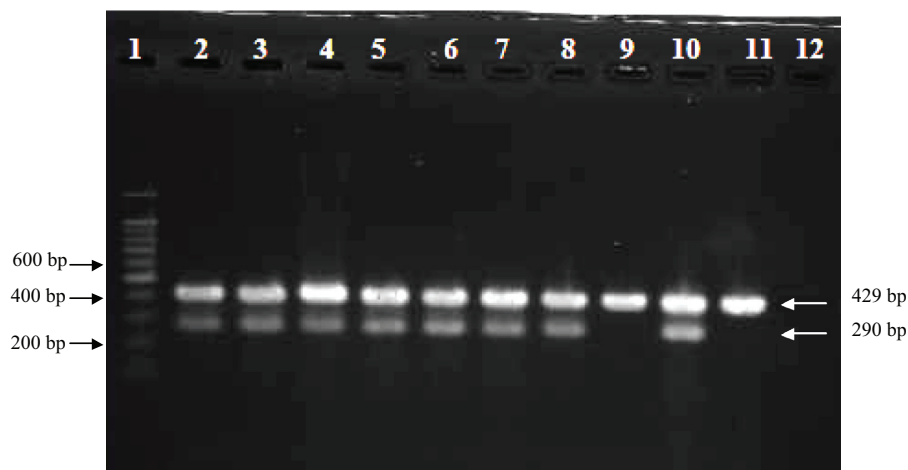
Fig 1–Representative electrophoretic gel of PCR amplicons employed 2 primer sets (primers SPB166/167 and ST11/15) for detection of *S.* Paratyphi B strains. Lane 1, 100 bp DNA marker; lanes 2-12, SPB 03/07, SPB 42/07, SPB 04/08, SPB 06/08, SPB 486/03, SPB QB9 M7, SPB 1961/95, SPB 45, SPB 85, SPB 117, negative control.

Table 1
Antibiograms of *S.* Paratyphi B strains (biotype Paratyphi B dT⁻ and Java dT⁺).

Antibiogram (n)	Resistant phenotypes	Year isolated	Biotype
Profile 1 (4)	A^R, C^R, S^R, Su^R	2002, 2003, 2008	Java
Profile 2 (2)	A^R, S^R, Su^R	1983, 2001	Java
Profile 3 (2)	CTX^R, CPD^R, CAZ^R	2002, 2004	Java
Profile 4 (1)	S^R, Su^R, T^R	1983	Paratyphi B
Profile 5 (1)	$A^{R,}$ W^R	1998	Java
Profile 6 (1)	CN^R, Su^R	2003	Java
Profile 7 (4)	S^R, Su^R	2001, 2003, 2008	Java
Profile 8 (3)	A^R	1982, 1999, 2005	Java
Profile 9 (7)	S^R	2002-2005	Java
Profile 10 (1)	Su^R	2005	Java
Profile 11 (2)	T^R	1982	Paratyphi B
Profile 12 (72)	Sensitive and intermediate	1982-2007	Java

A, ampicillin; C, chloramphenicol; CN, gentamicin; S, streptomycin; Su, sulphonamides; T, tetracycline; W, trimethoprim; CTX, cefotaxime sodium; CPD, cefpodoxime; CAZ, ceftazidime

ampicillin 10%, chloramphenicol 4%, tetracycline 3%, cefotaxime 2%, cefpodoxime 2%, ceftazidime 2%, gentamicin 1% and trimethoprim 1%. None showed resistance towards amoxicillin-clavulanic acid, ceftiofur, ciprofloxacin, nalidixic acid and trimethoprim-sulphamethoxazole.

Of particular interest was that the strains resistant to cefotaxime, cefpodoxime and ceftazidime were first observed
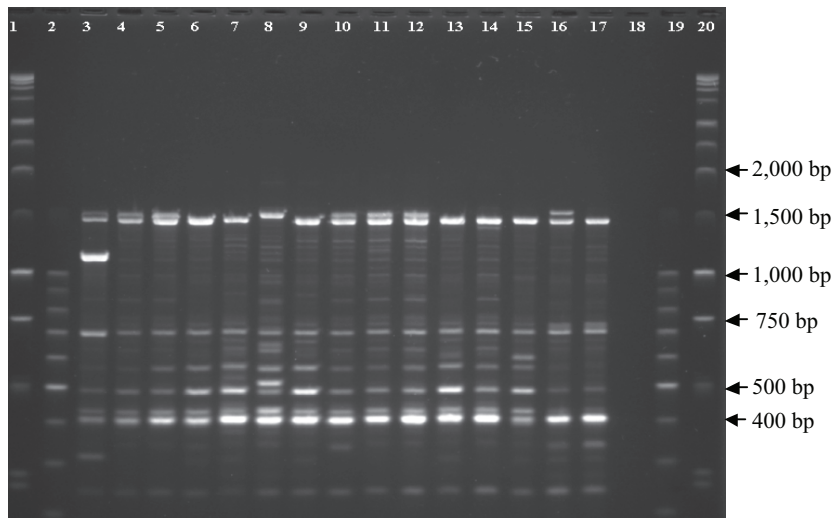
Fig 2–Representative REP profiles of *S.* Paratyphi B strains. REP primer employed was 5'-GCGCC-GICA TGCGGCATT-3'. Lanes 1 and 20, 1'kb DNA ladder; lanes 2 and 19, 100 bp DNA marker; lanes 3-17, SPB180, SPB181, SPB212, SPB70001, SPB80433, SPB ATCC 8759, SPB 311/03, SPB 9428/01, SPB 4714/95, SPB4, SPB5, SPB6, SPB7, SPB MOB 2442/05, SPB MOB 2587/05; lane 18, negative control.

in 2002, while strains resistant towards the older antimicrobials, such as ampicillin, tetracycline, streptomycin and sulphonamides, were observed since 1982. Two clinical biotype Java strains (SPB 702/02 and SPB 164/04) isolated in 2002 and 2004 showed resistance against cefpodoxime, cefotaxime and ceftazidime (third generation cephalosporin). Seven clinical biotype Java strains isolated from 1983 to 2008 from different localities in Malaysia showed multiple drug resistance towards 3 or more classes of antimicrobial agents. Four clinical biotype Java strains (SPB 63/02, SPB 661/03, SPB 05/08, SPB 06/08) showed resistance against ampicillin, chloramphenicol, streptomycin and sulphonamides. Of the 2 biotype Paratyphi B dT⁻ strains, 1 had resistance against streptomycin, sulphonamides and tetracycline.

Genetic diversity of *Salmonella* ser. Paratyphi B by REP-PCR

REP-PCR subtyped the 98 biotype Java strains into 32 REP profiles (F = 0.48 - 1.0) consisting of 4 to 9 fragments, ranging in size from approximately 350 bp to 1,600 bp (Fig 2). All the profiles were reproducible when analysis was repeated twice. However, both biotype Paratyphi B dT⁻ strains could not be typed as no distinct bands were obtained. The predominant REP profile was REP019 (n = 18), which was shared by 16 clinical and 2 food strains isolated in 2002 - 2007. Twenty REP profiles were unique and were represented by one strain each.

Cluster analysis of the REP profiles gave 2 major clusters (A and B) based on 80% similarity (Fig 3). Cluster A consisted of 13 REP profiles (REP001 - REP013) from
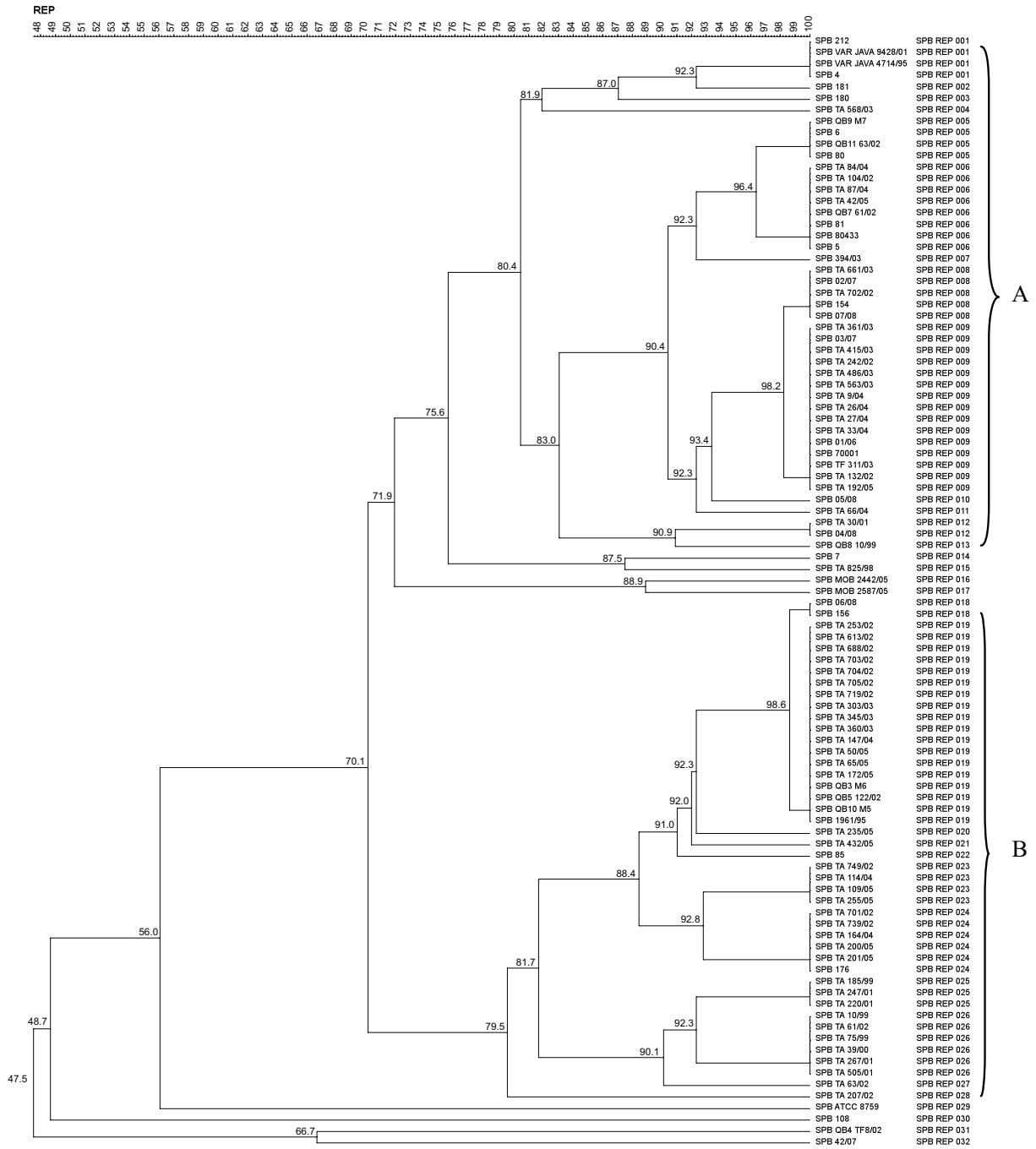
Fig 3–Dendrogram generated using DICE coefficient based on REP profiles of 98 *S*. Paratyphi B isolates, and constructed using UPGMA algorithm.
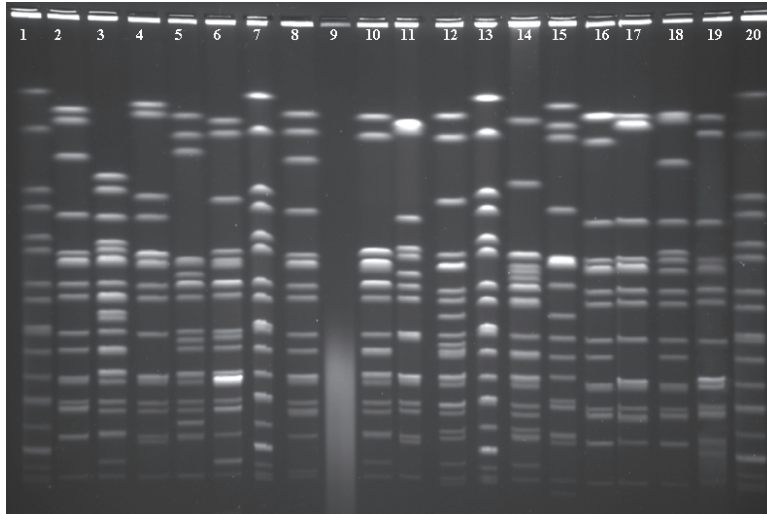
Fig 4–Representative electrophoretic gel of *Xba*I-PFGE profiles of *S.* Paratyphi B strains. Lanes 1, 7, 13 and 20, *Salmonella* Braendup H9812; lanes 2-6, SPB 661/03, SPB 164/04, SPB 235/05, SPB 255/05,SPB 432/05; lanes 8-12, SPB QB3 M6, SPB 85, SPB 176, SPB TF 311/03, SPB 9428/01; lanes 14-19, SPB4, SPB MOB 2587/05, SPB 07/08, SPB 02/07, SPB 05/08, SPB 303/03.

45 biotype Java strains (38 clinical, 5 food and 2 zoonotic strains) isolated over 18 years period (1982 - 2008) from different localities in Malaysia. Cluster B consisted of 11 REP profiles (REP018 - REP028) from 44 biotype Java dT^+ strains (41 clinical and 3 food) isolated in 1983 - 2008 from different localities.

Genetic diversity of *Salmonella* ser. Paratyphi B by PFGE

PFGE analysis subtyped the 99 *Xba*I-digested *Salmonella* ser. Paratyphi B strains (97 biotype Java dT^+ and 2 biotype Paratyphi B dT^-) into 76 different pulsotypes (F = 0.15 - 1.0) consisting of 11 to 19 fragments, ranging in size from approximately 30 kb to 1,130 kb (Fig 4). All the pulsotypes were reproducible when analysis was repeated twice. One biotype Java dT^+ strain, SPB 85, could not be typed by PFGE in spite of repeated analysis (lane 9, Fig 4).

No predominant pulsotype was observed. Among 76 different pulsotypes, 74 were observed among the 97 biotype Java strains and 2 pulsotypes for the 2 biotype Paratyphi B strains. There were 62 unique pulsotypes, which were represented by one strain each. Two pulsotypes, XBA021 and XBA041 were shared by strains from humans and food. Both the biotype Paratyphi B dT^- strains were distinguishable (F = 0.72) and were distinctly different from the biotype Java strains (F = 0.51).

Based on 80% similarity, cluster analysis of the pulsotypes gave 5 clusters (A, B, C, D and E) (Fig 5). The major Cluster B comprised 36 pulsotypes (XBA018 - XBA053) from 50 biotype Java dT^+ strains (43 clinical and 7 food strains) isolated over 18 years period (1982 - 2008) from different localities. Cluster A consisted of 13 pulsotypes (XBA004 - XBA016) from 15 biotype Java dT^+ strains (13 clinical and 2 zoonotic strains). Cluster C consisted of 4 pulsotypes (XBA055 - XBA058) from 4 biotype Java strains (3 clinical and 1
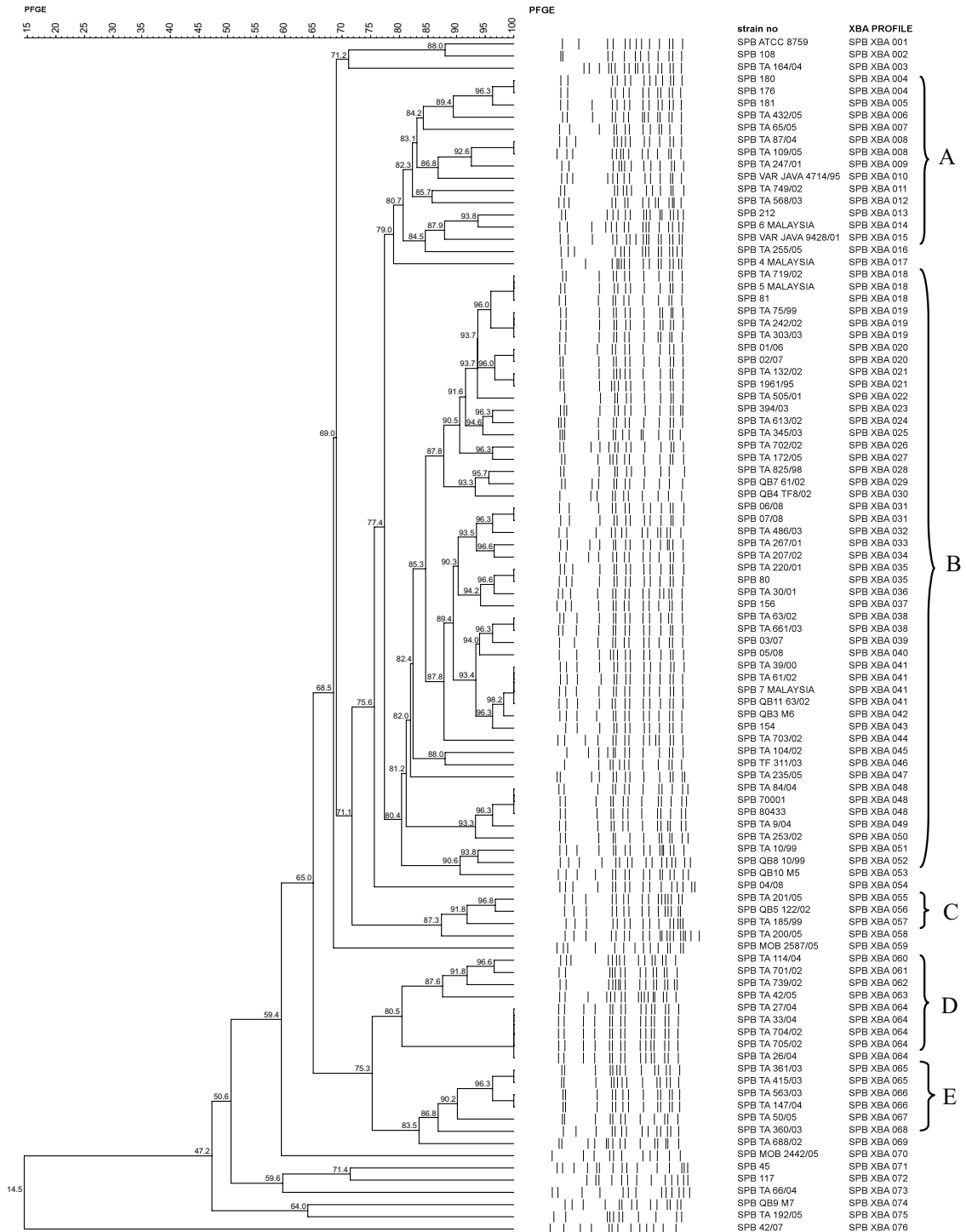
PFGE2 (98 entries)

Fig 5–Dendrogram generated using DICE coefficient based on PFGE profiles of 99 *S*. Paratyphi B isolates digested with *Xba*I, and constructed using UPGMA algorithm.

food). Cluster D consisted of 5 pulsotypes (XBA060 - XBA064) from 9 clinical biotype Java strains. Cluster E consisted of 5 pulsotypes (XBA065 - XBA069) from 7 clinical biotype Java strains.

DISCUSSION

The differentiation of two closely related biotypes of *Salmonella* ser. Paratyphi B is important as they cause different disease outcome. Malorny *et al* (2003) reported a better concordance (100 %) between the 6-days incubation using the lead acetate test with the PCR results compared to the 3-days incubation which gave only 78 % concordance. However, Martinez *et al* (2006) reported no difference between the 3- and 6- days' incubation period. In this study, we adopted the 6-days incubation period (to ensure completion of bacterial activity) and it was 100% concordant with the PCR result.

PCR-based method was much faster and convenient as results could be obtained within 4 hours and did not require additional media preparation. This approach is useful to augment the conventional method. In this study, 98% of the *Salmonella* ser. Paratyphi B was d-tartrate fermenters (dT$^+$). This is a common finding as reported in Canada (Mulvery *et al*, 2004), Germany (Miko *et al*, 2002), Spain (Martinez *et al*, 2006) and France (Weil *et al*, 2005). This is not surprising as gastroenteritis is a more common disease syndrome of *Salmonella* rather than paratyphoid fever. Most cases of paratyphoid fever are caused by *S.* Paratyphi A. In more recent years in Malaysia, most of the *Salmonella* ser. Paratyphi B are of biotype Java while biotype Paratyphi B was last seen in early 1980s (Goh *et al,* 2003). Therefore it is recommended to further subtype *Salmonella* ser. Paratyphi B using

the simpler PCR approach to provide a more precise and rapid information on the causative agent of paratyphoid fever or gastroenteritis.

A previous study by Goh *et al* (2003) showed that *Salmonella* ser. Paratyphi B has high resistance towards ampicillin, chloramphenicol, streptomycin, and tetracycline. In this study, similar results were observed except for resistance rates to chloramphenicol and tetracycline, which were relatively lower. Increased resistance towards first line antimicrobials, ampicillin, chloramphenicol and trimethoprim-sulphamethoxazole, has led to the use of newer antimicrobials such as quinolones and cephalosporins for treatment of invasive salmonellosis. Although the incidence of *S*.Paratyphi B biotype Java resistant to third generation cephalosporins was low in this study (0.03 %), continued monitoring is necessary as these resistance phenotypes seemed to have emerged since 2002.

Overall, PFGE (D = 0.99) was more discriminative than REP-PCR (D = 0.93) and antimicrobial susceptibility test (D = 0.48) in subtyping the 100 *Salmonella* ser. Paratyphi B strains (Table 2). REP-PCR exhibited less unique profiles compared to PFGE (62 unique pulsotypes). PFGE was more reproducible and generated clear distinct banding patterns that were easy to visualize while REP-PCR, though simpler to perform, yielded less discernable banding patterns.

The two Paratyphi B biotypes strains were not typeable by REP-PCR. This could be due to the loss of specific sites for REP primer binding in the genome of these strains. Most REP-PCR profiles have high similarity and shared the majority of their fragments. Robertson *et al* (2003) reported that REP-PCR by using REP1R-Dt

Table 2
Relationship among antibiograms, REP profiles and pulsotypes of *S*. Paratyphi B.

PFGE cluster	Source (No.)	Period isolated	No. of antibiogram profiles (D = 0.48)	No. of REP profiles (D = 0.93)	No. of pulsotypes (D = 0.99)
A	Clinical (*n* = 13) Animal (*n* = 2)	1983 -2005	2	11	13
B	Clinical (*n* = 43) Food (*n* = 7)	1982 -2008	7	18	36
C	Clinical (*n* = 3) Food (*n* = 1)	1999 -2005	2	3	4
D	Clinical (*n* = 9)	2002 -2005	1	5	5
E	Clinical (*n* = 7)	2002 -2005	2	2	5

and REP-2D and ERIC-PCR were less discriminative than plasmid profiling in genotyping of *Salmonella* ser. Paratyphi B. The use of PFGE with *Xba*I has been widely applied in *Salmonella* fingerprinting (Thong *et al*, 1995). This study shows that PFGE has the highest discriminatory power compared to REP-PCR and antimicrobial susceptibility test.

The concordance between DNA profiling methods and antimicrobial susceptibility test was low. The specific genes that contribute to antibiotic resistance might be located on plasmids, which can be easily lost (Hopkins *et al*, 2007). Antibiotic resistance that are caused by minor changes on the genomic DNA are inadequate to alter the DNA profiles unless the changes occur within REP-PCR primer binding sites or recognition sites for PFGE restriction enzyme (Thong *et al*, 2003). Therefore, REP-PCR and PFGE might not be able to distinguish multidrug-resistant from the susceptible strains. Similar observation was reported by Robertson *et al* (2003).

In summary, biotype Java was the most common biotype of *Salmonella* ser. Paratyphi B in Malaysia. It is necessary to further differentiate *Salmonella* ser. Paratyphi B into its biotypes in order to provide more information for *Salmonella* surveillance. The PCR approach is highly recommended due to its simplicity, specificity and ease of operation. The level of antimicrobial agent resistance among *Salmonella* ser. Paratyphi B remained relatively low in Malaysia but the emergence of resistance to cephalosporins is a cause for concern. PFGE had a higher discriminatory power compared to REP-PCR to differentiate the two closely related biotypes of *Salmonella* ser. Paratyphi B.

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