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Original Article

Macrorestriction Analysis and Antimicrobial Susceptibility Profiling of *Salmonella enterica* at a University Teaching Hospital, Kuala Lumpur

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SUMMARY: The genetic diversity and antimicrobial resistance rates of clinical *Salmonella* isolates (2007–2008) at the University of Malaya Medical Centre, Kuala Lumpur, were investigated and the genetic diversity of the isolates was determined by pulsed-field gel electrophoresis (PFGE) and repetitive extragenic palindromic (REP)-PCR. *Xba*I-PFGE analysis generated 57 profiles (Dice coefficient, F = 0.08–1.00), whereas REP-PCR using the REP primer generated only 35 (F = 0.34–1.00). PFGE was therefore the more discriminative and reproducible method for assessing the genetic diversity of salmonellae. The antibiograms of 78 *Salmonella* isolates were assessed against 19 antimicrobials using the disk diffusion method. Twenty serotypes were identified, with the most common being *S.* Enteritidis (18%) followed by *S.* Typhimurium (14%), *S.* Paratyphi B var Java (9%), *S.* Weltevreden (9%), and *S.* Corvallis (9%). A total of 38 resistant profiles were defined, with 53.8% of the isolates being resistant to three or more antimicrobials. The highest resistance rates were observed for cephalothin (55.1%), tetracycline (47.4%), and nalidixic acid (35.9%). The presence of multidrug-resistant *Salmonella* strains is a cause for concern as it may limit the treatment of severe salmonellosis. One multidrug-resistant *S.* Enteritidis strain was a putative extended-spectrum beta-lactamase producer, based on a double disk diffusion analysis, and was resistant to ceftriaxone (MIC $> 32\ \mu$g/mL). The data generated by this study will contribute towards epidemiological monitoring and investigations of *Salmonella* infections in Malaysia.

INTRODUCTION

Non-typhoidal salmonellosis (NTS) continues to be a major public health problem in both developed and developing countries. A recent study at the University of Malaya Medical Centre (UMMC), Kuala Lumpur showed that, between 1978 and 1997, non-typhoidal *Salmonella* was the most common bacterial pathogen (57%) isolated from Malaysian children under 16 years of age with diarrhea (1).

Salmonella enterica serovar Typhimurium was the most common *Salmonella* serovar isolated at UMMC between 1973 and 1982, although the relative dominance of certain serovars of *S. enterica* in Malaysia was found to vary with time. *S.* Typhi was the most common serotype recovered from humans in Malaysia between 1983 and 1992 (2), whereas *S.* Weltevreden was reported to be the most common non-typhoidal *Salmonella* between 1989 and 1994 (3). In the period 2003 to 2005, the National Public Health Laboratory of Malaysia (4) reported *S.* Enteritidis (28.1% of 552 isolates) to be the most common non-typhoidal *Salmonella*, followed by *S.* Weltevreden (25.7%), *S.* Corvallis (10.3%), and *S.* Typhimurium (6.7%). All these serovars are able to cause disease.

Additionally, the emergence of multidrug-resistant (MDR) *Salmonella* is a public health concern. The prophylactic or chemotherapeutic use of antimicrobials in food-producing animals has been linked to the emergence of antimicrobial-resistant strains (5) and MDR *Salmonella* have been isolated from many Asian regions, including Malaysia (6).

The surveillance and subtyping of *Salmonella* serotypes are important for epidemiological investigations of *Salmonella* outbreaks, and various methods exist for this purpose. Conventional typing methods based on phenotypic characteristics, such as biotyping, serotyping, and phage typing, have been used widely, although these methods tend to be less discriminative. In contrast, molecular approaches, such as pulsed-field gel electrophoresis (PFGE), amplified-fragment length polymorphism (AFLP), and multilocus variable-number tandem-repeat analysis (MLVA) offer a much higher discrimination. PFGE is widely regarded to be the "gold standard" for *Salmonella* subtyping (7).

In this study, clinical isolates of *Salmonella* (2007–2008) at UMMC, a tertiary teaching hospital in Kuala Lumpur, Malaysia were subtyped using PFGE and repetitive extragenic palindromic (REP)-PCR and their rates and patterns of antimicrobial resistance were determined.

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MATERIALS AND METHODS

Bacterial strains: Seventy-eight non-repeat clinical isolates (2007–2008) were identified biochemically to the genus *Salmonella* by the Microbiology Laboratory at UMMC. All except one (blood culture) were from stool cultures. The majority of patients were diagnosed with acute gastroenteritis or diarrhea. Dysentery was reported in 4 patients, pyrexia of unknown origin (PUO) in 2 patients, from whom *S.* Typhi was cultured, and *S.* Braenderup and *S.* Enteritidis were recovered from 2 patients with sepsis. Confirmation of the serotypes was carried out by the Salmonella Reference Centre at the Institute for Medical Research according to standard procedures.

REP-PCR: A boiled suspension of bacterial cells was used as DNA template, and REP-PCR was carried out using the primer 5′ GCG CCG ICA TGC GGC ATT 3′. Cycling conditions were taken from Gallardo et al. (8) and Bennasar et al. (9) with slight modifications. The conditions were: 2 cycles of 94°C for 5 min, 33°C for 5 min, and 68°C for 5 min, followed by 30 cycles of 94°C for 1 min, 45°C for 1 min, and 68°C for 2 min, and a single final extension at 68°C for 16 min. The 25 μL reaction contained 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μM of dNTP, 0.4 μM of each primer, 1.5 U *Taq* DNA polymerase (Promega, Madison, Wis., USA) and 5 μL (approximately 100 ng) of crude DNA. The PCR products were resolved on a 1.5% w/v agarose gel, stained with ethidium bromide, visualized under UV light and analyzed using a Gel Doc system (Bio-Rad Laboratories, Hercules, Calif., USA).

PFGE: Genomic DNA was prepared and embedded in agarose plugs, as described previously (10). Slices of these plugs were digested with 10 U of *Xba*I (Promega) restriction enzyme overnight at 37°C and then electrophoresed on a CHEF Mapper (Bio-Rad) for 26 h at 6 V/cm, 120°C, with an initial pulse time of 2.2 s and final pulse of 63.8 s at 14°C. *Xba*I restricted-*Salmonella* serotype Braenderup H9812 was used as both control and size marker. The macrorestricted fragments were resolved on a 1% w/v agarose gel, stained with ethidium bromide, then destained and photographed under UV illumination. DNA fragment patterns were assessed visually and distinct profiles assigned an arbitrary restriction endonuclease analysis pattern. Analysis of the restricted fragments was carried out using the Gel Compar II software (Applied Maths, Kortrijk, Belgium). A dendrogram based on the Dice coefficient was generated using the unweighted pair group method with arithmetic mean (UPGMA) algorithm and 1% position tolerance.

Antimicrobial susceptibility test: All isolates were tested for their susceptibility to 19 antimicrobial agents using the agar disk diffusion technique on Mueller-Hinton agar, as recommended by the Clinical and Laboratory Standards Institute (CLSI) (11). The following antimicrobial agents were used: streptomycin (10 μg), kanamycin (30 μg), amikacin (30 μg), gentamicin (10 μg), amoxicillin/clavulanic acid (20/10 μg), cephalothin (30 μg), ceftiofur (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg) trimethoprim/sulfamethoxazole (1.25/23.75 μg), imipenem (10 μg), ampicillin (10 μg), chloramphenicol (30 μg), nalidixic acid (30 μg), gatifloxacin (5 μg), ciprofloxacin (5 μg), levofloxacin (5 μg), and tetracycline (30 μg). All disks were obtained from Oxoid (Hampshire England) except gatifloxacin and ceftiofur (Becton Dickinson, Sparks, Md., USA). The *Escherichia coli* ATCC 25922 strain was used as quality control. Inhibition zones, in millimeters, were recorded and interpreted as susceptible, intermediate, or resistant by reference to the breakpoints, as recommended by CLSI. All antimicrobial susceptibility tests were repeated twice.

A double disk diffusion assay was carried out to screen for extended spectrum beta-lactamase (ESBL) producers against the third-generation cephalosporins (ceftiofur, ceftazidime, ceftriaxone, and cefotaxime). An amoxicillin/clavulanic acid disk and a third-generation cephalosporin disk were placed 15 mm apart, as measured from the disk edge, and the results were interpreted as per CLSI guidelines.

RESULTS

The 78 *Salmonella* isolates were subtyped into 20 serotypes: Enteritidis (n = 14), Typhimurium (n = 11), Paratyphi B var Java (n = 7), Weltevreden (n = 7), Corvallis (n = 7), Stanley (n = 5), Braenderup (n = 5), Albany (n = 5), Thompson (n = 3), Richmond (n = 2), Lexington (n = 2), Typhi (n = 2), and one each from serotypes Newport, Paratyphi A, Bovismorbificans, Larochelle, Okatie, Matopeni, Muenchen, and Infantis. *S.* Okatie, *S.* Lexington, *S.* Matopeni, and *S.* Weltevreden were each recovered from 4 patients with dysentery.

DNA fingerprinting via PFGE and REP-PCR: All 78 *Salmonella* isolates were typeable using PFGE and REP-PCR. Overall, PFGE was more reproducible and discriminative than REP-PCR in subtyping these strains.

With PFGE, *Xba*I restriction of the isolates yielded 12 to 19 DNA-restricted fragments with sizes ranging from 33.4 to 1135.0 kb (Dice coefficient, F = 0.08–1.00; Fig. 1). Fifty-seven distinct pulsotypes arbitrarily designated as Xba001 to Xba057 were defined. PFGE was able to distinguish isolates from different serotypes as no 2 serotypes shared the same profile. Members of certain serotypes were very diverse with 6 pulsotypes being obtained from 7 *S.* Paratyphi B var Java isolates (F = 0.7–1.0) and 7 *S.* Weltevreden isolates gave 6 pulsotypes (F = 0.39–1.0). Members of *S.* Enteritidis and *S.* Typhimurium were less diverse. Thus, 14 *S.* Enteritidis isolates were subtyped to 6 pulsotypes with only a 5-band difference (F = 0.89–1.0), and 11 *S.* Typhimurium isolates were subtyped to 5 pulsotypes (F = 0.86–1.0).

Each of the 11 clusters (A–K) in the dendrogram (Fig. 1) was represented by a serotype. For example, clusters A, B, and D contained only *S.* Corvallis (F = 0.77–1.0), *S.* Lexington (F = 0.91), and *S.* Enteritidis isolates (F = 0.89–1.0), respectively. The only exceptions were observed for isolates of *S.* Weltevreden and *S.* Stanley, whose isolates were distributed in more than one cluster (F = 0.39–1.0 and F = 0.15–0.95, respectively; Fig. 1).

REP-PCR generated between 9 and 18 DNA bands ranging from 0.1 to 1.5 kb, thereby yielding 37 distinct profiles. The REP profiles of isolates from the same serotype were relatively more homogenous than in

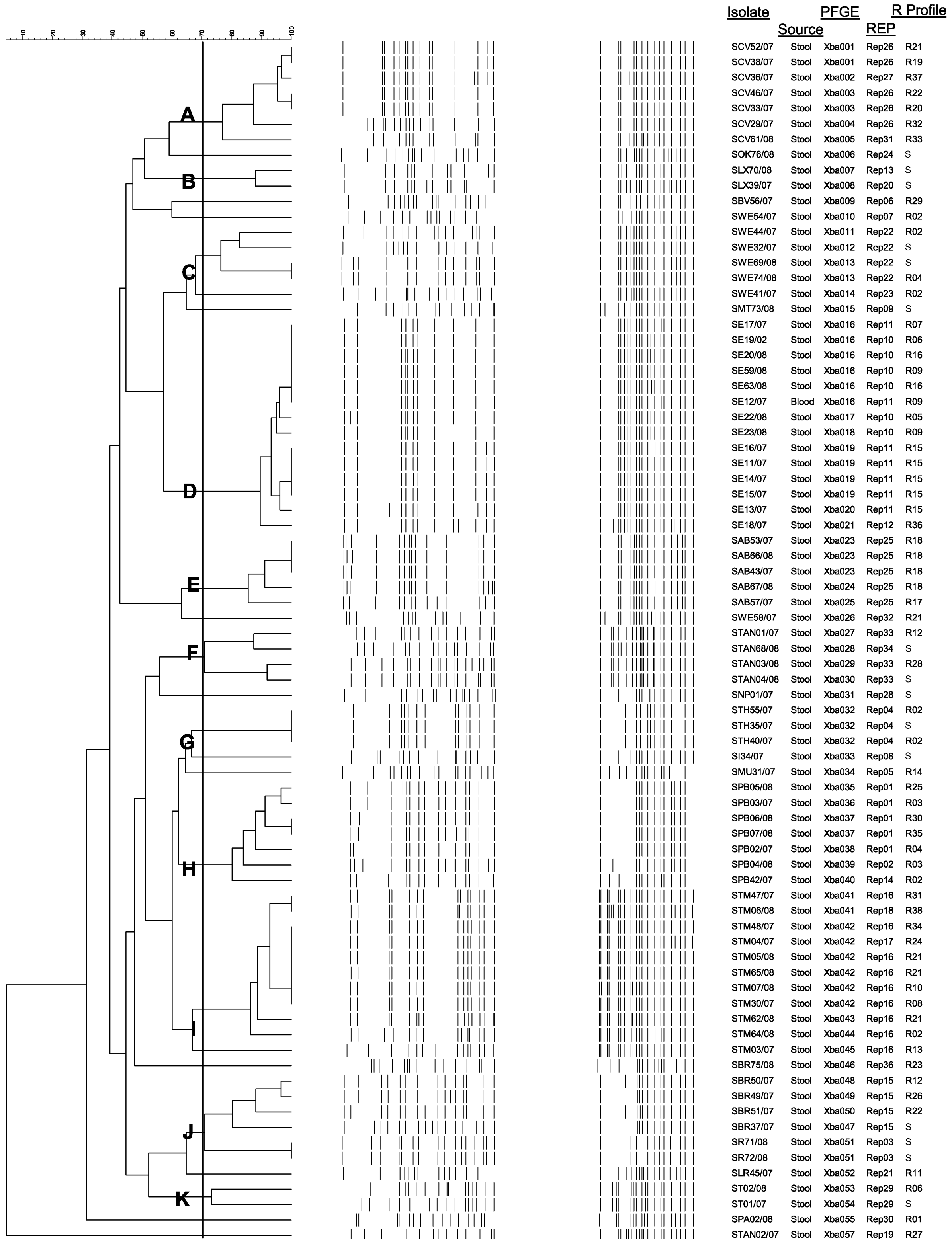

Fig. 1. Dendrogram generated using the Dice coefficient based on PFGE profiles of the 78 *Salmonella* isolates restricted with *Xba*I, constructed using UPGMA algorithm (Gel ComparII). Resistance profiles, R as described in Table 2. S, sensitive. Codes used to indicate serotype: Enteritidis (SE), Typhimurium (STM), Paratyphi B var Java (SPB), Weltevreden (SWE), Corvallis (SCV), Stanley (STAN), Braenderup (SBR), Albany (SAB), Thompson (STH), Richmond (SR), Lexington (SLX), Typhi (ST), Newport (SNP), Paratyphi A (SPA), Bovismorbificans (SBV), Larochelle (SLR), Okatie (SOK), Matopeni (SMT), Muenchen (SMU), and Infantis (SI).

Table 1. Percentage of antimicrobial resistance of 78 *Salmonella* isolates evaluated using the disk diffusion method

Antimicrobial agent	Resistance % (no.)
Cephalothin	55.1 (43)
Tetracycline	47.4 (37)
Nalidixic acid	35.9 (28)
Streptomycin	32.1 (25)
Ampicillin	23.1 (18)
Trimethoprim/sulfamethoxazole	19.2 (15)
Chloramphenicol	14.1 (11)
Ceftiofur	12.8 (10)
Kanamycin	10.3 (8)
Gatifloxacin	6.4 (5)
Ceftazidime	3.8 (3)
Amoxicillin/clavulanic acid	2.6 (2)
Amikacin	2.6 (2)
Gentamicin	2.6 (2)
Levofloxacin	2.6 (2)
Ciprofloxacin	1.3 (1)
Ceftriaxone	1.3 (1)
Cefotaxime	1.3 (1)
Imipenem	0 (0)

PFGE. For example, there were only 3 REP-PCR profiles for the 14 *S.* Enteritidis isolates compared to 6 PFGE profiles (Fig. 1). Similarly, REP-PCR generated 3 distinct profiles compared to 5 PFGE profiles for the 11 *S.* Typhimurium isolates. Overall, REP-PCR (F = 0.34–1.00) showed a lower discriminatory ability than PFGE, although it is a simpler and faster technique.

Antimicrobial susceptibility testing: Eighty-one percent (n = 63) of the 78 isolates exhibited resistance to at least one antimicrobial, with 42 isolates (53.8%) being MDR (defined as resistance to three or more antimicrobial agents). The highest resistance rates were found to be to cephalothin (55.1%, n = 43), tetracycline (47.4%, n = 37), and nalidixic acid (35.9%, n = 28). Table 1 summarizes the rates of resistance of the isolates tested, and Table 2 shows the 38 different resistance profiles arbitrarily designated as R01 to R38. Of the 20 serotypes, *S.* Newport, *S.* Typhi, *S.* Paratyphi A, *S.* Thompson, *S.* Infantis, *S.* Okatie, *S.* Lexington, *S.* Larochelle, *S.* Matopeni, *S.* Richmond, and almost all *S.* Weltevreden isolates were either entirely susceptible or resistant to one or two antimicrobials. In contrast, more than 50% of the *S.* Typhimurium, *S.* Enteritidis, *S.* Paratyphi B var Java, *S.* Braenderup, *S.* Corvallis, *S.* Albany, *S.* Stanley, *S.* Bovismorbificans, and *S.* Muenchen serotypes were resistant to at least 3 antimicrobials.

Overall, *S.* Typhimurium isolates were resistant to the highest number of antimicrobials. Isolate STM06/08 (Xba041R38) was resistant to 13 antimicrobial agents, and STM47/07 (Xba041R31) was resistant to 11 agents. However, although these 2 isolates shared the same pulsotype, they were isolated 8 months apart from different patients.

An *S.* Enteriditis isolate (SE18/07, Xba021R36) was found to be resistant to all four third-generation cephalosporins as well as to cephalothin, ampicillin, and nalidixic acid. The pulsotype of this isolate was different from all other *S.* Enteritidis isolates. Analysis using the double disk diffusion method showed a synergistic action when amoxicillin/clavulanic acid disks were placed 15 mm from both cefotaxime and ceftazidime disks, thus suggesting that this isolate is a putative ESBL producer. This synergism was not observed in any of the other 77 isolates.

DISCUSSION

PFGE is the preferred molecular typing approach over REP-PCR due to its higher discriminatory power and reproducibility. The discriminative ability of REP-PCR was limited by use of a single primer (12), although other studies that used more than one primer set reported a high discriminative ability (13,14). Weigel et al. (14) found REP-PCR profiles to show greater variation than PFGE profiles when using the restriction enzymes *Avr*II, *Spe*I, and *Xba*I to genotype *Salmonella* isolates.

The National Public Health Laboratory of Malaysia (4) reported the five most prevalent non-typhoidal *Salmonella* serotypes to be *S.* Enteritidis, *S.* Weltevreden, *S.* Corvallis, *S.* Typhimurium, and *S.* Tshiongwe. Lee et al. (15) reported that *S.* Enteritidis was the most common non-typhoidal *Salmonella* serotype isolated from Malaysian children between 1991 and 2001. The predominant serotypes isolated at UMMC, including *S.* Enteritidis, *S.* Typhimurium, *S.* Weltevreden, *S.* Corvallis, *S.* Albany, and S. Stanley, were reflected in the official bulletin. Rarer serotypes, such as *S.* Richmond, *S.* Okatie, *S.* Matopeni, and *S.* Larochelle, were observed only once during the study period. The diversity of *Salmonella* serovars and their dissimilar DNA fingerprint profiles is not surprising as isolates were derived from sporadic cases of salmonellosis. Multiple PFGE genotypes are often associated with sporadic cases in endemic areas, whereas single PFGE genotypes are generally associated with outbreak cases (16). The Xba016 pattern seen in this study was also the dominant pattern generated by *Xba*I-restricted *S.* Enteritidis isolates in a 1995 outbreak in Kuala Lumpur (17), thus suggesting that *S.* Enteritidis isolates with this particular profile are endemic in this part of Malaysia and have been genetically quite stable for more than a decade. On the other hand, the *Xba*I profiles of *S.* Weltevreden isolates in this study were different from the clinical isolates from different parts of Malaysia isolated between 1996 and 2001 (10).

Although PFGE is widely recognized as the subtyping tool of choice for *Salmonella* due to its high discriminatory power (18), this method requires a high degree of technical skill and careful standardization of the protocol. Furthermore, it has a limited discriminatory ability for serotypes such as *S.* Typhimurium (19) and *S.* Enteritidis (17) in certain cases.

Antibiogram typing is commonly used in epidemiological studies of MDR *Salmonella*, and it was encouraging that the less common serotypes such as *S.* Infantis, *S.* Okatie, *S.* Richmond, *S.* Lexington, *S.* Matopeni, and *S.* Larochelle did not show resistance to the antimicrobials tested. However, the high percentage (53.8%) of the 78 isolates found to exhibit resistance to three or more antimicrobial agents may be a cause for concern as this may limit treatment options for severe

Table 2. Resistance profiles (R) observed in this study

Profile	Resistance profile[1)]																			No. of isolates
Sensitive																				15
R01	–	–	–	–	–	–	–	–	–	–	–	–	–	Nx	–	–	–	–	–	1
R02	–	–	C^1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7
R03	–	–	–	–	–	–	–	–	–	–	S	–	–	–	–	–	–	–	–	2
R04	–	–	–	–	–	–	–	–	–	K	–	–	–	–	–	–	–	–	–	2
R05	–	–	–	C^2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
R06	–	–	C^1	–	–	–	–	–	–	–	–	–	–	Nx	–	–	–	–	–	2
R07	–	–	C^1	–	–	–	–	–	–	–	–	T	–	–	–	–	–	–	–	1
R08	–	–	–	–	–	–	–	–	–	–	–	T	–	–	–	–	GA	–	–	1
R09	–	–	–	–	–	–	–	–	–	–	–	T	–	–	–	–	–	TS	–	3
R10	–	–	–	C^2	–	–	–	–	–	–	–	–	–	Nx	–	–	–	–	–	1
R11	–	–	C^1	C^2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1
R12	–	–	C^1	–	–	–	–	–	–	–	–	T	–	Nx	–	–	–	–	–	2
R13	–	–	C^1	–	–	–	–	–	–	–	–	–	–	Nx	–	L	–	–	–	1
R14	–	–	–	–	–	–	–	–	–	–	–	T	–	Nx	–	–	–	–	–	1
R15	–	–	C^1	–	–	–	–	–	–	–	–	–	A	Nx	–	–	–	–	–	5
R16	–	–	C^1	–	–	–	–	–	–	–	–	T	–	–	–	–	–	TS	–	2
R17	–	–	C^1	–	–	–	–	–	–	–	–	T	–	Nx	–	–	–	TS	–	1
R18	–	–	C^1	–	–	–	–	–	–	–	–	T	A	Nx	–	–	–	TS	C	4
R19	–	–	–	–	–	–	–	–	–	–	S	T	–	Nx	–	–	–	–	–	1
R20	–	–	–	–	–	–	–	–	–	–	S	T	–	Nx	CP	–	–	–	–	1
R21	–	–	C^1	–	–	–	–	–	–	–	S	T	–	–	–	–	–	–	–	5
R22	–	–	C^1	–	–	–	–	–	–	–	S	T	–	Nx	–	–	–	–	–	2
R23	–	–	–	–	–	–	–	–	–	–	S	T	–	–	–	–	GA	–	–	1
R24	–	–	C^1	–	–	–	–	–	–	–	S	T	–	–	–	–	–	TS	–	1
R25	–	–	–	–	–	–	–	–	–	–	S	T	A	–	–	–	–	–	C	1
R26	–	–	C^1	–	–	–	–	–	–	K	–	T	–	Nx	–	–	–	–	–	1
R27	–	–	C^1	–	–	–	–	–	–	K	S	–	–	–	–	–	–	–	–	1
R28	–	–	–	–	–	–	–	–	–	K	S	T	A	–	–	–	–	TS	C	1
R29	–	–	C^1	–	–	–	–	–	–	K	S	T	A	–	–	–	–	TS	C	1
R30	–	–	–	–	–	–	–	N	–		S	T	A	–	–	–	–	–	C	1
R31	–	–	C^1	–	–	–	–	N	G	K	S	T	A	Nx	–	–	GA	TS	C	1
R32	–	–	–	C^2	–	–	–	–	–	–	S	T	–	Nx	–	–	–	–	–	1
R33	–	–	C^1	C^2	–	–	–	–	–	–	S	T	–	–	–	–	–	–	–	1
R34	–	–	C^1	C^2	–	–	–	–	–	–	S	T	–	Nx	–	L	GA	–	–	1
R35	–	–	–	C^2	–	–	–	–	–	–	S	–	A	–	–	–	–	–	C	1
R36	–	–	C^1	C^2	C^3	C^4	C^5	–	–	–	–	–	A	Nx	–	–	–	–	–	1
R37	–	AC	C^1	C^2	–	C^4	–	–	–	–	S	T	A	–	–	–	–	–	–	1
R38	–	AC	C^1	C^2	–	C^4	–	–	G	K	S	T	A	Nx	–	–	GA	TS	C	1
Total																				78

1): The resistance profiles excluded those that exhibited intermediate resistance.
AC, amoxicillin/clavulanic acid; A, ampicillin; N, amikacin; C^1, cephalothin; C^2, ceftiofur; C^3, cefotaxime; C^4, ceftazidime; C^5, ceftriaxone; C, chloramphenicol; CP, ciprofloxacin; G, gentamicin; GA, gatifloxacin; L, levofloxacin; K, kanamycin; S, streptomycin; T, tetracycline; TS, trimethoprim/sulfamethoxazole; Nx, nalidixic acid.

and invasive salmonellosis.

The Institute for Medical Research, Malaysia recently released the National Surveillance on Antibiotic Resistance Report for 2008 (20). Of the 914 isolates, 1.2% were ceftriaxone-resistant. Lee et al. (15) previously reported a high sensitivity to antimicrobials, including 100% sensitivity to ceftriaxone, for *Salmonella* isolates from Malaysian children between 1991 and 2001. However, ceftriaxone resistance was observed in this study.

In the 2000–2004 survey of antimicrobial susceptibility in 10 European countries performed by Meakins et al. (21), the most significant increase in resistance was to nalidixic acid (14 to 20%), particularly in *S.* Enteritidis (10 to 26%). In this study, nalidixic acid resistance, which has been reported to be indicative of fluoroquinolone resistance (22), was found in isolates of *S.* Enteritidis (n = 7/14), *S.* Albany (n = 5/5), *S.* Typhimurium (n = 5/11), *S.* Corvallis (n = 4/6), *S.* Braenderup (n = 2/5), *S.* Stanley (n = 1/5), *S.* Muenchen (n = 1/1), *S.* Typhi (n = 1/2), and *S.* Paratyphi A (n = 1/1). In this study, isolates resistant to fluoroquinolones were generally resistant to nalidixic acid as well, such as the case of ciprofloxacin resistance in an *S.* Corvallis isolate and levofloxacin/gatifloxacin resistance in 4 *S.* Typhimurium isolates. However, even in the presence of an apparent susceptibility to other fluoroquinolones, nalidixic acid resistance is associated with failure to respond to

fluoroquinolones in extraintestinal *Salmonella* infections (11). It is therefore of concern that 28 isolates (35.9%) in this study were resistant to nalidixic acid, although 5 of these showed susceptibility to the other fluoroquinolones in vitro. The use of common fluoroquinolones such as ciprofloxacin must be monitored very carefully in such cases. Further characterization of these nalidixic-resistant strains will be the subject of a future study.

Although streptomycin is not used in the treatment of *Salmonella* infection, streptomycin susceptibility is widely used as an epidemiological marker (23). For instance, streptomycin resistance is part of the characteristic phenotype of resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracyclines (ACSSuT) associated with *S.* Typhimurium DT 104. The highly MDR *S.* Typhimurium isolates STM47/07 and STM06/08 and *S.* Stanley isolate STAN03/08 were resistant to these antimicrobials.

However, a major limitation of in vitro susceptibility testing is that it cannot detect ''silent'' antimicrobial resistance genes and cannot accurately predict the in vivo response to therapy (24).

In conclusion, this study has shown the presence of MDR *Salmonella* strains circulating in the Kuala Lumpur area, which presents an increasing public health problem. Frequent antimicrobial surveillance of such strains and the rational use of antimicrobial agents, is therefore important. PFGE has been shown to be a useful and discriminative method for assessing the genetic diversity of salmonellae, and the data reported herein will contribute towards epidemiological monitoring and investigations of *Salmonella* infections in Malaysia.

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Conflict of interest None to declare.

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