EPIDEMIOLOGICAL TYPING

Analysis of *Salmonella enterica* serotype Typhimurium by phage typing, antimicrobial susceptibility and pulsed-field gel electrophoresis

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Three typing methods commonly used for bacteria – phage typing, antimicrobial susceptibility and pulsed-field gel electrophoresis (PFGE) – were used to characterise 64 *Salmonella enterica* serotype Typhimurium isolates from individual adult patients from Nairobi, Kenya. The isolates encompassed 11 definitive phage types (DTs), which fell into eight PFGE clusters; 31.3% of isolates were either untypable or reacted non-specifically with the phages used for typing and 26.6% were of DT 56. Plasmids of *c.* 100 kb were responsible for self-transferable multiresistance among the isolates. Analysis by PFGE and phage type demonstrated that multiresistant Typhimurium strains causing diarrhoea and invasive disease were multiclonal.

Introduction

Non-typhoidal salmonella infections are primarily associated with food-borne outbreaks of infection world-wide [1]. Among the *Salmonella* serotypes that cause food poisoning, *S. enterica* serotype Typhimurium has remained the most important world-wide. Other commonly encountered serotypes include Enteritidis, Agona, Anatum, Hadar, Heidelberg, Indiana and Newport. In developed countries, outbreaks of non-typhoidal salmonella infection have been caused mainly by serotypes Enteritidis and Typhimurium [2]. Studies have shown that only a few phage types tend to dominate within a geographical region for a period of time. Hence, traditionally phage typing has been employed to study the epidemiology of the most common *Salmonella* serotypes. For example, throughout the 1980s Typhimurium definitive phage type (DT) 204c was the predominant cause of salmonellosis in man and livestock in the UK [3].

However, other Typhimurium phage types, especially multiresistant Typhimurium DT 104, have emerged recently as important causes of food-borne salmonellosis in the UK [4]. Similarly, until recently, Typhimurium infections in livestock in Denmark, were mainly of

DT 12 and DT 135 [5]. However, since 1996 several outbreaks caused by multiresistant strains of DT 104 associated with pork meat of Danish origin have been reported [6]. In both the UK and Denmark, multi-resistance phenotype in DT 104 was attributed to chromosomally located integrons [7, 8]. The reservoirs of these strains are food animals, particularly cattle, but also poultry, pigs and sheep [9]. Recent surveys in the USA [10] and Canada [11] have also indicated that, since 1994, DT 104 has been a major cause of salmonellosis – often associated with the consumption of unpasteurised dairy products and contact with livestock.

In developing countries, non-typhoidal salmonellae account for a steadily increasing proportion of human infections and represent 20–30% of *Salmonella* isolates from systemic infections. In particular, multiresistant Typhimurium strains cause serious outbreaks. For example, in Zaire [12] and Rwanda [13] multiresistant Typhimurium strains were the predominant cause of bacteraemic illness in children, while in Kenya and Malawi [14, 15] multiresistant strains were found to be important causes of bacteraemia in immunosuppressed adults. Similarly, studies from India have implicated multiresistant Typhimurium strains containing 15-MDa plasmids as important causes of severe illness in adults [16]. In Kenya, Typhimurium isolates resistant to commonly used drugs such as chloramphenicol, ampicillin, co-trimoxazole and tetracyclines were found to account for 36% of all cases of nosocomial

Received (resubmission) 2 March 1999; accepted 3 April 1999.

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diarrhoea [17]. Large self-transferable plasmids of *c.* 60 MDa were found to be responsible for multi-resistance in isolates from Kenya [14]. Most Typhimurium infections in HIV-1 seropositive patients from Kenya presented as bacteraemic rather than gastrointestinal illness [18]. Indeed, recurrent bacteraemia with Typhimurium and other non-typhoidal salmonellae is now regarded as a diagnostic criterion for AIDS and HIV-infected individuals [19]. Studies from India have also shown that large plasmids of 58–114 MDa encoded multiresistance in Typhimurium outbreaks [20].

In developing countries, person-to-person transmission of non-typhoidal salmonellae has been assumed to be important, but the natural reservoirs for non-typhoidal salmonella infection remain unknown. Moreover, non-typhoidal salmonella infections in these countries cause high morbidity and mortality, particularly in the young [21] and in immunosuppressed individuals, thus often necessitating medical intervention [1]. To enhance understanding of outbreaks of disease due to *Salmonella* serotypes, molecular techniques such as pulsed-field gel electrophoresis (PFGE) and plasmid analysis have been used widely in conjunction with phenotypic methods such as phage typing to aid epidemiological analysis of these outbreaks. For example, PFGE of macrorestricted chromosomal DNA was used recently to confirm that multiresistant DT 104 strains from both man and animals from various countries in three different continents were from an epidemic clone [22]. In the present study, phage typing, antimicrobial susceptibility and PFGE of macrorestriction digests of chromosomal DNA were used to characterise 64 Typhimurium isolates from individual adult patients admitted to hospital in Nairobi.

Materials and methods

Isolates

Bacterial isolates were obtained during 1990–1994 from adult patients admitted to the medical wards at the Kenyatta National Hospital with symptoms of fever, with or without diarrhoea. Specimens were obtained from patients within 4 h of admission and processed routinely to isolate significant bacterial pathogens. Initially, bacteria were characterised by biochemical tests (API 20E, bioMérieux, Basingstoke) and then serotyped with agglutinating antisera (Murex Diagnostics, Dartford). Isolates were stored at -70°C on Protect Beads (Technical Service Consultants, Heywood) until analysed.

Phage typing

Bacterial isolates confirmed as serotype Typhimurium were sent to the Laboratory of Enteric Pathogens, Central Public Health Laboratory, 61 Colindale Avenue, London, for phage typing.

Antimicrobial susceptibility

Susceptibility tests with commonly used antimicrobial agents were performed on IsoSensitest Agar (Oxoid) by the disk diffusion technique. *Escherichia coli* ATCC 25922 was used as the sensitive control. The antibiotic disks (Oxoid) used were ampicillin 10 μg , tetracycline 30 μg , trimethoprim 5 μg , sulphamethoxazole 100 μg , chloramphenicol 30 μg , streptomycin 10 μg , gentamicin 10 μg , co-amoxiclav 20:10 μg , ceftazidime 30 μg , ciprofloxacin 3 μg and nalidixic acid 10 μg . Disk zone sizes were interpreted according to the NCCLS guidelines [23].

Conjugation experiments

Conjugation experiments were performed in broth by the method of Walia *et al.* [24] with *E. coli* K12 (nalidixic acid-resistant) as recipient. Transconjugants were selected on MacConkey Agar (Oxoid) supplemented with nalidixic acid 32 mg/L and ampicillin/chloramphenicol 32 mg/L. Plasmid DNA was extracted from the exconjugants by an alkaline lysis method [25]. Plasmids were separated by electrophoresis on horizontal agarose 0.8% gels at 100 V for 2 h. Plasmid sizes were determined by co-electrophoresis with plasmids of known sizes from *E. coli* strains V517 (35.8, 4.8, 3.7, 2.6, 2.0, 1.8, 1.4 MDa) and 39R861 (98, 42, 24, 4.6 MDa). DNA bands were visualised with an ultraviolet transilluminator (UVP, San Gabriel, CA, USA) after staining with ethidium bromide 0.05%.

PFGE of macrorestricted chromosomal DNA

Chromosomal DNA was prepared in agarose plugs as described by Thong *et al.* [26] from an overnight bacterial culture in Luria broth. Equal volumes of the bacterial cell culture and CleanCut Agarose (BioRad Laboratories, Richmond, CA, USA) 2% were mixed in a mould to form plugs. The agarose plugs were incubated overnight at 37°C in lysis solution (lysozyme 25 mg/ml in 0.01 M Tris, pH 7.2, 0.05 M NaCl, sodium deoxycholate 0.2%, sarkosyl 0.5%). The plugs were then deproteinated by incubating overnight at 50°C in proteinase K solution (proteinase K 25 mg/ml in 0.1 M EDTA, pH 8.0, sodium deoxycholate 0.2%, sarkosyl 1%). Cell debris and any excess proteinase K were removed by washing once with PMSF (phenylmethylsulphonylfluoride in iso-propanol 1.7%) and twice with a Tris-EDTA buffer (0.02 M Tris, pH 8.0, 0.05 M EDTA) for 1 h each at room temperature.

Agarose plugs were first equilibrated for 1 h in 0.5 ml of REACT2 and REACT3 buffers (Life Technologies, UK), respectively, for *Xba*I and *Spe*I restriction endonucleases. The plugs were then incubated overnight at 37°C in fresh buffer (300 μl), containing 25 units of *Xba*I or 20 units of *Spe*I. PFGE of agarose plug inserts was then performed in a CHEF-DR II system (BioRad) on a horizontal agarose 1% gel for 22 h at 120 V, pulse time of 1–40 s, at 14°C .

A λ DNA digest consisting of a ladder (*c.* 22 fragments) of increasing size from 48 kb to *c.* 1000 kb was included as a DNA size standard. The gel was stained with ethidium bromide 0.05% and photographed on an UV transilluminator (UVP). The restriction endonuclease digest patterns were interpreted by considering migration distance and intensity of all visible bands and by the use of guidelines described by Tenover *et al.* [27]. According to these criteria, isolates that gave indistinguishable PFGE banding patterns were assumed to be from a single outbreak strain. Isolates that gave banding patterns showing differences in fewer than four bands were assumed to be closely related, as they may represent isolates differing by a single genetic event. Furthermore, isolates with differences of four-to-six bands may be part of the outbreak. However, isolates showing a difference of more than seven bands in their banding patterns may represent more than three genetic events, in which case they would be considered epidemiologically unrelated. Genetic similarity between PFGE patterns of the isolates was calculated by the Dice coefficient and clustered by the unweighted pair group arithmetic averaging method (Molecular Fingerprinting Program version 1.4.1, BioRad).

Results

Isolates

A total of 320 blood cultures from consecutive adult admissions was examined; in addition, stools were obtained from 38 of the patients. Of the blood cultures, 55 contained serotype Typhimurium and 40 contained other pathogens – *Streptococcus pneumoniae* (32), Enteritidis (5) and Newport (3); 169 cultures were sterile and 46 were contaminated with skin flora. Amongst the 55 patients with blood cultures containing Typhimurium, 10 had the same Typhimurium strain isolated from stool. A further nine Typhimurium isolates were obtained from stools of patients whose blood cultures were sterile. Thus, 55 Typhimurium isolates came from blood and 19 from stool specimens. For the present study, only the 64 non-duplicate Typhimurium isolates that were obtained from blood or stools of individual patients were selected for further analysis.

Antimicrobial susceptibility

All 64 Typhimurium isolates were resistant to two or more of the commonly available drugs including

ampicillin, co-trimoxazole, streptomycin, tetracycline and chloramphenicol. A total of 36 isolates (56.3%) was resistant to four or five antimicrobial agents; 15 isolates were of DT 56 and eight isolates were of RDNC. The distribution of these and other multi-resistant phage types is shown in Table 1. A further 20 (31.3%) Typhimurium isolates showed reduced disk zone sizes for gentamicin, co-amoxiclav and nalidixic acid, but were still interpreted as susceptible. However, ciprofloxacin, nalidixic acid and ceftazidime were uniformly effective *in vitro* against all the Typhimurium isolates. In all cases of drug-resistant Typhimurium, plasmids of *c.* 100 kb, in addition to other smaller plasmids (15–45 kb), were isolated.

Conjugation experiments

In conjugation experiments, all 64 Typhimurium isolates transferred plasmids of *c.* 100 kb to *E. coli* K12. In all cases to 100-kb plasmids co-transferred resistance to ampicillin. In addition, resistances to co-trimoxazole, tetracycline and chloramphenicol were transferred together in 19 (29.7%) donor-to-*E. coli* K12 conjugation tests (Table 1).

PFGE patterns and phage types of isolates

All duplicate Typhimurium strains (from blood and stool of same patient) were shown to be indistinguishable by PFGE and phage typing and were, therefore, regarded as single strains during analysis.

The isolates encompassed 11 DTs – 56 (17 isolates; 26.6%), 193 (7; 10.9%), 135 (5; 7.8%); 16 (25%) isolates were in seven other smaller phage type groups; 10 (15.6%) were untypable and 9 (14.1%) were not specific to any of the standard phages used for typing (RDNC). The Typhimurium isolates were divided into eight PFGE clusters. Isolates of the most common phage type, DT 56, were distributed among six of the eight PFGE clusters (Table 2). Within each cluster, isolates had >75% coefficient of similarity. More than one-third of the isolates (24; 37.5%) were in PFGE cluster 2 which contained 11 different phage types and only two isolates in PFGE cluster 2 were untypable. However, there was no consistent pattern of association between a particular PFGE cluster and the phage type of the isolates.

Apart from PFGE cluster 7, which contained one untypable isolate, all other PFGE clusters contained

Table 1. Phage types of multiresistant Typhimurium isolates and antibiotic resistance phenotypes transferred to *E. coli* K12

Resistance phenotype in Typhimurium	Number of isolates	Resistance phenotype in exconjugants	Phage type (number of isolates)
Am, TmSu, Te, Cm	19	Am, TmSu, Te, Cm	56 (7), UNT (2), RDNC (4), 135 (1), 193 (5)
Am, TmSu, Cm	12	Am, Cm	56 (2), 2 (6), 52 (1)
Am, TmSu, Te, Cxm, St	17	Am, Te	56 (8), UNT (3), RDNC (4), 193 (2), 12 (2), 208 (1)
Am, Te	16	Am	UNT (5), RDNC (1), 135 (4), 204a (5), 49 (2), 29 (1)

UNT, isolates were untypable by the phages used for typing; RDNC, isolates reacted non-specifically with the phages used.

Table 2. PFGE group patterns (genotypes) and phage types of Typhimurium isolates

PFGE cluster (number of isolates)	Phage type (number of isolates)
1 (9)	56 (3), 135 (1), 204a (1), 193 (1) RDNC (1), UNT (2)
2 (24)	56 (6), 135 (1), 49 (2), 12 (1), 193 (1), 1 (2), 204a (1), 52 (1), 2 (1), 29 (1), RDNC (5), UNT (2)
3 (9)	56 (4), 1 (2), RDNC (1), UNT (2)
4 (2)	56 (1), UNT (1)
5 (8)	204a (2), 193 (2), 135 (1), 12 (1), 56 (1) UNT (1)
6 (9)	56 (2), 193 (2), 135 (2), 204a (1), RDNC (2)
7 (1)	UNT (1)
8 (2)	193 (1), UNT (1)

RDNC, isolates reacted non-specifically with the phages used for typing; UNT, isolates were untypable by the phages used for typing.

more than one phage type. As shown in Fig. 1, the isolates produced similar patterns of the smaller fragments of 50–120 kb, but differed in restriction endonuclease digest patterns by two-to-four bands of fragments >250 kb. For *Xba*I-digested DNA, the total number of fragments ranged from 10 to 16, with two-to-seven band differences. Similarly, the PFGE patterns from the *Spe*I digests showed significant relatedness of the smaller fragments of 40–200 kb, but were different in the patterns of the larger fragments. When digested

with either *Xba*I or *Spe*I, the 100-kb plasmids were shown to produce fragments <20 kb which were not scored during the analysis. To determine the reproducibility of PFGE fragment patterns obtained, a second set of agarose plugs was digested with *Xba*I and *Spe*I by the same procedure. Comparing the fragment patterns of chromosomal DNA obtained with the two enzymes, *Xba*I produced better resolution of the bands than *Spe*I under the same PFGE conditions.

Discussion

Three techniques commonly used for bacterial typing – phage typing, antimicrobial susceptibility and PFGE patterns of *Xba*I- and *Spe*I-digested chromosomal DNA – were used to characterise isolates of serotype Typhimurium which were epidemiologically unrelated. Antimicrobial susceptibility testing demonstrated a high prevalence of self-transferable antimicrobial resistance among Typhimurium isolates causing severe bacteraemic disease in adult patients from Nairobi. Multiresistance – particularly to the commonly available drugs including ampicillin, chloramphenicol, tetracycline, co-trimoxazole and streptomycin – is of great concern, as this further reduces effective therapeutic choices in life-threatening illnesses. Indeed,

Fig. 1. Restriction endonuclease fragment patterns of *Xba*I-digested DNA from representative Typhimurium isolates. Lanes 1 and 16, 48.5-kb DNA M_r standard; 2, NR150 (DT 56, cluster 2); 3, NR3916 (DT 56, cluster 2); 4, NR3344 (DT 56, cluster 2); 5, NR815 (DT 135, cluster 3); 6, NR7830 (DT 135, cluster 4); 7, NR338 (DT 193, cluster 4); 8, NR1487 (DT 135, cluster 4); 9, NR592 (DT 1, cluster 5); 10, NR710 (DT 1, cluster 5); 11, NR617 (DT 12, cluster 3); 12, NR844 (RDNC, cluster 4); 13, NR2981 (RDNC, cluster 4); 14, NR7118 (untypable, cluster 3); 15, NR235 (untypable, cluster 2). Note that lanes 2 and 3 contained Typhimurium isolates from blood and stool of same patient, and had a PFGE pattern indistinguishable from isolate NR235 in lane 15. RDNC, strain gave non-specific reaction with the phages used for typing.

in many primary health-care facilities in Kenya these may be the only drugs available for treatment of most bacterial infections.

Cases of multiresistance among Typhimurium isolates are not an isolated phenomenon. For example, previously in Kenya [15, 17] Typhimurium isolates from cases of bacteraemia were also shown to be multiresistant to most commonly available drugs. In addition, in the present study 31.3% of the isolates showed decreased susceptibility to co-amoxiclav, gentamicin and nalidixic acid. Although still effective *in vitro*, the trend for the three drugs is of concern. Similarly, a high prevalence of multiresistant Typhimurium was reported from Zaire [12], Central Africa [13] and Malawi [15]. In most multiresistant Typhimurium outbreaks in Africa, third-generation cephalosporins and ciprofloxacin were effective in treatment. However, these drugs are usually too expensive for most patients who would normally present to the public hospitals. Therefore, it is prudent that cheaper and still effective therapeutic choices including gentamicin and nalidixic acid be used rationally to prolong their effectiveness.

Although, in developed countries, only a single phage type has often been associated with outbreaks of multiresistant Typhimurium infection in both man and animals [4, 6, 28], the findings from the present study did not indicate a significant predominance of any one phage type. Also, there was no significant association between any one phage type and the multiresistance phenotype. In all, 69.3% of the Typhimurium isolates were found to be in 11 definitive phage types; 16.9% isolates were untypable and 13.8% gave non-specific reactions with the phages used for typing. Furthermore, there was no significant association between any phage type and PFGE pattern, as most phage types were distributed among different PFGE clusters. Several of the more common DT 56 and RDNC strains gave different PFGE patterns, indicating that PFGE is able to discriminate between isolates closely related by phage typing.

Isolates from blood and stool of the same patients gave identical PFGE patterns and were of the same phage types. These were assumed to be the same Typhimurium strain that had become invasive. One such isolate from PFGE cluster 2 had an identical PFGE pattern to one isolate from a different patient. It is likely that this was a case of person-to-person infection with the same strain of Typhimurium either in the community or within the hospital. The clustering of 24 (37.5%) Typhimurium isolates of 11 different phage types in PFGE cluster 2 suggests that these isolates were genetically closely related, although they were not epidemiologically linked. Other workers [5] made similar observations in which Typhimurium isolates of a total of 17 phage types were clustered into five PFGE subgroups. In their study, four of the five phage

types that were further investigated formed tight clusters of PFGE patterns joining at 67%. In the present study, a coefficient of similarity >75% was observed for Typhimurium strains that formed each cluster.

Most outbreaks of disease due to Typhimurium in the UK and the USA have been traced to consumption of contaminated foods of animal origin or contact with livestock [8, 9]. However, in Kenya, sources of salmonella infections remain unknown. More studies are needed to determine whether infections have a livestock reservoir or are due to person-to-person contact.

Although all Typhimurium isolates in the present study were multiresistant and had a common 100-kb R-plasmid, these isolates were not from a single clone, as demonstrated by the existence of the different PFGE patterns and phage types. Therefore, Typhimurium isolates from cases of bacteraemia in adults in the present study may not be outbreak-related and are likely to be from sporadic cases of infection spreading only to a limited extent among human patients during each episode.

We are grateful to the Director, Kenya Medical Research Institute, for permission to publish this work. S.K. is supported by a Wellcome Trust Research Development Award in Tropical Medicine.

References

1. Pegues DA, Miller SI. Salmonellosis, including typhoid fever. *Curr Opin Infect Dis* 1994; 7: 616–623.
2. Threlfall EJ, Hampton MD, Chart H, Rowe B. Use of plasmid profile typing for surveillance of *Salmonella enteritidis* phage type 4 from humans, poultry and eggs. *Epidemiol Infect* 1994; 112: 25–31.
3. Wray C, McLaren IM, Jones YE. The epidemiology of *Salmonella typhimurium* in cattle: plasmid profile analysis of definitive phage type (DT) 204c. *J Med Microbiol* 1998; 47: 483–487.
4. Fone DL, Barker RM. Associations between human and farm animal infections with *Salmonella typhimurium* DT104 in Herefordshire. *Commun Dis Rep CDR Rev* 1994; 4: R136–R140.
5. Olsen JE, Skov MN, Angen Ø, Threlfall EJ, Bisgaard M. Genomic relationships between selected phage types of *Salmonella enterica* subsp. *enterica* serotype *typhimurium* defined by ribotyping, IS200 typing and PFGE. *Microbiology* 1997; 143: 1471–1479.
6. Baggesen DL, Aarestrup FM. Characterisation of recently emerged multiple antibiotic-resistant *Salmonella enterica* serovar Typhimurium DT104 and other multiresistant phage types from Danish pig herds. *Vet Rec* 1998; 143: 95–97.
7. Threlfall EJ, Frost JA, Ward LR, Rowe B. Epidemic in cattle and humans of *Salmonella typhimurium* DT 104 with chromosomally integrated multiple drug resistance. *Vet Rec* 1994; 134: 577.
8. Sandvang D, Aarestrup FM, Jensen LB. Characterisation of integrons and antibiotic resistance genes in Danish multi-resistant *Salmonella enterica* Typhimurium DT104. *FEMS Microbiol Lett* 1998; 160: 37–41.
9. Anonymous. *Salmonella* in livestock production, 1997. London, MAFF.
10. Glynn MK, Bopp C, Dewitt W, Dabney P, Mokhtar M, Angulo FJ. Emergence of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 infections in the United States. *N Engl J Med* 1998; 338: 1333–1338.
11. Poppe C, Smart N, Khakhria R, Johnson W, Spika J, Prescott J.

- Salmonella typhimurium* DT104: a virulent and drug-resistant pathogen. *Can Vet J* 1998; **39**: 559–565.
12. Green SDR, Cheesbrough JS. *Salmonella* bacteraemia among young children at a rural hospital in western Zaire. *Ann Trop Paediatr* 1993; **13**: 45–53.
 13. Lepage P, Bogaerts J, Nsengumuremyi F *et al.* Severe multi-resistant *Salmonella typhimurium* systemic infections in Central Africa – clinical features and treatment in a paediatric department. *J Antimicrob Chemother* 1984; **14** Suppl B: 153–159.
 14. Kariuki S, Gilks C, Corkill J *et al.* Multi-drug resistant non-typhi salmonellae in Kenya. *J Antimicrob Chemother* 1996; **38**: 425–434.
 15. Leegaard TM, Van Gestel MH, Petit PLC, Van de Klundert JAM. Antibiotic resistance mechanisms in *Salmonella* species causing bacteraemia in Malawi and Kenya. *APMIS* 1996; **104**: 302–306.
 16. Bhatia R, Kaur P, John PC, Singh H. Transferable beta lactam resistance against cephalosporins in *Salmonella typhimurium* strains in India. *Indian J Med Res* 1994; **99**: 203–205.
 17. Paton S, Nicolle L, Mwongera M *et al.* *Salmonella* and *Shigella* gastroenteritis at a public teaching hospital in Nairobi, Kenya. *Infect Control Hosp Epidemiol* 1991; **12**: 710–717.
 18. Gilks CF, Brindle RJ, Otieno LS *et al.* Life-threatening bacteraemia in HIV 1 seropositive adults admitted to hospital in Nairobi, Kenya. *Lancet* 1990; **336**: 545–549.
 19. Gruenewald R, Blum S, Chan J. Relationship between human immunodeficiency virus infection and salmonellosis in 20- to 59-year old residents of New York City. *Clin Infect Dis* 1994; **18**: 358–363.
 20. Mohan VP, Agarwal DS, Sharma KB, Ghose P, Pillai PK. Plasmid profiles, adhesive and invasive properties of *Salmonella* ser. Typhimurium strains: phage types 99 & 36. *Indian J Med Res* 1993; **97**: 196–201.
 21. De Wit S, Taelman H, Van de Perre P, Rouvroy D, Clumeck N. *Salmonella* bacteremia in African patients with human immunodeficiency virus infection. *Eur J Clin Microbiol Infect Dis* 1988; **7**: 45–47.
 22. Ridley A, Threlfall EJ. Molecular epidemiology of antibiotic resistance genes in multiresistant epidemic *Salmonella typhimurium* DT 104. *Microb Drug Resist* 1998; **4**: 113–118.
 23. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4, 1997. Wayne, PA, USA, National Committee for Clinical Laboratory Standards.
 24. Walia SK, Madhavan T, Chugh TD, Sharma KB. characterization of self-transmissible plasmids determining lactose fermentation and multiple antibiotic resistance in clinical strains of *Klebsiella pneumoniae*. *Plasmid* 1987; **17**: 3–12.
 25. Birnboim HC, Doly J. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res* 1979; **7**: 1512–1523.
 26. Thong K-L, Ngeow Y-F, Altwegg M, Navaratnam P, Pang T. Molecular analysis of *Salmonella enteritidis* by pulsed-field gel electrophoresis and ribotyping. *J Clin Microbiol* 1995; **33**: 1070–1074.
 27. Tenover FC, Arbeit RD, Goering RV *et al.* Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; **33**: 2233–2239.
 28. Low JC, Angus M, Hopkins G, Munro D, Rankin SC. Antimicrobial resistance of *Salmonella enterica* Typhimurium DT104 isolates and investigation of strains with transferable apramycin resistance. *Epidemiol Infect* 1997; **118**: 97–103.