Antimicrobial Susceptibility, Phage Typing and Plasmid Profile of *Salmonella enterica* Serotype *paratyphi* A Strains Isolated in Kuwait

D. Panigrahi^a T.D. Chugh^b P.W.J. West^a T.Z. Dimitrov^c S. Groover^c
G. Mehta^d

^aDepartment of Medical Laboratory Sciences, Faculty of Allied Health Sciences, ^bDepartment of Microbiology, Faculty of Medicine, Kuwait University, Kuwait, ^cInfectious Disease Hospital, Ministry of Health, Kuwait; ^dDepartment of Microbiology, L.H. Medical College, New Delhi, India

Key Words

Salmonella enterica serotype *paratyphi* A ·
Antimicrobial susceptibility · Phage type · Plasmid
profile

Abstract

Objective: To determine the antimicrobial susceptibility, phage type and plasmid profile pattern of *Salmonella enterica* serotype *paratyphi* A strains isolated in Kuwait. **Material and Methods:** From January 1995 to December 1999, 106 strains of *S. enterica* serotype *paratyphi* A isolated from an equal number of cases of enteric fever, attending the Infectious Disease and Mubarak Al-Kabeer Hospitals in Kuwait were investigated. The isolates were tested for antimicrobial susceptibility to 8 commonly used antimicrobial agents. Their phage type and plasmid profile patterns were determined using an international set of phages and Qiagen plasmid mini kit, respectively. **Results:** All of the isolates were susceptible to ciprofloxacin, cefuroxime, ceftazidime, piperacillin and co-trimoxazole. One hundred isolates were susceptible to ampicillin, 99 to chloramphenicol and 98 to tetracycline. None of the isolates was multidrug resistant. Sixty-six percent of the isolates were phage type I, 27.4% phage type II and

6.6% were untypable. All phage type I and untypable strains had 3 plasmids of 2.2, 5 and 20 kb, whereas phage type II strains had only 1 plasmid of 20 kb. **Conclusion:** The findings indicate that while all of the isolates of the *S. enterica* serotype *paratyphi* A were susceptible to 4 of the drugs tested, some were resistant to ampicillin, chloramphenicol or tetracycline, thereby indicating the need for continued surveillance and monitoring of antimicrobial susceptibility of these isolates.

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Introduction

Enteric fever is a common infectious disease prevalent in many countries of the world. The most important causative pathogen, *Salmonella enterica* serotype *typhi*, is estimated to cause 12–21 million cases of typhoid fever with 700,000 deaths annually [1–3]. *S. enterica* serotype *paratyphi* A, a serotype host adapted to man, is the second leading cause of enteric fever in Asia, the Middle East, Africa and South America after serotype *typhi* [4], and it is responsible for 3–20% of all enteric fever cases [5–7]. In a large community-based study in an urban slum in Delhi from October 1995 to October 1996, Kumar et al. [8]

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Dr. D. Panigrahi, MD
Professor of Microbiology, Faculty of Allied Health
Kuwait University, PO Box No. 31470
Sulaibikhat-90805 (Kuwait)
Tel. +965 4833631, Fax +965 4830937, E-Mail debadatta@kuc01.kuniv.edu.kw

reported that the *S. enterica* serotype *paratyphi A* caused 25% of the cases of enteric fever in the region. Sood et al. [9] in a retrospective study of the laboratory records of the All India Institute of Medical Sciences, New Delhi, India, showed that the proportion of serotype *paratyphi A* isolates increased from 6.5% in 1994 to 44.9% in 1998. This increase may be due to a high degree of clinical suspicion, changing test susceptibility or even a change in the virulence of the organism. In Kuwait, serotype *typhi* is common, as reported by Panigrahi et al. [10].

Various epidemiological typing methods that included antimicrobial susceptibility testing, phage typing, biotyping and various molecular typing methods have been used to identify the relatedness of *S. enterica* serotypes isolated from outbreaks. Molecular typing methods based on characterization of plasmid DNA that have been used for the differentiation of *S. enterica* serotypes include plasmid profile typing, plasmid fingerprinting and the identification of plasmid-mediated virulence genes [11–15]. Such typing methods are extensively reported for serotype *typhi* and other salmonellae. Only one report is available for *S. enterica* serotype *paratyphi A* [2]. The present study was carried out to determine the antimicrobial susceptibility pattern, phage typing and plasmid profile of *S. enterica* serotype *paratyphi A* strains isolated in Kuwait.

Materials and Methods

A total of 106 *S. enterica* serotype *paratyphi A* strains sequentially isolated from blood from January 1995 to December 1999 at the Infectious Diseases and Mubarak Al-Kabeer Hospitals, Kuwait, were included in the study. The isolates were identified by API-20E and confirmed by serotyping with appropriate antisera (Difco, USA). In vitro antimicrobial susceptibility against ampicillin, tetracycline, chloramphenicol, co-trimoxazole, ciprofloxacin, cefuroxime, ceftazidime and piperacillin was done by the Kirby-Bauer disc diffusion test [16]. Phage typing was carried out at the National Phage Typing Centre, L.H. Medical College, New Delhi, India, using an international set of phages. Plasmid DNA profiling was done using the Qiagen plasmid mini kit, according to the manufacturer's instructions (Qiagen Inc., Chatsworth, Calif., USA), followed by electrophoresis on agarose gel at room temperature for 2 h. The molecular weights were estimated in relation to λ DNA/*Hind* III marker (Gibco Life Technology, Paisley, UK).

Results

Of the 106 *S. enterica* serotype *paratyphi A* strains tested, 5 each were isolated from Kuwaiti and Egyptian patients. The remaining 96 strains were isolated from patients coming from the Indian subcontinent (42 from

Table 1. Details of patterns of resistance of *S. paratyphi A* strains

Resistance pattern	Total number of resistant strains
C	3
A and T	5
C and T	3
A and C	1
Total	12

A = Ampicillin; C = chloramphenicol; T = tetracycline.

India, 30 from Bangladesh, 24 from Pakistan). All isolates were susceptible to ciprofloxacin, cefuroxime, ceftazidime, piperacillin and co-trimoxazole. Seven strains (6.6%) were resistant to chloramphenicol of which 3 were resistant to it alone and 3 and 1 were also resistant to tetracycline and ampicillin, respectively. Five strains were also resistant to ampicillin and tetracycline. None of the isolates was multidrug resistant (more than 2 drugs) (table 1). All of the resistant strains were isolated from patients coming from the Indian subcontinent. All of the 10 isolates from the Kuwaiti and Egyptian patients were susceptible to the 8 antimicrobial agents tested. Phage typing showed that 70 isolates (66%) were of phage type I, 29 (27.4%) phage type II and the remaining 7 (6.6%) were untypable. Plasmid profile analysis demonstrated that all of the phage type I and untypable strains had 3 plasmids of 2.2, 5 and 20 kb, whereas phage type II strains had only 1 plasmid of 20 kb (fig. 1).

Discussion

S. enterica serotype *paratyphi A* has always been an important causative agent of enteric fever, next only to *S. enterica* serotype *typhi*. Drug resistance to *S. enterica* serotype *paratyphi A* has not been reported in Kuwait. All of the 106 isolates were susceptible to ciprofloxacin, cefuroxime, ceftazidime, piperacillin and co-trimoxazole. None of the isolates was multidrug resistant. In an unpublished study, one of the present authors (Mehta at the National Salmonella Phage Typing Centre, New Delhi, India) showed that 215 strains of *S. enterica* serotype *paratyphi A* obtained during 2001 were susceptible to

Fig. 1. Plasmid profiles of *S. enterica* serotype *paratyphi A*. Lane 1: molecular weight markers; lanes 2–4, and lanes 5 and 6 represent plasmids in phage type 1, untypable and phage type 2 isolates, respectively.

ciprofloxacin, cotrimoxazole, aminoglycosides and cephalosporins (cefuroxime, ceftazidime), but 64 (29.7%) were resistant to tetracycline, 8 (3.7%) to ampicillin and 4 (1.8%) to chloramphenicol. In this study, a similar pattern of drug resistance was observed with one exception: higher resistance to tetracycline, probably due to the fact that tetracycline is very rarely used in Kuwait. It is also noteworthy that all of the resistant strains were isolated from patients from the Indian subcontinent. In another study, Mahanta [17] reported very high resistance (92%) in *S. enterica* serotype *paratyphi A* strains to ampicillin.

However, these isolates were from an outbreak in north-east India.

Phage type I is the most common phage type of *S. enterica* serotype *paratyphi A* as reported by various workers, varying from 63 to 100% [7, 17, 18] and similar to 66% phage type 1 of this study. Although Mehta at the National Salmonella Phage Typing Centre, New Delhi, had phage type IV (22%) and untypable (35%) isolates, we did not encounter any phage type IV strains in Kuwait.

Application of plasmid profile analysis is a useful typing method for determining relatedness of salmonellae such as *S. dublin* [19], *S. typhimurium* [20], *S. enteritidis* [14] and *S. typhi* [21], but no such report is available for *S. enterica* serotype *paratyphi A* strains. Although we have demonstrated the presence of plasmids in the strains studied, this was not sufficient to establish the relatedness of the isolates. We plan to use restriction enzyme cleavage in future studies to resolve this problem.

Conclusion

The 106 strains of *S. enterica* serotype *paratyphi A* isolated in Kuwait were susceptible to ciprofloxacin, cefuroxime, ceftazidime, piperacillin and co-trimoxazole. However, several of the isolates were resistant to ampicillin, chloramphenicol or tetracycline, thereby indicating the need for continuous monitoring of the susceptibility pattern of *S. enterica* serotype *paratyphi A* to these drugs in Kuwait.

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References

- 1 Threlfall EJ, Hall MLM, Rowe B: *Salmonella* bacteraemia in England and Wales 1981–1990. *J Clin Pathol* 1992;45:34–36.
- 2 Thong KL, Cheong YM, Puthucherry S, Koh CL, Pang T: Epidemiologic analysis of sporadic *Salmonella typhi* isolates and those from outbreaks by pulsed-field gel electrophoresis. *J Clin Microbiol* 1994;32:1135–1141.
- 3 Mirza SH, Beeching NJ, Hart CA: Multi-drug resistant typhoid: A global problem. *J Med Microbiol* 1996;44:317–319.
- 4 Old DC, Threlfall EJ: *Salmonella*; in Balows A, Duerden BI (eds): *Topley & Wilson's Microbiology and Microbial Infections*, ed 9. London, Arnold, 1998, pp 969–997.
- 5 Matsubara Y, Murata M, Masuda G, Tsuji M, Negishi M: Clinical research on patients with typhoid and paratyphoid fever (1984–1987). *Research Group for Infectious Enteric Diseases, Japan. Kansenshogaku Zasshi* 1991;65: 710–717.
- 6 Hustinx WN, Nio Y, Speelman P: Abdominal typhus and paratyphoid fever in 2 academic hospitals: 1984–1990. *Ned Tijdschr Geneesk* 1993;137:291–294.
- 7 Kapil A, Sood S, Reddaiah VP, Das B, Seth P: Paratyphoid fever due to *Salmonella enterica* serotype *paratyphi A*. *Emerg Infect Dis* 1997;3: 407.
- 8 Kumar R, Sazawal S, Sinha A, Sood S, Bhan MK: Typhoid fever: Contemporary issues as related to the disease in India. *Round Table Conference Series on Water Borne Diseases*, ed 12. New Delhi, Ranbaxy Science Foundation, 1997, vol 2, pp 31–36.

- 9 Sood S, Kapil A, Dash N, Das BK, Goel V, Seth P: Paratyphoid fever in India: An emerging problem. *Emerg Infect Dis* 1999;5:483–484.
- 10 Panigrahi D, al-Aneziz AH, West PW: Plasmid mediated multidrug resistance in *Salmonella typhi* in Kuwait. *Trop Med Int Health* 1996;1: 439–442.
- 11 Taylor DN, Wachsmuth IK, Shangkuan YH, Schmidt EV, Barrett TJ, Schrader JS, Scherach CS, McGee HB, Feldman RA, Brenner DJ: Salmonellosis associated with marijuana. A multistate outbreak traced by plasmid fingerprinting. *N Engl J Med* 1982;306:1249–1253.
- 12 Threlfall EJ, Hall MLM, Rowe B: *Salmonella gold-coast* from outbreak of food-poisoning in the British Isles can be differentiated by plasmid profile. *J Hyg Camb* 1986;97:115–122.
- 13 Threlfall EJ, Powell NG, Rowe B: Differentiation of *Salmonellae* by molecular methods. *PHLS Microbiol Dig* 1994;11:199–202.
- 14 Rushdy AA, Wall R, Seng C, Wall PG, Stuart JM, Ridley AM, Threlfall EJ, Ward LR: Application of molecular methods to a nosocomial outbreak of *Salmonella enteritidis* phage type 4. *J Hosp Infect* 1997;36:123–131.
- 15 Ridley AM, Threlfall EJ, Rowe B: Genotypic characterization of *Salmonella enteritidis* phage types by plasmid analysis, ribotyping and pulsed-field gel electrophoresis. *J Clin Microbiol* 1998;36:2314–2321.
- 16 Bauer AW, Kirby WM, Sherris JC, Turck M: Antibiotic susceptibility testing by a standardized simple disk method. *Am J Clin Pathol* 1966;45:493–496.
- 17 Mahanta J: Drug sensitivity of *Salmonella paratyphi A* isolated from a suspected outbreak of enteric fever in Duliajan. *J Indian Med Assoc* 1994;92:49–50.
- 18 International Federation for Enteric Phage-typing. The geographical distribution of *Salmonella typhi* and *Salmonella paratyphi A* and *B* phage types during the period 1 January 1970 to 31 December 1973. *J Hyg* 1982;88:231–254.
- 19 Ferris KE, Andrews RE Jr, Thoen CO, Blackburn BO: Plasmid profile analysis, phage typing and antibiotic sensitivity of *Salmonella dublin* from clinical isolates in the United States. *Vet Microbiol* 1992;32:51–62.
- 20 Mohan VP, Sharma KB, Agarwal DS, Purnima G, Pillai PK: Plasmid profile and phage type of *Salmonella typhimurium* strains encountered in different regions on India. *Comp Immunol Microbiol Infect Dis* 1995;18:283–290.
- 21 Hamton MD, Ward LR, Rowe B, Threlfall EJ: Molecular fingerprinting of multidrug-resistant *Salmonella enterica* serotype *typhi*. *Emerg Infect Dis* 1998;4:317–320.