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

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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.

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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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 AI-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., Chatswofth, Calif., USA), followed by electrophoresis on agarose gel at room temperature for 2 h. The molecular weights were estimated in relation to λ DNA/*Hin*d 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 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).

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.

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

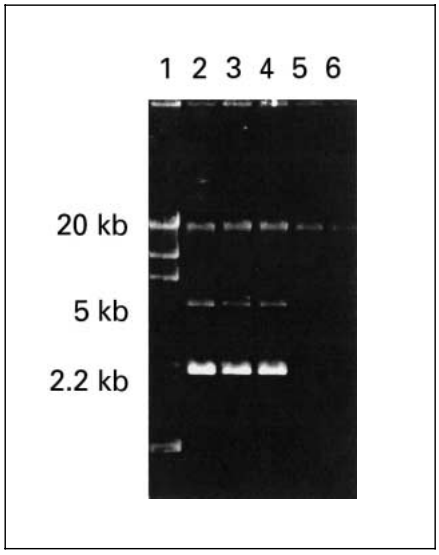

Fig. 1. Plasmid profiles of *S. enterica* serotype *paratyphi A*. Lane 1: molecular weight markers; lanes 2–4, and lanes 5 and 6 represent plasmids in phage type 1, untypable and phage type 2 isolates, respectively.

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 northeast 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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