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Emergence of *Salmonella enterica* serotype Paratyphi A as a major cause of enteric fever in Kathmandu, Nepal

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Summary We performed pulsed-field gel electrophoresis (*Xba*I) on 114 bloodstream isolates of *Salmonella enterica* serotype Paratyphi A and *S. enterica* serotype Typhi collected from febrile patients in Kathmandu, Nepal. Of the 56 *S.* Paratyphi A isolates, 51 (91%) were indistinguishable, which suggests the emergence of a single clone. In contrast, only 21 (36%) of the 58 *S.* Typhi isolates exhibited a common genotype, which is consistent with endemic disease from multiple sources.

1. Introduction

Enteric fever is the most commonly identified cause of febrile illness among adults in urban Nepal (Murdoch et al., 2004). In addition to *Salmonella enterica* serotype Typhi (*S.* Typhi), *Salmonella enterica* serotype Paratyphi A (*S.* Paratyphi A) has emerged as an important cause of enteric fever in South Asia during the past decade (Kapil et al., 1997; Ochiai et al., 2005; Rodrigues et al., 2003; Sood et al., 1999; Tankhiwale et al., 2003). Antibiotic resistance is occurring with increased frequency among isolates of both serotypes throughout Asia (Chandel et al., 2000a; Crump et al., 2003; Lewis et al., 2005). Furthermore, molecular analysis has indicated that outbreaks of *S.* Paratyphi A infection in India are due to a restricted number of clones (Chandel et al., 2000b; Goh et al., 2002; Thong et al., 1998). To assess whether the same pattern exists in Nepal, we performed susceptibility testing and subtyped bloodstream isolates of

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S. Paratyphi A and S. Typhi collected from febrile adults presenting to a hospital in Kathmandu.

2. Materials and methods

We studied consecutive febrile adults presenting to Patan Hospital, Kathmandu, Nepal, during two seasons (January–March 2001 [winter] and July–August 2001 [monsoon]). From each patient, 5 ml of blood was inoculated into a Myco/F LyticTM blood culture bottle (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) and incubated for 7 days at 35 °C. Gram-negative bacterial isolates were identified in Nepal by a triple sugar iron agar screen and manual review of a commercial biochemical system (BBL^{tm} Crystal^{tm} Enteric/Non-fermenter ID kit; Becton Dickinson Microbiology Systems). All identification was confirmed in the Clinical Microbiology Reference Laboratory at Duke University Medical Center using another commercial system (Dade Microscan Negative Combo Panel Type 12; Dade Behring Inc., West Sacramento, CA, USA). Disk diffusion for ampicillin, ceftriaxone, ciprofloxacin, nalidixic acid, and trimethoprim-sulfamethoxazole was performed and interpreted according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 2000, 2003).

For strain typing, chromosomal DNA from S. Paratyphi A and S. Typhi isolates was digested with *Xba*I before pulsed-field gel electrophoresis (PFGE) was performed on a CHEF-DR II/III system (Bio-Rad Laboratories, Richmond, CA, USA). Strain characterization among PFGE patterns was determined visually by the method of Tenover et al. (1995). In addition, the coefficient of similarity was calculated using the Jaccard method (Sneath and Sokol, 1973), and clustering was based on the unweighted pair group average method (UPGMA) (GelCompar, Applied Maths, Kartrijk, Belgium).

For comparison of demographic characteristics and clinical symptoms, two-sided *P* values were calculated using the χ^2 test or Fisher's exact test for dichotomous and ordinal variables. Continuous variables were compared using the two-sided Wilcoxon rank-sum test and Student's *t*-test.

The Human Research Board of the Centers for Disease Control and Prevention and the Nepal Health Research Council approved this study.

3. Results

Of the 876 febrile adults (age $\geq$ 14 years) enrolled in the study, 60 (21 in winter and 39 during monsoon) had blood cultures that grew S. Typhi and 57 (7 in winter and 50 during monsoon) had blood cultures that grew S. Paratyphi A (Table 1). The clinical presentation of patients, including frequency of admission and baseline laboratory values, did not differ between the two serotypes (Table 2). Patients received ciprofloxacin as empirical therapy in 81% of cases.

Fifty-eight of the S. Typhi isolates and 56 of the S. Paratyphi A isolates were available for analysis by PFGE. Of the S. Paratyphi A isolates, 51 (91%) were indistinguishable, with the remaining five isolates exhibiting two distinct restriction

Table 1 Bacteria isolated from febrile patients at Patan Hospital, Kathmandu, Nepal during January–March 2001 (dry season) and July–August 2001 (monsoon season)

	Dry season (%), *n* = 370	Wet season (%), *n* = 506	Total (%), *n* = 876
Total bacterial isolates	37 (10)	100 (20)	137 (16)
Salmonella Typhi	21 (6)	39 (8)	60 (7)
S. Paratyphi A	7 (2)	50 (10)	57 (7)
Escherichia coli	4 (1)	5 (1)	9 (1)
Streptococcus pneumoniae	2 (<1)	0 (0)	2 (<1)
Staphylococcus aureus	2 (<1)	5 (1)	7 (1)
Enterobacter cloacae	0 (0)	1 (<1)	1 (<1)
Neisseria meningitidis	1 (<1)	0 (0)	1 (<1)

Table 2 Characteristics of febrile patients with *Salmonella* Typhi and S. Paratyphi A during two seasons in Kathmandu, Nepal, 2001

	S. Typhi, *n* = 60	S. Paratyphi A, *n* = 57	*P* value
Monsoon season (%)	42 (70)	50 (89)	0.02
Median age (range)	20 (15–72)	26 (15–72)	0.2
Male gender (%)	41 (68)	41 (72)	0.7
Admission to hospital (%)	2 (2)	1 (1)	0.7
Enteric fever diagnosis (%)	46/58 (80)	38/48 (80)	0.9
Median heart rate (range)	108 (68–128)	105 (68–144)	0.9
Median white blood cell count (range) (10^{-9} per l)	6 (2.9–19.5)	5.5 (3.0–13.1)	0.2