International Journal of Infectious Diseases (2006) **10**, 434—438

http://intl.elsevierhealth.com/journals/ijid

Multidrug-resistant and extended-spectrum beta-lactamase (ESBL)-producing *Salmonella enterica* (serotypes Typhi and Paratyphi A) from blood isolates in Nepal: surveillance of resistance and a search for newer alternatives☆

Bharat M. Pokharel [a], Janak Koirala [b,*], Rajan K. Dahal [a], Shyam K. Mishra [a], Prem K. Khadga [a], N.R. Tuladhar [a]

[a] *Department of Microbiology, Tribhuvan University Teaching Hospital, Kathmandu, Nepal*
[b] *Division of Infectious Diseases, Southern Illinois University School of Medicine, PO Box 19636, Springfield, Illinois 62794-9636, USA*

Received 5 December 2005; received in revised form 12 May 2006; accepted 4 July 2006
Corresponding Editor: J. Peter Donnelly, Nijmegen, The Netherlands

KEYWORDS
Salmonella enterica;
Enteric fever;
Multidrug resistance (MDR);
Extended-spectrum beta-lactamase (ESBL);
Typhi;
Paratyphi A

Summary
Objectives: We evaluated the prevalence of multidrug resistance (MDR) and production of extended spectrum beta-lactamase (ESBL) by *Salmonella enterica* (serotypes Typhi and Paratyphi A) in a teaching hospital in Nepal. The MDR strains of *S. enterica* were also tested for susceptibility to newer antibiotics.
Methods: Blood cultures were obtained from 4105 patients with febrile illnesses. Isolates of *S. enterica* were serotyped and antibiotic susceptibility testing was carried out using disk diffusion (Kirby—Bauer) and E-tests. ESBL screening and phenotype confirmation were done following National Committee for Clinical Laboratory Standards (NCCLS) recommendations for *Escherichia coli*.
Results: A total of 541 isolates of *S. enterica* serotypes Typhi (47%) and Paratyphi A (53%) were grown. Twenty-eight isolates (5%) of *S. enterica* were resistant to two or more antibiotics (MDR isolates), with a greater prevalence among serotype Paratyphi A (7%). All ESBL producers (three isolates) were serotype Paratyphi A. Most of the MDR *S. enterica* showed reduced susceptibility to ampicillin, chloramphenicol, trimethoprim—sulfamethoxazole, ofloxacin, and ciprofloxacin, and had good susceptibility to extended-spectrum cephalosporins and carbapenems. Among the fluoroquinolones, gatifloxacin demonstrated better in vitro activity compared to levofloxacin, ciprofloxacin, and ofloxacin.

☆ Presented at the Ninth Conference of the International Society of Travel Medicine (CISTM), Lisbon, Portugal, May 1—5, 2005.

* Corresponding author. Tel.: +1 217 545 0190/+1 217 545 0181; fax: +1 217 545 8025.
E-mail address: jkoirala@siumed.edu (J. Koirala).

doi:10.1016/j.ijid.2006.07.001

Conclusions: A greater prevalence of *S. enterica* serotype Paratyphi A with higher rates of multidrug resistance and ESBL production is concerning for natives as well as travelers in Nepal since the current typhoid vaccines do not provide protection against this serotype.

Introduction

Enteric fever continues to be a major health problem in underdeveloped countries including South Asian nations. It afflicts local inhabitants as well as travelers to endemic areas. Increasing multidrug resistance in *Salmonella enterica* serotype Typhi has been reported from various parts of the world.[1–4] Similarly, the causative agent of a less severe variety of enteric fever, *S. enterica* serotype Paratyphi A, has also been reported to have developed resistance to multiple antibiotics.[5] Epidemiological studies using the pulsed field gel electrophoresis (PFGE) technique have established that multidrug-resistant *S. enterica* serotype Typhi isolates circulating in Asia are not derived from a single clone.[6]

Enteric fever is endemic in Nepal. *S. enterica* serotype Typhi and *S. enterica* serotype Paratyphi A have been reported as the most common culture isolates from patients with febrile illnesses needing hospital admission.[7,8] Over the past decade, increasing antibiotic resistance in *S. enterica* has lead to a shift in the antibiotics used against this organism from chloramphenicol and ampicillin to trimethoprim–sulfamethoxazole, fluoroquinolones (ofloxacin, ciprofloxacin), and ceftriaxone. Even with the use of these antibiotics, the positive response to treatment has only been in the range of 16–40% in Nepal.[8] The oral Ty21a and parenteral Vi polysaccharide typhoid vaccines provide protection against the serotype Typhi only. Currently there is no vaccine available for the Paratyphi serotypes.

The primary objective of this study was to determine the prevalence of multidrug resistance (MDR) and extended-spectrum beta-lactamase (ESBL)-producing phenotypes among the bloodstream isolates of *S. enterica* serotypes Typhi and Paratyphi A. We also evaluated the antibiotic susceptibility pattern of these MDR and ESBL-producing isolates to three relatively new antibiotics – gatifloxacin, levofloxacin, and ertapenem – in a search for an appropriate alternative. This is the first study done in Nepal exploring the presence of MDR and ESBL-producing strains of *S. enterica*.

Materials and methods

This study was conducted from January to September 2004, at Tribhuvan University Teaching Hospital, a referral center with 450 beds in Kathmandu, Nepal. Blood samples were obtained from febrile patients with clinically suspected enteric fever. Identification of bacteria was done using standard microbiological techniques.[9–11] Serotyping of *S. enterica* was done using polyvalent O-antisera A–G and individual O and H-antisera (Denka Seiken, Japan).

Susceptibility tests for *S. enterica* serotype Typhi and Paratyphi A were performed using standard disk diffusion (Kirby–Bauer) methods and following National Committee for Clinical Laboratory Standards (NCCLS) recommendations.[9–12] The antibiotics tested included: ampicillin, chloramphenicol, ciprofloxacin, ofloxacin, trimethoprim–sulfamethoxazole, cefotaxime, ceftriaxone, ceftazidime, and imipenem. We labeled isolates as MDR if they were resistant to at least two classes of first-line agents including ampicillin, chloramphenicol, trimethoprim–sulfamethoxazole, fluoroquinolones (ciprofloxacin and ofloxacin), and cephalosporins (cefotaxime, ceftriaxone, and ceftazidime). *Escherichia coli* ATCC 25922 was used for quality control.

All the isolates of *S. enterica* were screened for ESBL production using both ceftazidime and cefotaxime discs (Becton, Dickinson & Company, USA) following the NCCLS criteria for *E. coli* and *Klebsiella pneumoniae*.[12] The organisms showing zones of inhibition (ZOI) $\leq$22 mm and $\leq$27 mm for ceftazidime and cefotaxime, respectively, were also tested in combination with clavulanic acid. The organisms were phenotypically confirmed as ESBL producers when they showed an increase in ZOI by greater than or equal to 5 mm when evaluated in combination with clavulanic acid. Quality control was performed by testing *Escherichia coli* ATCC 25922.

The MDR isolates of *S. enterica* were also tested against newer antibiotics including gatifloxacin, levofloxacin, and ertapenem. The minimum inhibitory concentrations (MICs) of these antibiotics were determined by using the E-test (AB Biodisk, Sweden) following standard procedures as recommended by the manufacturer. Antibiotic breakpoints per NCCLS guidelines were used to determine the susceptibilities.[12]

Statistical comparisons of prevalence rates between the two serotypes and differences in resistance rates against antibiotics were done by Fisher's exact tests and Chi-square tests using SPSS software version 12.0 (SPSS Inc., Chicago, USA).

Results

Between January and September 2004, we collected 4105 blood culture samples from patients with a febrile illness visiting Tribhuvan University Teaching Hospital, Kathmandu. Blood cultures obtained from 667 (16%) patients were positive for bacterial growth with 541 non-duplicate isolates (81% of positive cultures) of *S. enterica*. Serotyping showed that 253 (47%) of these isolates were *S. enterica* serotype Typhi and 288 isolates (53%) were *S. enterica* serotype Paratyphi A ($p = 0.03$). Among these 541 isolates of *S. enterica*, 28 (5%) were resistant to two or more antibiotics and were classified as MDR isolates. A greater proportion of *S. enterica* serotype Paratyphi A was MDR (21 isolates, 7%) compared to *S. enterica* serotype Typhi (seven isolates, 3%) ($p = 0.02$).

The MDR isolates of *S. enterica* serotype Typhi demonstrated poor susceptibility to oral antibiotics including ampicillin (43%), chloramphenicol (29%), trimethoprim–sulfamethoxazole (29%), ofloxacin (57%), and ciprofloxacin