Etiological Agents of Bacteraemia and Antibiotic Susceptibility Pattern in Kathmandu Model Hospital

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ABSTRACT

The presence of bacteria in blood is simply known as bacteraemia. The main aim of this study is to determine the bacteraemia in patients visiting Kathmandu Model Hospital and antibiotic susceptibility pattern of isolates with special interest on ciprofloxacin. This prospective study was carried out in microbiology laboratory, Kathmandu Model Hospital from April 2005 to June 2005.

Standard procedure was followed for blood sample collection. The bacteria were isolated and identified by standard microbiological procedure. Further, antibiotic susceptibility test was determined by NCCLS recommended Kirby-Bauer disc diffusion method.

Out of 532 culture requests, 123 samples showed evidential microbial growth. The number of isolate of *Salmonella typhi*, *Salmonella paratyphi* A and *Escherichia coli* were 78, 44 and one respectively. The antibiotic susceptibility test demonstrated that chloramphenicol was the foremost drug of choice among the tested antibiotics with its sensitive rate of 98.4%. All the isolates of *Salmonella typhi* were susceptible to ceftriaxone and all isolates of *Salmonella paratyphi* A were susceptible to chloramphenicol, cotrimoxazole and amoxycillin. Ciprofloxacin resistant serotype of *Salmonella* was not isolated but out of 16 isolated serovar of Typhi and 10 serovar of Paratyphi A screened with nalidixic acid, 10 serovar of Typhi and all serovar of Paratyphi A were found to be resistant. Three isolates of *Salmonella typhi* were found as multidrug resistant (MDR) whereas no MDR was found in *Salmonella paratyphi A*.

From this it can be concluded that *Salmonella* bacteraemia is more than other. Although nalidixic acid resistant serovars were isolated, ciprofloxacin resistant serovar were not present.

Key words: *Bacteraemia, Ciprofloxacin resistant, Multidrug resistant, Salmonella.*

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INTRODUCTION

Bacteraemia and septicaemia are potentially life threatening condition. The illness associated with bacteraemia ranges from self limiting condition to life threatening condition such as sepsis which is an uncontrolled reaction of the human immune system to a severe bacterial infection.[1] Nepal being a South Asian country, *Salmonella* bacteraemia is the most common problem.[2,3] It is the most common isolate from blood specimen of patients needing hospital visit but other blood stream infections (BSI) are not so defined.[4] *Salmonella enterica* subspecies *enterica* serovar Typhi and *Salmonella paratyphi* A are human adopted bacterial pathogens that adheres to and invades the distal ileum and subsequently disseminates to cause systemic diseases, collectively called enteric fever or typhoid.[1] However, *S. typhi* appears to be avirulent in small animals.

Typhoid fever is distressingly prevalent in developing countries, where it remains a major health problem. In developing countries, its annual incidence ranges from 12 to 622/100000 persons. *Salmonella enterica* serovar Typhi is responsible for the majority of cases followed by *Salmonella enterica* serovar Paratyphi A that causes 20% of the cases.[5] In Nepal, typhoid fever is prevalent in mountains, valleys and southern belts as an endemic disease with its peak incidence in May to August.[3]

Typhoid fever is a major cause of morbidity and mortality with an estimated global incidence of 16-33 million cases with an estimated and 500,000 to 600,000 deaths annually. In the last outbreak in the Democratic Republic of Congo, from 27 September 2004 and early January 2005, no less than 42,564 cases of typhoid fever were reported, including 214 deaths and 696 cases of peritonitis and intestinal perforations. In virtually all endemic areas, the incidence of typhoid fever is highest in children from 5–19 years.[6] The last outbreak of typhoid fever in Nepal was in Bharatpur during 2002.[3]

The early diagnosis and appropriate treatment of BSI are important clinical concerns to substantially reduce the frequency of shock and increased survival, regardless of the underlying disease but it is complicated by increasing the antibiotic resistant in worldwide.[7] There are substantial increases in proportion of reports of *Staphylocccus aureus* resistant to Methicillin, *Streptococcus pneumoniae* resistant to Penicillin and Erythromycin, *Enterococcus faecalis* and *Enterococcus faecium* resistant to Vancomycin. Similarly in case of gram negative bacteria especially in Enterobacteriaceae family and glucose non fermenting bacteria such as *Pseudomonas* spp., *Acinetobacter* spp., *Stenotrophomonas maltophilia*, *Burkholderia* spp. have shown the "Extended Spectrum Beta Lactamase" [ESBL] which have ability to degrade third generation cephalosporin and monobactams.

Over the last decade, fluoroquinolones have emerged as the mainstay of therapy for enteric fever. At the same time, the increasing incidence of infection with *salmonellae* resistant to nalidixic acid, which usually display decreased susceptibility to fluoroquinolones, has raised considerable global concern.[8] Mounting clinical evidence suggests that infections due to serovar Typhi and nontyphoidal strains resistant to nalidixic acid but susceptible to ciprofloxacin by National Committee for Clinical Laboratory Standards (NCCLS) criteria may not respond to fluoroquinolone therapy.[9] The incidence of infection with nalidixic acid-resistant *S. enterica* serotype Typhi has been reported to be as high as 23.2% by the National Antimicrobial Resistance Monitoring System of the Centers for Disease Control and Prevention.[10] The vast majority of nalidixic acid-resistant strains remain within the current susceptible range for ciprofloxacin (1 μg/ml) as recommended by the NCCLS. However, the probability of clinical response to fluoroquinolone therapy in patients with invasive *Salmonella* infection is lower in those with nalidixic acid-resistant than with -susceptible strains.[11]

MATERIALS AND METHODS

This prospective study was conducted in Kathmandu Model Hospital on patients attending for the treatment of suspected bacteraemia. This study included 532 cases from April 2005 to June 2005. The clinical history and examination finding were recorded on the standard form before preceding the blood culture.

The evidential microbiological diagnosis of bacteraemia was made by isolation of bacteria from blood culture. Total 5ml blood sample was drawn aseptically from adult patients and inoculated in 45 ml of Brain heart infusion (BHI) (Hi Media, India) broth containing 0.03% of SPS as anticoagulant. For children, 3ml of blood was inoculated in 20 ml of BHI so that blood-to-broth ratio 1:10 was maintained. The culture bottle was incubated for four days at 37^0 C. All blood culture bottles were examined daily. If the bottle showed any visible sign of growth such as uniform turbidity with gas bubble formation, haemolysis of blood with greenish tinge or cottony ball formation, subculture was done on blood agar and MacConkey agar plate (Hi Media, India). The plates were then incubated at 37^0 C for 24 hrs. Before discarding the culture bottle, blind subculture was done after 96 hours of incubation.

Table 1. Culture request pattern

Provisional diagnosis	Frequency	%
Enteric fever	**523**	**98.2**
Pneumonia	3	0.6
Inf. Endocarditis	2	0.4
Meningitis	1	0.2
Others	3	0.6
Total	**532**	**100**

The isolates were identified by adopting standard microbiological procedure which includes colony morphology, Gram stain reaction and biochemical reaction such as catalase, oxidase, citrate agar slant, Sulphide Indole Motility test, Methyl red test, Voges Proskauer test, Triple Sugar Iron agar test and urease test. The isolate of *Salmonella* were further confirmed by agglutination with Polyvalent O-antisera A-S and Individual H antisera (Denka Seiken, Japan). Further, antibiotic susceptibility tests of all isolates were performed by NCCLS recommended Kirby-Bauer disc diffusion method. The antibiotic incorporated plates were incubated at 37^{0} C and zone of inhibition around the antibiotic were measured after 18hrs and within 24 hours of incubation. The routinely used antibiotics, all from Hi Media, were amoxycillin (30 μg), cefixime (5 μg), ceftriaxone (30 μg), cephotaxime (30 μg), ofloxacin (5 μg), ciprofloxacin (5 μg), cotrimoxazole (1.25/23.75 μg) and chloramphenicol (30 μg). The isolated were considered as multidrug resistant if they were resistant to at least two classes of antibiotics. Few isolates of *Salmonella* were screened for nalidixic acid susceptibility test.

RESULTS

Within April to June 2005, 532 blood samples were collected with febrile illness in patients visiting Kathmandu Model Hospital in which 98.2 % (523/532) cultures were for enteric fever. (Table 1)

Of all cultures, evidential microbial growth was shown by 123 (23.12%) cultures. The most frequently isolated bacteria was *Salmonella typhi,* 78 (63.4%) in number followed by *Salmonella paratyphi* A, 44 (35.8%) in number and *Escherichia coli,* 1 (0.8%) in number. No gram-positive microorganism was isolated during the study period.

Of the 123 isolates tested against chloramphenicol, 121(98.4%) isolates were sensitive. The resistant pattern of isolates exhibited decreased susceptibility to ofloxacin (5.7%), cotrimoxazole (2.4%), amoxycillin (1.6%), chloramphenicol (1.6%) and cefixime (0.8%). Ciprofloxacin, cephotaxime and ceftriaxone resistant bacteria were not isolated. (Table 2)

Ceftriaxone was the principal antibiotic of choice since all isolates of *S. typhi* were susceptible towards ceftriaxone. Ciprofloxacin was the least susceptible drug with its susceptibility rate 93.6% but ciprofloxacin resistant typhi were not isolated. Increased number of cotrimoxazole and ofloxacin (3.8%) resistant serovars were isolated. 16 isolates of *S. typhi* tested against nalidixic acid susceptibility test showed 62.5% isolates were resistant. Again 78 isolates, three isolates were determined as multidrug resistant and they were resistant to chloramphenicol, amoxycillin, ofloxacin, cefixime and cotrimoxazole. (Table 3)

All strains of *Salmonella paratyphi* A were susceptible to three antibiotics chloramphenicol, amoxycillin and cotrimoxazole. Cefixime showed decreased susceptibility; of the 44 serovars tested against cefixime, 33 (75%) serovars showed full susceptibility. Only ofloxacin resistant

Table 2. Antibiotic susceptibility pattern of Gram-negative isolates

Antibiotics Used	Antibiotic susceptibility pattern						Total isolates
	Susceptible		Intermediate		Resistant		
	Number	%	Number	%	Number	%	
Amoxycillin	120	97.6	1	0.8	2	1.6	123
Cephotaxime	116	94.3	7	5.7	0	0	123
Ceftriaxone	120	97.6	3	2.4	0	0	123
Cefixime	108	87.8	14	11.4	1	0.8	123
Ciprofloxacin	109	88.6	14	11.4	0	0	123
Ofloxacin	111	90.2	5	4.1	7	5.7	123
Cotrimoxazole	120	97.6	0	0	3	2.4	123
Chloramphenicol	**121**	**98.4**	**0**	**0**	**2**	**1.6**	**123**
Nalidixic acid	6	23	0	0	20	77	26

serovars were isolated and it covered 9.1% and no multidrug resistant *S. paratyphi* A was isolated. Ten isolates of *S. paratyphi* A were tested against nalidixic acid in which all the isolates were found to be resistant. (Table 4)

Only one strain of *Escherichia coli* was isolated and the isolate was susceptible to all the tested antibiotics.

The antibiotic susceptibility pattern of multidrug resistant (MDR) *S. typhi* demonstrated that cotrimoxazole was the least effective followed by chloramphenicol and amoxycillin; however, ceftriaxone and cephotaxime were susceptible to the MDR isolates. (Table 5)

All together, there were 20 nalidixic acid (30 μg) resistant (inhibition zone equal or less than 13 mm) *S. typhi* and *S. paratyphi* A in which all the *S. typhi* were susceptible to ciprofloxacin but in case of *S. paratyphi* A 50% were susceptible and other 50% were intermediate.(Table 6)

DISCUSSION

This study reflected heavy burden of enteric fever in Kathmandu Valley although it is hospital based study. Commonly isolated pathogens were *Salmonella typhi* and *Salmonella paratyphi* A. The number of isolates of *S. typhi* and *S. paratyphi A* were 78 (63.4%) and 44 (35.8%) respectively. Only one (0.8%) strain of *Escherichia coli* was isolated.

Chloramphenicol, ampicillin, tetracycline, cotrimoxazole were the first line drugs of choice for *Salmonella* spp. but there are shocking reports of development of resistance against these drugs. Chloramphenicol in 1948 became the gold standard drug for the treatment of typhoid fever. Resistance to chloramphenicol was reported in *S. typhi* in 1950 but it was not until 22 years later that the first outbreaks of chloramphenicol-resistant typhoid fever occurred.[12] It reduced mortality rate around 1% as well as length of fever from 14 to 28 days to 3 to 5 days but it was no longer in use due to emergence of resistance, a high relapse rate, a high rate of continued and chronic carriage and its adverse effect.[13] In this study, 97.4% (76/78) *S. typhi* were susceptible to chloramphenicol whereas 2.6% (2/78) were resistant. These results are supported by Sharma et al[3] who showed that 92.7% of isolates were susceptible to chloramphenicol and 3.6% of isolates were resistant to chloramphenicol. Although all the isolates of *S. paratyphi* A were susceptible to chloramphenicol and no MDR *S. paratyphi* A was isolated in present study, recently Pokharel et al[2] showed MDR *S. paratyphi* A with 5% resistant to chloramphenicol. Among the multidrug resistant isolates of *S. typhi*, 66.7% were resistant to chloramphenicol which is supported by Pokharel et al.[2] Their finding showed that among the MDR *S. typhi*, 57% isolates were resistant to chloramphenicol.

Cotrimoxazole is the second cost effective antibiotics used to treat enteric fever. Present study shows that 96.1% (75/78) typhi isolates were susceptible while 3.8% (3/78) isolates were resistant to cotrimoxazole. Similar results were also shown by Murdoch et al[4] as 5% isolates were resistant to cotrimoxazole. Similarly, all isolates of *S. paratyphi* A were susceptible to cotrimoxazole which was also shown by Murdoch et al.[4] Among the MDR *S. typhi;* all the isolates were resistant to cotrimoxazole. This work was supported by Nagesha *et al*.[14] who showed that among MDR *Salmonella typhi*, 97% of the isolates were resistant to cotrimoxazole.

Among the isolates of serovar typhi and paratyphi A, 96.1% and 100% were susceptible to amoxycillin respectively. Besides that, the third generation cephalosporins; cephotaxime, ceftriaxone and cefixime were also tested against the typhoid and paratyphoid bacilli. Third generation cephalosporin now have gained importance for the treatment of enteric infections. Parenterally administered third generation cephalosporins are showing their efficacy in the treatment of typhoid fever.[15] Hence, it is now considered as the first antibiotic of choice for the treatment of enteric fever unless the in vitro susceptibility tests prove otherwise. In this study, ceftriaxone is the gold standard antibiotic as all the isolates were susceptible. This result is supported by other researches which showed 100% susceptibility rate of ceftriaxone to *S. typhi.*[2,3,16,17] However, ceftriaxone resistant *S. typhi* was detected in Bangladesh in 1999.[18] Besides that 93.2% (41/44) isolates of *S. paratyphi* A were susceptible to ceftriaxone. In case of other two cephalosporins cefixime and cephotaxime, 94.9% and 98.7% of typhi isolates were susceptible respectively and 75% and 86.4% of paratyphi A isolates were susceptible respectively. This means these antibiotics have shown decreased susceptibility towards the isolates. In case of MDR isolates, ceftriaxone and cephotaxime were susceptible.

Antibiotic resistance in *S. typhi* particular to chloramphenicol, ampicillin and cotrimoxazole is often encoded by large (~180 kb) conjugative plasmids belonging to the incompatibility complex group *Inc*HI.[19] The genetic basis of MDR in serovar Paratyphi A has

Table 3. Antibiotic susceptibility pattern of Salmonella typhi

Antibiotics Used	Antibiotic susceptibility pattern						Total isolates
	Susceptible		Intermediate		Resistant		
	Number	%	Number	%	Number	%	
Amoxycillin	75	96.1	1	1.3	2	2.6	78
Cephotaxime	77	98.7	1	1.3	0	0	78
Ceftriaxone	**78**	**100**	**0**	**0**	**0**	**0**	**78**
Cefixime	74	94.9	3	3.8	1	1.3	78
Ciprofloxacin	73	93.6	5	6.4	0	0	78
Ofloxacin	74	94.9	1	1.3	3	3.8	78
Cotrimoxazole	75	96.1	0	0	3	3.8	78
Chloramphenicol	76	97.4	0	0	2	2.6	78
Nalidixic acid	6	37.5	0	0	10	62.5	16

Table 4. Antibiotic susceptibility pattern of Salmonella paratyphi A

Antibiotics Used	Antibiotic susceptibility pattern						Total isolates
	Susceptible		Intermediate		Resistant		
	Number	%	Number	%	Number	%	
Amoxycillin	**44**	**100**	**0**	**0**	**0**	**0**	**44**
Cephotaxime	38	86.4	6	13.6	0	0	44
Ceftriaxone	41	93.2	3	6.8	0	0	44
Cefixime	33	75	11	25	0	0	44
Ciprofloxacin	35	79.5	9	20.5	0	0	44
Ofloxacin	36	81.8	4	9.1	4	9.1	44
Cotrimoxazole	**44**	**100**	**0**	**0**	**0**	**0**	**44**
Chloramphenicol	**44**	**100**	**0**	**0**	**0**	**0**	**44**
Nalidixic acid	0	0	0	0	10	100	10

Table 5. Antibiotic susceptibility pattern of MDR *Salmonella typhi*

Antibiotics Used	Antibiotic susceptibility pattern						Total isolates
	Susceptible		Intermediate		Resistant		
	Number	%	Number	%	Number	%	
Amoxycillin	0	0	1	33.3	2	66.7	3
Cephotaxime	**3**	**100**	**0**	**0**	**0**	**0**	**3**
Ceftriaxone	**3**	**100**	**0**	**0**	**0**	**0**	**3**
Cefixime	2	66.7	0	0	1	33.3	3
Ciprofloxacin	2	66.7	1	33.3	0	0	3
Ofloxacin	2	66.7	0	0	1	33.3	3
Chloramphenicol	1	33.3	0	0	2	66.7	3
Cotrimoxazole	0	0	0	0	3	100	3
Nalidixic acid	0	0	0	0	3	100	3

Table 6. Ciprofloxacin susceptibility pattern of nalidixic acid resistant *Salmonella typhi* and *S. paratyphi* A

Antibiotics Used	Antibiotic susceptibility pattern			Total
	Susceptible	Intermediate	Resistant	
S. typhi	10	0	0	10
S. paratyphi A	5	5	0	10

remained predominantly undefined, however, the DNA sequence of an *IncHI*1 plasmid, encoding MDR in a serovar Paratyphi A strain shares a common *IncHI*1 associated DNA backbone with the serovar Typhi plasmid.[20] Reports from Nepal shows that MDR *S. typhi* isolates contained a class 1 integron with a single cassette, *dfrA7*, conferring resistance to trimethoprim and resistance to ampicillin and chloramphenicol but not ciprofloxacin was mediated by bla_{TEM}-like and *catA* genes respectively.[21]

Due to emergence of first line of drugs, quinolones became alternative drugs of choice to treat enteric fever. They are very effective and reduce the duration of treatment as shown in various randomized control trials.[22] The fluoroquinolone particularly ciprofloxacin was the most frequently used antibiotic. Ciprofloxacin resistant typhi was not isolated in present study which is supported by other researches.[10,19,21] But their susceptibility rate was found to be 93.6% that means its susceptibility rate was slowly decreasing in trend. Similarly, no ciprofloxacin resistant paratyphi A was isolated but its susceptibility rate was greatly reduced to 79.5%. This may be due to over use of ciprofloxacin. Another fluoroquinolone ofloxacin shows decreased susceptibility; 94.9% in typhi and 81.8% in paratyphi A. A part from this, 3.8% and 9.1% of the typhi and paratyphi A isolates were resistant respectively.

There have been increasing reports of treatment failure using ciprofloxacin for patient whose serovar typhi isolated are susceptible to fluoroquinolone and resistant to nalidixic acid. In those ciprofloxacin susceptible *S. enterica* isolates, nalidixic acid resistance has been proposed as an indicator that infection with such a strain may not respond to fluoroquinolone treatment. The presence of nalidixic acid resistance has been suggested as a laboratory marker of isolates with reduced susceptibility to fluoroquinolones and an indicator that invasive infections may fail to respond to fluoroquinolone therapy it is suggested that all *S. typhi* isolates should be screened for nalidixic acid resistance along with ciprofloxacin.[11] Shirakawa et al showed the presence of *gyr*A gene mutations in 30 clinical strains of *S. typhi* and 39 of *S. paratyphi* A, all of which were isolated in Kathmandu in 2003, showed the resistance to nalidixic acid and decreased susceptibility to ciprofloxacin and levofloxacin. They reported 73.3% and 94.9% of *S. typhi* and *S. paratyphi* A strains contained *gyr*A gene mutation respectively.[23] In our study, 62.5% (10/16) typhi isolates were resistant to nalidixic acid and these strains are called Nalidixic acid resistant *Salmonella typhi* [NARST] and paratyphi A strain tested against nalidixic acid, all were resistant. A number of resistance mechanisms to quinolones have been identified, including point mutations that result in amino acid substitutions in the topoisomerases, reduced outer membrane permeability, increased efflux of antibiotics and other harmful compounds, and the plasmid-encoded *Qnr* genes. In most strains, acquired fluoroquinolone resistance was attributed to point mutations in chromosomal "Quinolone Resistance Determining Region" (QRDR) genes encoding DNA gyrase (*gyr*A, *gyr*B) or DNA topoisomerase IV (*par*C, *par*E) but the major one is *gyr* A in which at position 83 of the DNA gyrase enzyme, serine is mutated to tyrosine or phenylalanine or alanine and position 87 aspartate is mutated to asparagine or glycine or tyrosine either alone or both.[24, 25] The most common amino acid substitution reported in *par*C is threonine in position 57 changed to serine, with threonine in position 66 changed to isoleucine or serine in position 80 changed to arginine.[24] In case of *Salmonella paratyphi* A also, the resistance is caused by change in genetic sequence of QRDR region especially *gyr* A and *par* C.[25] In present study, all isolates of *Salmonella paratyphi* A were resistant to nalidixic acid.

The choice of oral antimicrobial regimens for uncomplicated typhoid fever caused by isolates of *S. enterica* serovar Typhi that are both MDR and Nalidixic acid is not established. They are now quite common in Asian countries. A fluoroquinolone given in a high dose for 7 days is the most affordable first-line option for MDR with nalidixic acid resistant.[26] Here only one strain of *Escherichia coli* was isolated and it was susceptible to all the tested antibiotic.

These antibiotic resistance problems are rapidly growing up that threatens our ability to treat common infections. Bacteria become more and more resistant to newly used drugs like third and fourth generation cephalosporins as well as fluoroquinolones. These critical points ask "Are we on the verge of a medical disaster?" As there is rapid antibiotic resistancet problem,we recommended the conscientious and prudent use of antibiotic as well as tailoring the choice of antibiotic before starting the treatment. Again the MIC test of ciprofloxacin should be done if any isolate of typhi and paratyphi A shows resistance to nalidixic acid and it should be reported as intermediately susceptible to ciprofloxacin.

CONCLUSION

Our data showed that *Salmonella* was the most generally isolated bacteria in blood stream infection. Upon antibiotic susceptibility test, chloramphenicol still remain the drug

of choice and in case of *Salmonella typhi* ceftriaxone was the drug of choice having susceptibility rate of 100%; for *Salmonella paratyphi* A drug of choice were amoxicillin, chloramphenicol and cotrimoxazole having susceptibility rate of 100%. Although ciprofloxacin and ceftriaxone resistant bacteria were not isolated in our study, many case reports and journals show about resistance. Hence continuous monitoring of antimicrobial resistance among blood isolated should be done.

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