Etiological agents of bacteraemia and antibiotic susceptibility pattern of isolates in Kathmandu Model hospital, Kathmandu

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Abstract

Introduction Blood stream infection is the one of the maior infection and its rate of infection increases progressively in Nepal. This is further intensified by antibiotic resistant of different classes complicating the therapeutic management.

Objectives To determine the pathogenic microorganism in blood specimen collected from patients visiting Kathmandu Model Hospital and antibiotic susceptibility pattern of isolates with main focus on Ciprofloxacin.

Methods The requested clinical samples were subjected to routine processing following standard protocol. The bacteria isolated were first identified by following standard microbiological operating process and the antimicrobial susceptibility pattern of isolated bacteria was then determined by NCCLS recommended Kirby-Bauer disc diffusion method.

Results Total 532 sample cultures were processed: 123 samples (23.12%) were found to be bacterial growth in which 78 isolates were *Salmonella typhi*, 44 isolates were *Salmonella paratyphi* A and one isolate was *Escherichia coli*. The antibiotic susceptibility test of isolates showed that Chloramphenicol was the principal drug of choice among the tested antibiotics with the efficacy rate of 98.37 percent. Ceftriaxone being 100 percent susceptibility was the drug of choice for *S. typhi* whereas Amoxycillin, Chloramphenicol and Cotrimoxazole were the drug of choice for *S. paratyphi* A. Ciprofloxacin resistant serotypes of *Salmonella* was not isolated but out of 16 isolated serovar of Typhi and 10 serovar of Paratyphi A were screened with Nalidixic acid, 10 serovar of Typhi and all serovar of Paratyphi A were found to be resistant respectively. Three isolates of *S. typhi* were found as multidrug resistant (MDR) whereas no MDR *S. paratyphi A* was identified.

Conclusion The rate of isolation of *Salmonella* from blood stream infection is quite high rather than other bacteria. This is due to unhygienic practices since Nepal is within endemic region of typhoid fever and these isolated bacteria further create problems about antibiotic resistant.

Key words Bacteraemia, Ciprofloxacin, *Salmonella*, Antibiotic pattern.

Introduction

Bacteraemia is potentially life-threatening condition. The illness associated with bacteraemia ranges from self-limiting condition to life threatening condition sepsis[1]. Therefore, it requires rapid, immediate and aggressive antimicrobial treatment. Nepal being South Asian country, *Salmonella* bacteraemia is the most common problem[2,3] and it is the most common culture isolates from blood specimen of patients needing to hospital visit but other blood stream infections (BSI) are not so well defined[4]. The typhoid fever is prevalent in mountains, valleys and southern belts of Nepal as an endemic disease with its peak incidence in May to August. The last out break of Typhoid fever in Nepal was in Bharatpur during 2002[3] and now due to recent inundation, it is now again reported.

Typhoid fever remains a serious public health problem throughout the world, with an estimated 16–33 million cases and 500000 to 600000 deaths annually[5]. Hence, it remains maior causes of morbidity and mortality worldwide. In the last

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outbreak in the Democratic Republic of Congo, between 27 September 2004 and early January 2005, no less than 42564 cases of typhoid fever were reported, including 214 deaths and 696 cases of peritonitis and intestinal perforations[5].

The early diagnosis and proper treatment of BSI are important clinical concerns to substantially reduce the frequency of shock and increased survival, regardless of the underlying disease[6] but it is problematical by increasing the antibiotic resistant in worldwide. There is substantial raise in proportion of reports of *Staphylococcus aureus* resistant to Methicillin, *Streptococcus pneumoniae* resistant to Penicillin and Erythromycin, *Enterococcus faecalis* and *E. faecium* resistant to Vancomycin[7]. Similarly, in case of gram negative bacteria especially in enterobacteriaceae 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 of Cephalosporin and Monobactams. Although Fluoroquinolones are the mainstay therapy for the infection of typhoid fever, there are growing incidences of infection with *Salmonella enterica* serovar Typhi strains resistant to Nalidixic acid, which usually demonstrate decreased susceptibility to Fluoroquinolones, have raised considerable global concern[8]. The incidence of infection with Nalidixic acid resistant *S. enterica* serotype Typhi has been reported to be as high as 23.2 percent by the National Antimicrobial Resistance Monitoring System of the Centers for Disease Control and Prevention[9]. Furthermore, several clinical treatment failures after the administration of Ciprofloxacin and other Fluoroquinolones to patients with typhoid fever due to strains with decreased susceptibilities to Fluoroquinolones have also been reported[10]. The emergence and spread of these organisms have been reported from developing countries. There is evidence that the incidence of strains that are resistant to Nalidixic acid and that exhibit decreased susceptibilities to the most recent Fluoroquinolones used for the treatment of typhoid fever is increasing[10]. Hence, in Ciprofloxacin susceptible *Salmonella enterica* isolates, Nalidixic acid resistance has been proposed as an indicator that infection with such a strain may not respond to Fluoroquinolone treatment[11].

Methodology

Study Population:

This prospective study is conducted in Kathmandu Model Hospital on patients attending for the treatment of suspected bacteraemia case. The clinical history and examination finding were recorded on the standard form before preceding the blood culture. This study included total 532 cases from 3rd April 2005 to 26th June 2005 requested for blood culture by physician.

Sample Processing:

Approximately 5ml of blood sample was drawn aseptically from adult patients and inoculated in 45ml of Brain heart infusion (BHI) (Hi Media, India) broth containing 0.03% of SPS. In case of children, 3ml of blood was inoculated in 20ml of BHI so that blood-to-broth ratio 1:10 was maintained.

The blood culture bottles were incubated for four days at 37^0C. They were examined daily and if any bottle showed visible sign of growth such as uniform turbidity with gas bubble formation, haemolysis of blood with greenish tinge or cottony ball formation, sub-culture was done on blood agar and MacConkey agar plate (Hi Media, India). The plates were incubated at 37^0C for 24 hrs. Before discarding the culture bottle, blind sub-culture was done after 96 hr incubation. The isolates from blood culture were identified by following standard microbiological technique. The isolate of *Salmonella* were further confirmed by agglutination with antisera polyvalent Oantisera A-S and individual H antisera (Denka Seiken, Japan).

Antimicrobial susceptibility testing:

The antibiotic susceptibility test of the all isolates was performed by Kirby-Bauer Disc diffusion method. Routinely used antibiotics all from Hi Media were Amoxycillin (30µg), Cefixime (5µg), Ceftriaxone (30µg), Cephotaxime (30µg), Ofloxacin (5µg), Ciprofloxacin (5µg), Cotrimoxazole (1.25/23.75µg) and Chloramphenicol (30µg). The isolated were considered as multidrug resistant if they were resistant to at least two classes of drugs 2. Few isolated of *Salmonella* were screened for Nalidixic acid susceptibility.

Results

The age of the study population ranged from 9 month to 82 years old with mean ± standard deviation of 24.63 ± 112.83 respectively. The male female ratio of study population was 1.97:1. Seventy seven percent of the patients visiting hospital were of below 30 years old. During the study period, 532 blood samples were processed in which 98.3 percent (523/532) sample cultures were for enteric fever as shown in table 1.

Table 1: Clinical history and culture pattern.

Clinical history	Number	%
Enteric fever	523	98.30
pneumonia	3	0.56
Inf. Endocarditis	2	0.37
Meningitis	1	0.18
others*	3	0.56
Total	**532**	**100**

* Includes patients with symptomsi severe headache and abdominal pain

Out of 532 cultures, 123 (23.12%) sample cultures showed bacterial growth in which maximum growth was found in age group between 20-30. The ratio of culture positive in male to female was 2:1. The results are revealed in table 2.

Table 2: Age and sex wise distribution of culture positive and culture negative pattern.

	Sex						
	Male			Female			
Age group	Culture positive	Culture negative	Total	Culture positive	Culture negative	Total	Grand Total
0-10	5	23	28	2	11	13	41
10-20	28	55	83	14	31	45	128
20-30	38	131	169	21	51	72	241
30-40	7	29	36	2	25	27	63
40-50	2	17	19	0	10	10	29
50-60	2	7	9	0	5	5	14
60-70	0	6	6	1	2	3	9
70-80	0	2	2	1	3	4	6
80-90	0	1	1	0	0	0	1
Total	**82**	**271**	**353**	**41**	**138**	**179**	**532**

The most frequently isolated bacteria from blood culture was *Salmonella typhi*, 78 isolates (63.41%) followed by *Salmonella paratyphi* A, 44 isolates (35.77%) and *Escherichia coli*, one isolates (0.82%). The *Escherichia coli* was isolated from female of age 78 years old. No gram-positive bacteria were isolated during the study period. The results are shown in table 3.

Table 3: Patten of bacterial isolates

Bacteria	Number	%
Salmonella typhi	78	63.41
Salmonella paratyphi A	44	35.77
Escherichia coli	1	0.82
Total	**123**	**100**

The antibiotic susceptibility test of the isolates showed that Chloramphenicol was the principle drug of choice and its efficacy rate in-vitro was 98.37 percent. The least effective drug was Ofloxacin having efficacy rate of 87.81 percent. The resistant pattern of isolates showed increased resistant to Ofloxacin (5.7%), Cotrimoxazole (2.44%), Amoxycillin (2.44%), Chloramphenicol (1.63%) and Cefixime (0.81%) as shown in table 4.

Table 4: Antibiotic susceptibility pattern of Gram-negative isolates

	Antibiotic susceptibility pattern						
	Susceptible		Intermediate		Resistant		
Antibiotics Used	Number	%	Number	%	Number	%	Total isolates
Amoxycillin	120	97.56	1	0.81	2	1.63	123
Cephotaxime	116	94.3	7	5.7	0	0	123
Ceftriaxone	120	97.56	3	2.44	0	0	123
Cefixime	108	87.81	14	11.38	1	0.81	123
Ciprofloxacin	109	88.62	14	11.38	0	0	123
Ofloxacin	111	90.24	5	4.06	7	5.7	123
Cotrimoxazole	120	97.56	0	0	3	2.44	123
Chloramphenicol	121	98.37	0	0	2	1.63	123
Nalidixic acid	6	23	0	0	20	77	26

For *Salmonella typhi*, Ceftriaxone was the principle drug of choice as all the isolates of *Salmonella typhi* was susceptible towards Ceftriaxone. Ciprofloxacin was the least susceptible drug and its efficacy rate was found to be 93.6 percent among the tested antibiotics. It was noted that no Ciprofloxacin resistant typhi was isolated but increased number of Cotrimoxazole and Ofloxacin (3.85%) resistant serovars were isolated. Sixteen isolates of *Salmonella typhi* were tested against Nalidixic acid susceptibility in which 62.5 percent of the isolates were resistant. Among 78 isolates, three isolates were found as multidrug resistant and they were resistant to Amoxycillin, Ofloxacin, Chloramphenicol and Cotrimoxazole as shown in table 5.

Table 5: Antibiotic susceptibility pattern of *Salmonella typhi*

	Antibiotic susceptibility pattern						
	Susceptible		Intermediate		Resistant		
Antibiotics Used	Number	%	Number	%	Number	%	Total isolates
Amoxycillin	75	96.15	1	1.28	2	2.56	78
Cephotaxime	77	98.72	1	1.28	0	0	78
Ceftriaxone	78	100	0	0	0	0	78
Cefixime	74	94.87	3	3.85	1	1.28	78
Ciprofloxacin	73	93.6	5	6.41	0	0	78
Ofloxacin	74	94.87	1	1.28	3	3.85	78
Cotrimoxazole	75	96.15	0	0	3	3.85	78
Chloramphenicol	76	97.44	0	0	2	2.56	78
Nalidixic acid	6	37.5	0	0	10	62.5	16

For *Salmonella paratyphi* A, three drugs Amoxycillin, Chloramphenicol and Cotrimoxazole was the principle drug of choice based on antibiotic susceptibility pattern. The least susceptible drug was Cefixime whose efficacy rate was only 75 percent. Only Ofloxacin resistant serovars were isolated, it covered 9.1 percent, and no multidrug resistant *Salmonella paratyphi* A was isolated. Ten isolates of *Salmonella paratyphi* A were tested against Nalidixic acid in which all the isolates were found to be resistant as shown in table 6.

Table 6: Antibiotic susceptibility pattern of *Salmonella paratyphi* A

	Antibiotic susceptibility pattern						
	Susceptible		Intermediate		Resistant		
Antibiotics Used	Number	%	Number	%	Number	%	Total isolates
Amoxycillin	44	100	0	0	0	0	44
Cephotaxime	38	86.36	6	13.64	0	0	44
Ceftriaxone	41	93.18	3	6.82	0	0	44
Cefixime	33	75	11	25	0	0	44
Ciprofloxacin	35	79.54	9	20.46	0	0	44
Ofloxacin	36	81.82	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

Only one strain of *Escherichia coli* was isolated and the isolate was susceptible to all the tested antibiotics. The antibiotic susceptibility pattern of MDR *Salmonella typhi* showed that Cotrimoxazole was the least effective followed by Chloramphenicol and Amoxycillin, however, the tested Cephalosporin groups were sensitive to the multi-drug resistant isolates. The results are shown in table 7.

Table 7: Antibiotic susceptibility pattern of MDR *Salmonella typhi*

	susceptibility pattern						
	Susceptible		Intermediate		Resistant		
Antibiotics Used	Number	%	Number	%	Number	%	Total
Amoxycillin	0	0	1	33.33	2	66.67	3
Cephotaxime	3	100	0	0	0	0	3
Ceftriaxone	3	100	0	0	0	0	3
Cefixime	3	100	0	0	0	0	3
Ciprofloxacin	2	66.67	1	33.33	0	0	3
Ofloxacin	2	66.67	0	0	1	33.33	3
Chloramphenicol	1	33.34	0	0	2	66.33	3
Cotrimoxazole	0	0	0	0	3	100	3

All together, there were 20 Nalidixic acid (30µg) resistant (Inhibition Zone Diameter d"13 mm) *Salmonella typhi* and *Salmonella paratyphi* A in which all the *Salmonella typhi* were susceptible to Ciprofloxacin but in case of *Salmonella paratyphi* A, 50 percent were resistant and other 50 percent were intermediate to the tested antibiotic Nalidixic acid.

Table 8: Ciprofloxacin susceptibility pattern of nalidixic acid resistant *Salmonella typhi* and *S. paratyphi* A

Microorganism	Sensitive	Intermediate	Resistance	Total
S. typhi	10	0	0	10
S. paratyphi A	5	5	0	10

Discussion

Although it is hospital-based study, it reflected the heavy burden of enteric fever in Kathmandu Valley. The most common pathogen isolated was *Salmonella typhi* and *Salmonella paratyphi* A. In this study, out of 123 isolates 78 isolates (63.41%) were *Salmonella typhi*, 44 (35.77%) isolates were *Salmonella paratyphi* A and only one (0.8%) isolate was *Escherichia coli* and the male female ratio of *Salmonella* bacteraemia was 2.05:1. This result was supported by Guha et al. who found that out of 114 isolates, 76 isolates (66.7%) were *Salmonella typhi*, 38 (33.3%) isolates were *Salmonella paratyphi* A and male female ratio of *Salmonella* bacteraemia was 1.8:1[12].

Chloramphenicol, Ampicillin, Tetracycline, Cotrimoxazole were the foremost drug of choice for *Salmonella* spp. but there are shocking reports of development of resistant of these antibiotics. Chloramphenicol in 1948 became the gold standard drug for the treatment of typhoid fever[13] and it successfully reduced the mortality rate around 1 percent as well as length of fever from 14 to 28 days to three to five days. However, due to emergence of resistant, a high relapse rate, a high rate of continued and chronic carriage and its adverse effect, Chloramphenicol was no longer in use[14]. In this study, 97.44 percent (76/78) of *Salmonella typhi* were susceptible to Chloramphenicol and 2.56 percent (2/78) were resistant to Chloramphenicol. These results are supported by Sharma et al.[3] who showed that 92.7 percent of isolates were susceptible to Chloramphenicol and 3.6 percent of isolates were resistant to Chloramphenicol. In case of *Salmonella paratyphi* A, all the isolates were susceptible to Chloramphenicol and no multidrug resistant *Salmonella paratyphi* A was isolated. Among the multidrug resistant isolates of *Salmonella typhi*, 66.67 percent were resistant to Chloramphenicol, which is supported by Dahal et al.[2]. That report showed that among the MDR *Salmonella typhi*, 57 percent of isolates were resistant to Chloramphenicol.

Cotrimoxazole is the second cost effective drug used to treat typhi and paratyphi infection. Present study showed that 96.15 percent (75/78) of typhi isolates were susceptible to this drug and 3.85 percent (3/78) of typhi isolates were resistant to this drug. Similar results are shown by Murdoch et al[4]. as 5 percent isolates were resistant to Cotrimoxazole. Similarly, in case of *Salmonella paratyphi* A, all the isolates were susceptible to Cotrimoxazole, which is also shown by Murdoch et al.[4]. Among the multidrug resistant *Salmonella typhi*, all the isolates were resistant to Cotrimoxazole. This report was supported by Nagesha et al.[15] who showed that among the multidrug resistant *Salmonella typhi*, 97 percent of the isolates were resistant to Cotrimoxazole.

Among the isolates of both typhi and paratyphi A, 96.15 percent and 100 percent isolates were susceptible to Amoxycillin respectively. Besides that, the third generation Cephalosporins, Cephotaxime, Ceftriaxone and Cefixime were also tested against the typhoid and paratyphoid bacilli. Now a days, third generation Cephalosporin have gained importance for the treatment of enteric infections. Parenterally administered third generation, Cephalosporins are showing their efficacy in the treatment of typhoid fever[16]. Hence, it is now considered as a first drug of choice for the treatment of enteric fever unless the *in vitro* susceptibility tests prove otherwise. In this study, Ceftriaxone is the gold standard drug as all the isolates were susceptible. This result is supported by Sharma et al.[3], Dahal et al.[2], Dutta et al.[17], Madhulika et al.[18] in which the researcher showed the 100 percent susceptibility rate of Ceftriaxone to *Salmonella typhi*. However, Ceftriaxone resistant *Salmonella typhi* was detected in Bangladesh in 1999[19]. In case of *Salmonella paratyphi* A 93.18 percent (41/44) of isolates were susceptible to Ceftriaxone. In case of other two Cephalosporins Cefixime and Cephotaxime, 94.87 percent and 98.72 percent of typhi isolates were susceptible and 75 percent and 86.36 percent of paratyphi A isolates were susceptible. This means these drugs have shown decrease susceptibility towards the isolates. In case of multidrug resistant isolates, all the tested Cephalosporins were susceptible.

Since the emergence of first line of drug against Quinolones became the alternative drugs of choice to treat the typhi and paratyphi infection. They are very effective drug and reduce the duration of treatment as shown in various randomized control trials[20]. The Fluoroquinolone particularly Ciprofloxacin was most frequently used antibiotic. In this study, no Ciprofloxacin resistant typhi was isolated which is supported by Shanahan et al. 21 Ackers et al. 9 but their susceptibility rate was found to be 93.59 percent 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 79.54 percent. This is due to over use of Ciprofloxacin. Another Fluoroquinolone, Ofloxacin also shows decreased susceptibility as 94.87 percent in typhi and 81.82 percent in paratyphi A. Apart from this, 3.85 percent and 9.1 percent of the typhi and paratyphi A isolates were resistant to Ofloxacin 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. Hence, Nalidixic acid resistance is a marker for predicting low-level resistance to Ciprofloxacin among *S. typhi* and it is suggested that all *S. typhi* isolates should be screened for Nalidixic acid resistance test along with Ciprofloxacin[10]. In this study, 62.5 percent (6/16) typhi isolates were resistant to Nalidixic acid. These strains are called as Nalidixic acid resistant *Salmonella typhi*. In most strains, the acquired Fluoroquinolone resistance was attributed to

mutations in the quinoloneresistance–determining region genes encoding DNA *gyrase* (*gyr*A, *gyr*B) or DNA *topoisomerase* IV (*par*C, *par*E)[22] but the principle one is *gyr* A in which at position 83 of the DNA gyrase enzyme, the amino acid serine is changed in to another amino acid Phenylalanine and position 87 aspartate change to glycine[23]. In case of *Salmonella paratyphi* A also, these resistant are caused by change in genetic sequence of ORDR region especially *gyr* A and *par* C[22]. In present study all the tested isolates were resistant to Nalidixic acid. The effective treatment for these Nalidixic acid resistant as well as multidrug resistant bacteria was either Azithromycin or Ceftriaxone[17, 24]. Here only one strain of *Escherichia coli* was isolated and it was susceptible to all the tested antibiotic.

These antibiotic resistance problems are in rapidly growing up that threatens our ability to treat common infections. Bacteria become more and more resistant to newly used drug like third and fourth generation Cephalosporins as well as Fluoroquinolone. These critical points ask, "Are we on the verge of a medical disaster?" As there is rapidly antibiotic resistant problem, we recommended the conscientiously 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 frequently isolated bacteria from blood stream infection. Upon antibiotic susceptibility test, Chloramphenicol still remain the major drug of choice and in case of *Salmonella typhi* Ceftriaxone was the drug of choice having susceptibility rate of 100 percent; for *Salmonella paratyphi* A the drug of choices were Amoxicillin, Chloramphenicol and Cotrimoxazole having susceptibility rate of 100 percent. Although Ciprofloxacin and Ceftriaxone resistant bacteria were not isolated in our study, resistance may be found in others' study. Hence, continuous monitoring of antimicrobial resistance among blood isolated should be monitored.

Acknowledgement

We would like to thank the Directors of the Kathmandu Model Hospital, Kathmandu and the staff of the microbiology laboratory Kathmandu Model Hospital, for their support in this study.

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