ORIGINAL ARTICLE

Bacterial Spectrum and Antimicrobial Profile of Pediatric Blood Stream Infection at a Tertiary Care Hospital in Pakistan

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Objectives Blood stream infections (BSIs) are the main cause of morbidity and mortality in children worldwide. The present study was carried out to determine the prevalence of BSI with a focus on the identification of the causative agent of BSI, and to further evaluate the antibiotic susceptibility profile of the causing bacterial pathogens.

Methods A cross-section study was carried out at the tertiary care hospital in Peshawar, Pakistan from January to December, 2018. Blood samples were collected in BACTEC™ bottles and standard microbiological protocols were applied for the isolation and identification of bacterial strains. The antibiotic susceptibility tests were performed using disc diffusion method as per the 2014 guideline of Clinical Laboratory Standard Institute (CLSI).

Results Of 567 blood cultures in total, 111(19.6%) were positive for BSI. Male children were 64 % (71/111) and female children were 36% (40/111). For the causative predominant group of microorganisms, Gram-negative bacteria were identified in 79 (71.1%) isolates, and Gram-positive bacteria in 32 (28.9%) isolates. The common bacteria of isolates were *S. typhi* (n=35, 31.5%), *E. coli* (n=19, 17.1%), *S. aureus* (n=18, 16.2%), *K.*

pneumonia ($n=12$, 10.8%), and *Enterococcus* species ($n=7$, 6.3%). The 36.7% (29/79) isolates of Gram-negative bacteria were ESBL producers, and 61.1% (11/18) of *S. aureus* isolates were methicillin resistant. Overall, 72.9% isolates were multidrug resistant.

Conclusions Gram-negative bacteria were the main cause of pediatric BSIs, where the predominant microorganism was *S. typhi*. Remarkably, majority of the *S. typhi* isolates were resistant to ciprofloxacin.

INTRODUCTION

Blood stream infection (BSI) in children is reported to be the major cause of morbidity and mortality worldwide.^[1] The BSI refer to the presence of pathogenic bacteria in blood stream, and the onset of BSI is confirmed by the bacterial culture in combination with signs and symptoms of infection.^[2] Inadequate treatment of BSI may lead to sepsis; a dysregulated host response against infection may lead to life-threatening organ dysfunctions. BSIs are only associated with 25%-30% of sepsis cases.^[2] Furthermore, it has been reported that sepsis is responsible for approximately 60%-80% of the estimated 10 million childhood deaths every year.^[3] The clinical symptoms of both BSI and sepsis vary and are non-specific; however, they share commonalities, like chills, fever, and malaise. The high risk factor for developing BSI in children is indwelling vascular catheter, which is used for parenteral nutrition or chemotherapy.^[4]

BSIs are often caused by gram-negative bacteria, *Staphylococci* and group B *Streptococci*.^[3] For effective treatment and elimination of bacterial pathogens, antibiotics are commonly used.^[5] Resistance against antimicrobials has increased worldwide alarmingly.^[3, 6] It has been reported that in infants, almost half of the pathogens causing complicated BSIs are resistant to the first and second line antibiotics.^[7] Of particular concern is the emergence of multidrug resistant (MDR) bacterial strains. Globally, it was estimated that 31% of the 690,000 neonatal sepsis deaths were due to MDR pathogens each year.^[8] The increase of MDR bacteria is a growing threat and is considered as a high risk of infection-associated deaths in children.^[9]

Currently, the World Health Organization (WHO) has acknowledged the increased prevalence of antimicrobial resistance (AMR) in low and middle income countries (LMICs) as a widespread and endemic problem,^[10] which has posed a considerable challenge to the global health and infectious disease control, and become a potential disastrous problem. In developing

countries, the situation is far worse due to poor sanitation, over-the-counter sale of antibiotics, and lack of legislation.

The knowledge and understanding of local and regional AMR profile of the bacterial pathogens involved in BSI is crucial for empirical therapy and treatment guidelines. The variation in antibiotic susceptibility patterns of bacterial pathogens causing BSI has been observed locally, geographically and among hospitals. In this study, we investigated the proportion of BSI in children, the bacteria involved and their antimicrobial resistant profiles.

MATERIALS AND METHODS

Study design and clinical setting

This cross-section study was conducted at the department of microbiology, Khyber Teaching Hospital (KTH) in Peshawar, Pakistan. KTH is one of the largest tertiary care hospital of the country with the capacity of 1200 beds. The hospital has facilities such as emergency, intensive care unit, specialized pediatric, general pediatric, trauma, etc., serving a large population in northwest of Pakistan and Northeastern Afghanistan. The study enrolled all blood stream infected children admitted to the pediatric ward of KTH from January to December 2018 and suspicious for invasive infection. The criteria for performing blood culture^[11] were: 1) patient's body temperature was higher than 38°C or lower than 36°C; 2) the peripheral blood WBC $>10 \times 10^9/L$, or $<4 \times 10^9/L$; 3) respiratory rate exceeded 20 breaths /min; 4) other signs including coma, chills, hypotension, and skin or mucosal bleeding; 5) increased level of inflammatory proteins, e.g., procalcitonin, C reactive protein. All children with BSI who were subjected to applying antibiotics were excluded from the study. Although absorbent resin is used for neutralization of antibiotics in blood culture, some studies have reported that the resins present in blood culture bottle do not properly neutralize antimicrobial preparation of imipenem-cilastatin.^[12]

cline 30 µg; erythromycin 15 µg; linezolid 15 µg; penicillin 10 µg; rifampicin 30 µg; teicoplanin 30 µg; vancomycin 30 µg; oxacillin 5µg. The measurement and interpretation of the inhibition zone was carried out in accordance with CLSI guidelines, and the isolates that were interpreted as non-susceptible to specific antibiotic was identified as resistant to that antibiotic.^[14] Species of bacteria showing resistance to at least one antimicrobial drug in three or more antimicrobial categories were considered as MDR.

Phenotypic detection of ESBLs & MRSA

The phenotypic detection of Extended Spectrum Beta-lactamases (ESBLs) was performed by double disc diffusion method as recommended in the guideline.^[14] The discs used were amoxicillin-clavulanic acid, cefotaxime, ceftazidime (3rd generation), and meropenem (aztreonam). Furthermore, the methicillin resistant *S. aureus* (MRSA) strains were phenotypically detected using antibiotic discs of ceftazidime and oxacillin as per the guideline.

Characteristics of study participants

A total of 567 blood samples were processed during the study period. Of the total samples, 19.6% ($n=111$) showed bacterial growth, while the rest 80.4% ($n=456$) showed no growth. Of the positive blood cultures, 64% (71/111) were from male children, 36% (40/111) were from female. Overall, age distribution of positive blood culture were as follow: 1-11 months, 35.1% ($n=39$); 1-5 years, 33.3% ($n=37$); 6-10 years, 18.1% ($n=20$); 11-15 years, 5.4% ($n=6$); 16-18 years, 8.1% ($n=9$).

Bacterial spectrum among pediatric BSIs

Of all the 111 isolated bacteria, 79 (71.1%) were Gram-negative, whereas the remaining 32 (28.9%) were Gram-positive. The most predominant types among the isolated bacteria were *Salmonella typhi* (*S. typhi*) (31.5%, $n=35$), followed by *E. coli* (17.1%, $n=19$), and *S. aureus* (16.2%, $n=18$). The overall bacterial spectrum in pediatric BSIs is shown in **Table 1**.

Antimicrobial susceptibility

In order to ascertain the antibiotic susceptibility patterns of the isolated bacteria, we performed tests according to the protocol mentioned earlier. Remarkably,

Table 1. Prevalence of bacterial isolates of blood stream infections (BSI) in children ($n=111$)

Bacterial isolates	<i>n</i>	%
Gram-positive isolates	32	28.8
Methicillin resistant <i>Staphylococcus aureus</i> (MRSA)	11	9.9
<i>Staphylococcus aureus</i>	7	6.3
<i>Enterococcus</i> spp	7	6.3
<i>Streptococcus</i> spp	6	5.4
Coagulase negative <i>Staphylococcus</i> spp (CONS)	1	0.9
Gram-negative isolates	79	71.2
<i>Salmonella typhi</i>	35	31.5
<i>Escherichia coli</i>	19	17.1
<i>Klebsiella pneumonia</i>	12	10.8
<i>Pseudomonas aeruginosa</i>	6	5.5
<i>Salmonella paratyphi</i>	4	3.6
<i>Enterobacter aeruginosa</i>	2	1.8
<i>Citrobacter</i> spp	1	0.9

the predominant isolated strain *S. typhi* showed high level of resistance against ciprofloxacin (77.1%, $n=27$), followed by ampicillin, aztreonam, chloramphenicol, co-trimoxazole (31.4%, each $n=11$), azithromycin (28.6%, $n=10$), piperacillin-tazobactam (22.9%, $n=8$), cefotaxime, and ceftazidime (17%, each $n=6$). However, a very low level of resistance was observed against imipenem (2.9%, $n=1$). We also found that of the isolated, all 35 strains of *S. typhi* were sensitive to ceftriaxone. The amikacin showed highest susceptibility against *E. coli*, *P. aeruginosa*, and *Enterobacter*. Majority of the Gram-negative bacteria were resistant to ampicillin, ciprofloxacin, trimethoprim-sulphamethoxazole, ceftazidime, and cefepime, ranging from 71.1% to 100% (Table 2).

Prevalence of MRSA

Among all 32 Gram positive isolates, 34.4% ($n=11$) were MRSA, which showed high resistance against ceftazidime (100%, $n=11$), cefepime (100%, $n=11$), followed by ampicillin (90.9%, $n=10$), penicillin (90.9%, $n=10$), amoxicillin-clavulanic acid (81.8%, $n=9$), ciprofloxacin (81.8%, $n=9$), erythromycin (81.8%, $n=9$), and levofloxacin (72.4%, $n=8$). Overall, MRSA showed low level of resistance against chloramphenicol, rifampicin and clindamycin (18.2%-27.3%). All the Gram-positive isolates were susceptible to both vancomycin and linezolid, with no resistance observed. Details are shown in Table 3.

Prevalence of ESBL

Our data showed 36.7% (29/79) of Gram negative isolates were ESBL producers. Interestingly, within species, high percentage of ESBL were found among *K. pneumonia* (12/12, 100%), *Enterobacter* (2/2, 100%), followed by *E. coli* (12/19, 63%), and *P. aeruginosa* (3/6, 50%). No ESBLs were detected in isolates of *S. typhi*, *S. paratyphi*, and *Citrobacter* species.

Multi-drug resistance

The bacterial isolates from children with BSI were analyzed for multidrug resistance. Our data revealed that in total, 72.9% (81/111) isolates were MDR (Table 4). Within species, MDR were observed in 66.7% ($n=4$) of *Enterococcus* species, 33.3% ($n=2$) of *Streptococcus* species, 21.1% ($n=4$) of *E. coli*, 16.7% ($n=2$) of *K. pneumonia*, 16.6% ($n=1$) of *P. aeruginosa*, 15.8% ($n=3$) of *S. aureus*, and 8.6% ($n=3$) of *S. typhi*. No MDRs were detected in *S. paratyphi* and *Citrobacter* species.

DISCUSSION

The current study put emphasis on the prevalence of BSI, the causative bacterial pathogens and the antibiotic susceptibility profile of bacterial pathogens involved in BSI. A total of 567 blood cultures were processed during the study period. Of the total blood culture samples, 19.6% ($n=111$) showed bacterial growth, which was in accordance with the reports from Iran, whereas relatively low frequency of positive cases were reported in other studies.^[15-18] The low pathogens yield in other studies were possibly due to the low quantity of blood inoculation in culture media, variation in bacteriological techniques, and prior use of antimicrobials for the management of BSI.^[19-21]

The present study found that a wide range of bacterial pathogens were involved in BSI. The most frequently isolated micro-organisms were Gram-negative bacteria, including *Salmonella* spp, *E. coli*, *K. pneumonia*, followed by Gram-positive bacteria, which was in accordance with the previous report from Pakistan.^[15] Similarly, studies from India and South Africa reported Gram-negative bacteria as the predominant microbes involved in BSI and septicemia.^[21,22] Studies from other sites reported that the Gram-positive bacteria were the predominant pathogens causing BSI in children.^[3, 23-25] *E. coli* and *Klebsiella pneumonia* are the normal flora in gastrointestinal tract, but emerge as the predominant causing agents of community and hospital acquired

Table 2. Antibiotic resistance patterns of Gram-negative bacterial isolates from children with blood stream infections in Pakistan [*n*(%)]

Bacteria	Ampli- cillin	Aztreo- nam	Azithro- mycin	Amika- cin	Chloram- phenicol	Ciproflo- xacin	Co-Trim- oxazole	Ceftria- xone	Cefota- xime	Ceftazi- dime	Imipe- nam	Cefepime	Colistin/ Polymyxin B	Tazocin	Gentami- cin
<i>S. typhi</i> (<i>n</i> =35)	11 (31.4)	11 (31.4)	10 (28.6)	NT	11 (31.4)	27 (77.1)	11 (31.4)	0 (0)	6 (17.1)	NT	1 (2.9)	6 (17.1)	NT	8 (22.9)	NT
<i>S. paratyphi</i> (<i>n</i> =4)	0 (0)	1 (25)	0 (0)	NT	0 (0)	2 (50)	0 (0)	0 (0)	NT	NT	NT	3 (25)	NT	3 (75)	NT
<i>P. aeruginosa</i> (<i>n</i> =6)	NT	2 (33.3)	NT	0	NT	1 (16.7)	NT	2 (33.3)	2 (33.3)	2 (33.3)	5 (83.3)	2 (33.3)	NT	0 (0)	2 (33.3)
<i>K. pneumoniae</i> (<i>n</i> =12)	12 (100)	12 (100)	NT	9 (75)	NT	8 (66.7)	7 (58.3)	12 (100)	12 (100)	11 (92.7)	7 (58.3)	12 (100)	1 (8.3)	3 (25)	7 (58.3)
<i>Enterobacter</i> spp (<i>n</i> =2)	2 (100)	2 (100)	NT	0	NT	1 (50)	2 (100)	2 (100)	1 (50)	1 (50)	0 (0)	2 (100)	0 (0)	1 (50)	2 (100)
<i>Citrobacter</i> spp (<i>n</i> =1)	NT	1 (100)	NT	0	NT	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<i>E. coli</i> (<i>n</i> =19)	NT	15 (78.9)	NT	0	11 (13.9)	5 (73.3)	13 (68.4)	13 (68.4)	NT	13 (68.4)	1 (5.3)	NT	NT	3 (15.8)	3 (15.8)
Total (<i>n</i> =79)	25 (31.6)	44 (55.6)	10 (12.6)	9 (11.3)	22 (27.8)	44 (55.6)	34 (43.0)	30 (37.9)	21 (26.5)	27 (34.1)	14 (17.7)	25 (31.6)	1 (1.3)	18 (22.7)	14 (17.7)

NT, not tested; spp, species.

Table 3. Antibiotic resistance patterns of Gram-positive bacterial isolates from children with blood stream infections in Pakistan [*n*(%)]

Bacteria*	Amikacin	Ampicillin/ clavulanic acid	Ampicillin	Cefepime	Cefotaxime	Ceftriaxone	Cefoxitin	Chloram- phenicol	Ciproflo- xacin	Clarith- romycin	Clinda- mycin	Co-Tria- moxazole
<i>Staphylococcus aureus</i>												
MRSA (<i>n</i> =11)	6 (54.5)	9 (81.8)	10 (90.9)	11 (100)	NT	NT	11 (100)	2 (18.2)	9 (81.8)	5 (45.6)	4 (36.4)	5 (45.6)
MSSA (<i>n</i> =7)	0 (0)	6 (85.7)	6 (85.7)	NT	NT	NT	0 (0)	1 (14.3)	5 (71.4)	NT	0 (0)	4 (57.1)
<i>Streptococcus</i> spp (<i>n</i> =6)	NT	NT	5 (83.3)	6 (100)	3 (50)	3 (50)	NT	0 (0)	0 (0)	4 (66.7)	3 (50)	NT
<i>Enterococcus</i> spp (<i>n</i> =7)	NT	NT	7 (100)	NT	NT	NT	NT	5 (71.4)	6 (85.7)	5 (71.4)	NT	NT
Total (<i>n</i> =31)	6 (19.3)	15 (48.4)	28 (90.3)	6 (100)	3 (9.6)	3 (9.6)	11 (35.5)	8 (25.8)	20 (64.5)	14 (45.2)	7 (22.6)	9 (29.0)
<i>Bacteria*</i>	Doxycycline	Erythromycin	Gentamicin	Levofloxacin	Linezolid	Meropenem	Minocycline	Penicillin	Rifampicin	Teicoplanin	Vancomycin	
<i>Staphylococcus aureus</i>												
MRSA (<i>n</i> =11)	5 (45.6)	9 (81.8)	7 (63.6)	8 (72.2)	0 (0)	NT	6 (54.5)	10 (90.9)	2 (18.2)	NT	0 (0)	0 (0)
MSSA (<i>n</i> =7)	0 (0)	3 (42.9)	0 (0)	NT	0 (0)	NT	0 (0)	7 (100)	0 (0)	NT	0 (0)	0 (0)
<i>Streptococcus</i> spp (<i>n</i> =6)	4 (66.7)	4 (66.7)	2 (33.3)	4 (66.7)	0 (0)	6 (100)	0 (0)	3 (50)	3 (50)	0 (0)	0 (0)	0 (0)
<i>Enterococcus</i> spp (<i>n</i> =7)	NT	6 (85.7)	4 (57.1)	NT	0 (0)	NT	1 (14.3)	3 (42.9)	7 (100)	1 (14.3)	0 (0)	0 (0)
Total (<i>n</i> =31)	9 (29.0)	22 (70.9)	13 (41.9)	12 (38.7)	0 (0)	6 (100)	7 (22.6)	23 (74.2)	12 (37.7)	1 (3.2)	0 (0)	0 (0)

MRSA, methicillin resistant *S. aureus*; MSSA, methicillin sensitive *S. aureus*.

*The contaminant organism, coagulase negative staphylococcus (CONS), was excluded from the data.

Table 4. Multidrug resistant pattern of bacterial isolates from children with blood stream infections (BSI) in Pakistan [$n=111$, $n(\%)$]

Bacterial	Isolates	Number of antibiotics being resistant to				
		1	2	3	4	>4
<i>S. typhi</i>	35 (31.5)	18 (51.4)	13 (37.1)	3 (8.6)	1 (2.9)	0 (0)
<i>E. coli</i>	19 (17.1)	4 (21.1)	7 (36.8)	4 (21.1)	4 (21.1)	0 (0)
<i>S. aureus</i>	19 (17.1)	2 (10.5)	1 (5.3)	3 (15.8)	4 (21.1)	9 (47.4)
<i>K. pneumonia</i>	12 (10.8)	0 (0)	2 (16.6)	2 (16.7)	0 (0)	8 (66.7)
<i>P. aeruginosa</i>	6 (5.4)	2 (33.3)	2 (33.3)	1 (16.6)	1 (16.6)	0 (0)
<i>Enterococcus</i> spp.	7 (6.3)	1 (16.6)	2 (33.3)	0 (0)	0 (0)	4 (66.7)
<i>Streptococcus</i> spp.	6 (5.4)	0 (0)	0 (0)	2 (33.3)	3 (50.0)	1 (16.6)
<i>S. paratyphi</i>	4 (3.6)	2 (50.0)	2 (50.0)	0 (0)	0 (0)	0 (0)
<i>Enterobacter</i> spp.	2 (1.8)	0 (0)	0 (0)	1 (50.0)	0 (0)	1 (50.0)
<i>Citrobacter</i> spp.	1 (0.9)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)
Total	111 (100)	29 (26.1)	30 (27.0)	16 (14.4)	13 (11.7)	23 (20.7)

infections, i.e., septicemia, urinary tract infection, and pneumonia. The infections were more common in neonates than other populations.^[26] In the current study, 16.2% of the isolates were *S. aureus*, which resides on skin and is ubiquitous in the nature. It causes various type of infections in humans. During labor, a newborn baby can easily be contaminated by *S. aureus*.^[26] As a contaminant organism, coagulase negative *Staphylococci* (CONS) in our study showed a prevalence of 0.9%, which was very low as compared to international acceptable contamination rate (2-5%).^[27] Usually, contamination occurs due to lack of proper aseptic measures when obtaining blood specimen.

In our study, the predominant organisms were *S. typhi* and *S. paratyphi*, and <32% resistance has been observed to all the tested antibiotics except ciprofloxacin (77%), which is in accordance with the report from India.^[18] We found that *E. coli* was highly resistant to ciprofloxacin, aztreonam, co-triamoxazole, ceftriaxone and ceftazidime. There are multiple risk factors involved in the resistance of *E. coli* against antibiotics, such as recent administration of antibiotics, prophylactic therapy for urinary tract infection, and hospitalization.^[28] Also, *E. coli* may acquire the resistance gene encoded on plasmid and chromosome through horizontal gene transfer, which can play significant role in its resistance.^[26] *E. coli* is usually involved in sepsis and urosepsis in neonate. According to the WHO guidelines, combination of gentamicin and ampicillin are suggested for the empirical treatment for urosepsis.^[29] We found that 84.2% of the *E. coli* isolates were sensitive to gentamicin, which was the highest among all isolates of pathogens.

Gentamicin continues to be administered for empirical treatment in this particular context. However, in some settings, third generation cephalosporin are used for empirical treatment as a mono therapy.^[30]

Remarkably, the highest proportion of the *K. pneumonia* (100%) and *E. coli* (63.2%) strains isolated from BSI were ESBL producers. Previous studies reported the prevalence of ESBL *K. pneumonia* was 78.3%, whereas low prevalences have been reported for ESBL *E. coli* (30% and 11.2%).^[1,21] In this study, other Gram-negative bacteria such as *P. aeruginosa* and *Enterobacter* spp showed the same patterns of resistance as in previous studies.^[16,18]

The current study also showed that MRSA strains had high susceptibility to vancomycin, linezolid, chloramphenicol, and rifampicin, whereas they showed highest resistance against augmentin, ampicillin, cefepime, and ceftioxin. This result was consistent with the previous reports.^[16,28] In our study, the frequency of MRSA was 9.9%, which was also similar with the study from Australia.^[3] However, higher percentages of MRSA were reported in the studies from Pakistan and India.^[15, 18] Majority of the Gram-positive were resistant to ampicillin, augmentin and penicillin. Regarding MDR, both *Enterococcus* and *S. aureus* can acquire MDR genes through horizontal gene transfer from other strains. Gram-negative bacteria such as *E. coli* and *K. pneumonia* acquire drug resistance mainly due to beta-lactam enzymes encoded on plasmid and chromosome.^[26]

In conclusion, we identified *S. typhi*, *E. coli*, *S. aureus*, *K. pneumonia* and *Enterococcus* species as the important pathogens of BSIs in children of Pakistan.

The major proportion of the isolates were Gram-negative, which reflects the trend around the region (**Table S1**). Majority of the bacterial isolates were resistant to commonly used antibiotics. It is believed that *in vitro* antibiotic susceptibility can help in predication of the *in vivo* effectiveness. However, selection criteria for appropriate empirical antimicrobial agents must consider various factors, such as local resistance patterns, costs, pathogen involved, outcome related to cure of the infection, and the adverse effects. Furthermore, for effective empirical therapy, periodic surveillance of antibiotic resistance at local, regional and national level will be helpful for timely management of infectious diseases.

Conflict of interests

The authors declared no competing interests.

Supplementary materials

Table S1: Literature analyses on BSIs prevalence, bacterial spectrum and the antibiotic resistance profiles of the bacterial pathogens (with references).

Available online at <http://cmsj.cams.cn/EN/10.24920/003675>.

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