ORIGINAL ARTICLE

Emerging Trends of Bloodstream Infections: A Six-Year Study at a Paediatric Tertiary Care Hospital in Kabul

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Abstract

Objective: To determine the frequency of pathogens causing bloodstream infections and evaluate their trends and antibiogram patterns among in-patients in a paediatric tertiary care centre.

Study Design: Descriptive study.

Place and Duration of Study: French Medical Institute for Mothers and Children (FMIC), Kabul, Afghanistan in two phases, from January 2010 to December 2015.

Methodology: Results of blood cultures from suspected cases of sepsis admitted in the FMIC, from January 2010 to December 2012 (Period-1), and from January 2013 to December 2015 (Period-2) were completed. Standard microbiological methods were followed for blood culture and antibiotic sensitivity testing.

Results: Out of total 1,040 cases of culture proven sepsis, 528 (50.77%) Gram-negative bacilli (GNB), 474 (45.58%) Gram-positive cocci (GPC), and 38 (3.65%) *Candida* species were isolated during the entire study period. Out of 528 GNB isolates, 373 (70.64%) belonged to the Enterobacteriaceae and 155 (29.36%) were non-fermenters. Among Enterobacteriaceae, 168 (31.82%) were *Klebsiella* species (*K. pneumoniae*=124, *K. oxytoca*=44), 70 (13.26%) were Enterobacter species (*E. cloacae*=52, *E. aerogenes*=18), 65 (12.31%) were *E. coli*, 37 (7.01%) were *Serratia marcescens* and 31 (5.87%) were others. Out of 155 non-fermenters, 88 (16.67%) were *Pseudomonas aeruginosa*, 39 (7.39%) were *Burkholderia cepacia* and 18 (3.41%) were *Stenotrophomonas maltophilia*. There was a drop in the frequency of Enterobacteriaceae from 85% in Period-1 to 58.68% in Period-2. There was an increase in the frequency of non-fermenters from 15% to 41.32%, particularly 18 new cases of sepsis caused by *Stenotrophomonas maltophilia* during Period-2. Among GPC, there was an overall rise of 16.14% in the prevalence of *Staphylococcus epidermidis* during Period-2 and a drop of 9.64% in the frequency of *Staphylococcus aureus* during Period-2. The majority of Gram-negative isolates were multidrug-resistant to commonly used antibiotics. However, most of the isolates were sensitive to amikacin and imipenem (except *S. maltophilia*). The frequency of those producing ESBL reduced by 11.22% during the Period-2. Among Gram-positive cocci, the pattern of antibiogram did not show a significant change during both periods, and majority remained resistant to commonly used antibiotics. All *Staphylococci* were sensitive to vancomycin but resistant to penicillin. There was a substantial decline of 18.87% in the frequency of *Methicillin*-resistant *Staphylococci* (MRSA/MRSE) during Period-2.

Conclusion: *Staphylococci* and *Klebsiellae* remain the most important bacteria responsible for bloodstream infections in a tertiary healthcare facility in Kabul. Yet, there has been an increase in the prevalence of *Pseudomonas* and *Burkholderia cepacia*. Moreover, *Stenotrophomonas maltophilia* emerged as a new hospital acquired pathogen. This study could possibly help in suggesting choices eluding the misuse of appropriate antibiotics.

Key Words: *Antibiogram. Blood culture. Bloodstream infections. Paediatric hospital acquired infections. Kabul.*

INTRODUCTION

Nearly 30 years of armed conflict in Afghanistan has adversely affected the healthcare delivery system, especially for the children. Diagnostic capabilities are limited making diagnosis dependent on clinical skills and guesswork, which is also inadequate because the practice of rational standard of paediatric medicine is also perplexing.

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The changing bacteriologic profile of septicemia in children warrants the need for an ongoing review of the causative pathogens and their drug susceptibility pattern.[1,2] Though blood culture is not always positive in all cases of septicemia, yet it remains a gold standard for the diagnosis of infection. Infections with Gram-negative bacteria create a more serious therapeutic problem, especially in ICU patients, because of a high proportion of multidrug-resistant bacterial strains.[3] Hence, an updated knowledge of pathogens causing sepsis in neonates and infants, especially in developing countries, is vital to plan management strategies.[4] Early diagnosis and proper management of neonatal septicemia could drastically cut down the morbidity and mortality in this age group.[5,6] The infection caused by multidrug-resistant organism is more likely to lengthen the hospital stay, increase the risk of death, and need treatment with more costly antibiotics. In most of the cases,

antimicrobial therapy is started empirically before the results of blood culture are available and therefor, correct choice of empiric therapy is of much significance. There is a lack of equipped microbiology laboratories in Afghanistan and no organized surveillance system exists to determine antimicrobial resistance patterns for common bacteria. Only a few studies have been done in this field in the country and published data on antibiotic resistance is lacking.[7]

Thus, the present study was undertaken to determine the organisms causing septicemia and their antibiogram in paediatric age group in this region of the world.

METHODOLOGY

This study was the second phase of a 6-year comprehensive retrospective study conducted in two 3-year phases, in which blood culture samples were processed and analysed for microbiological characteristics. All positive blood cultures from in-patients, aged neonates to 18 years, clinically suspected to be having septicemia, were studied from January 2010 to December 2015, in the Microbiology Laboratory of French Medical Institute for Mothers and Children (FMIC), Kabul. To evaluate the trend of local microbial prevalence and their antibiotic sensitivity pattern, the results of two periods were compared: Period-1, from January 2010 to December 2012, and Period-2, from January 2013 to December 2015.

The standard microbiological methods were followed for blood culture and antibiotic sensitivity testing.[8] Blood samples were collected aseptically in culture bottles and incubated in BACTEC™ 9240 Blood Culture System from BD (Becton Dickinson, USA). Bacteria were identified to the species level using various API (Analytical Profile Index) identification strips (bioMérieux, France). The antimicrobial susceptibility of bacterial isolates was done against locally available antibiotics by the disc diffusion method according to the CLSI guidelines.[9] Drug resistant strains of Enterobacteriaceae were studied for extended spectrum beta lactamases (ESBL) by combination disk method. Cefoxitin disk diffusion method was used for testing methicillin-resistant *Staphylococci*. ATTC (American Type Culture Collection) strains were used for quality control including *E. coli* 25922, *Pseudomonas aeruginosa* 27853, *Staphylococcus aureus* 29213 and *Enterococcus faecalis* 29212.

Data were analysed using descriptive statistics including frequency, proportion, content analysis, and interpretation. Institutional Ethics Committee approved the study.

RESULTS

During the 6-year study period, 8,336 samples for blood cultures were received from intensive care unit (ICU) and in-patient wards. Out of all samples, 1,040 (12.48%) yielded monomicrobial growth. Out of those, 470 (45.19%) were isolated from 4,032 blood samples received during Period-1 and 570 (54.81%) were isolated from 4,304 blood samples during Period-2. Among patients with sepsis, 725 (69.71%) were males and 315 (30.29%) were females; hence, male to female patient ratio was 2.3:1. The frequency of infection was the highest i.e., 728 (70%) in neonates and infants.

Out of total 1,040 cases of culture proven sepsis, 528 (50.77%) were Gram-negative bacilli (GNB), 474 (45.58%) were Gram-positive cocci (GPC) and 38 (3.65%) were *Candida* species. Within 528 GNB isolates, 373 (70.64%) belonged to the family Enterobacteriaceae and 155 (29.36%) were non-fermenters. Among Enterobacteriaceae, 168 (31.82%) were *Klebsiella* (*K. pneumoniae*=124, *K. oxytoca*=44), 70 (13.26%) were *Enterobacter* (*E. cloacae*=52, *E. aerogenes*=18), 65 (12.31%) were *E. coli*, 37 (7.01%) were *Serratia marcescens* and 31 (5.87%) were other members of Enterobacteriaceae. Out of 155 non-fermenters, 88 (16.67%) were *Pseudomonas aeruginosa*, 39 (7.39%) were *Burkholderia cepacia*, 18 (3.41%) were *Stenotrophomonas maltophilia* and 10 (1.89%) were *Acinetobacter baumannii*. Among GNB, an overall decline of 26.32% in the prevalence of Enterobacteriaceae was observed from 85% (in Period-1) to 58.68% (in Period-2). However, there was a reciprocal increase in the prevalence of non-fermenters from 15% to 41.32% between Periods 1 and 2, respectively, including an increase in the frequency of *Pseudomonas aeruginosa* and *Burkholderia cepacia,* and particularly 18 new cases of sepsis caused by *S. maltophilia* during Period-2 (Table I). Among GPC, there was a rise of 16.14% in the prevalence of *Staphylococcus epidermidis* during Period-2. Conversely, decrease of 9.64% and 6.12% were observed in the frequencies of *S. aureus* and *Streptococci,* respectively during Period-2 (Figure 1). Besides bacterial isolates, a total of 38 (3.65%) *Candida* species (including 27 *Candida albicans* and 11 non-*albicans*) were isolated during both the periods. The occurrence of *C. albicans* was much higher (71%) than

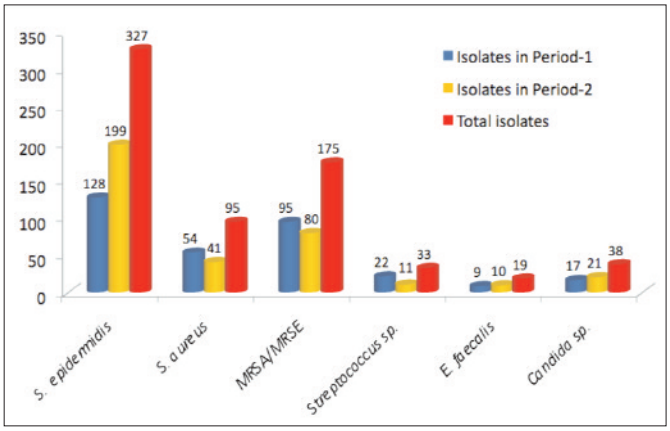

Figure 1: Types and frequency of GPC and *Candida* isolated during the two study periods

C. non-*albicans*. Majority (91.4%) of candidemia cases were observed in neonates and infants.

Table II shows the overall antibiogram pattern of Gram-negative isolates. The majority of GNB showed high percentage of resistance to the commonly used antimicrobials including ampicillin (78.18%), augmentin (66.14%), cefotaxime (67.05%), ceftazidime (50%), ciprofloxacin (30.11%), co-trimoxazole (67.27%), and gentamicin (47.35%). Least resistance for the GNB was observed against amikacin (14.39%), fosfomycin (21.21%) and imipenem (6.82%). Majority of Enterobacteriaceae i.e., 184 out of 373 (49.33%) were found to be ESBL producers (Table I). However, the frequency of ESBL producers reduced from 54.4% to 43.19% during the Period-2. Among GPC, the overall pattern of antibiogram was unremarkable during both the periods, except ciprofloxacin for which the resistance decreased by 19.1% during Period-2. The majority of GPC remained multidrug-resistant to commonly used antibiotics including ciprofloxacin (35.02%), co-trimoxazole (71.52%), erythromycin (61.18%), gentamicin (40.72%) and penicillin (94.51%). Majority of GPC were sensitive to fosfomycin (78.06%), fusidic acid (80.59%), pristinamycin (86.29%), rifampicin (80.38%) and vancomycin (99.79%). Only one strain of *Enterococcus faecalis* showed resistance to vancomycin during Period-1. All *Staphylococci* were sensitive to vancomycin but resistant to penicillin, whereas 41.47% were methicillin-resistant. However, there was a substantial decline of 18.87% in the frequency of MRSA/MRSE during Period-2 (Table III).

Table I: Types and frequency of GNB isolated during the two study periods .

Type and name of organism	No. (%) of isolates during Period-1	No. (%) of isolates during Period-2	Total (%) isolates in both periods
Gram-negative bacilli (GNB)	240 (23.08)	288 (27.69)	528 (50.77)
Klebsiella spp.	78 (32.50)	90 (31.25)	168 (31.82)
Enterobacter spp.	48 (20.00)	22 (7.64)	70 (13.26)
Escherichia coli	42 (17.50)	23 (7.99)	65 (12.31)
Serratia spp.	16 (6.67)	21 (7.29)	37 (7.01)
Other Enterobacteriaceae*	20 (8.33)	13 (4.51)	31 (5.87)
Total Enterobacteriaceae	204 (85.00)	169 (58.68)	373 (70.64)
ESBL producers	111 (54.41)	73 (43.19)	184 (49.33)
Pseudomonas aeruginosa	24 (10.00)	64 (22.22)	88 (16.67)
Burkholderia cepacia	8 (3.33)	31 (10.76)	39 (7.39)
Stenotrophomonas maltophilia	0 (0)	18 (6.25)	18 (3.41)
Acinetobacter baumannii	4 (1.67)	6 (2.08)	10 (1.89)
Total non-fermenters	36 (15.00)	119 (41.32)	155 (29.36)

* *Include Proteus* spp., *Citrobacter* spp., *Moraxella* spp., *Salmonella* spp.

DISCUSSION

Bloodstream infection (BSI) is a common life-threatening condition in hospitalised children, especially if associated with a multi-resistant microorganism; and carries a high morbidity and mortality worldwide.[10] Emerging antimicrobial resistance among bacterial pathogens causing

Table II: Antimicrobial susceptibility of Gram-negative bacilli during the two periods.

Antimicrobial agent	No. (%) of Isolates during Period-1		No. (%) of Isolates during Period-2		Total No. (%) of isolates in both periods	
	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive
Ampicillin	154/216 (71.30)	62/216 (28.70)	180/224 (80.36)	44/224 (19.64)	344/440 (78.18)	96/440 (21.82)
Augmentin	135/216 (62.50)	81/216 (37.50)	146/224 (65.18)	78/224 (34.82)	291/440 (66.14)	149/440 (33.86)
Amikacin	40/240 (16.67)	200/240 (83.33)	36/288 (12.50)	252/288 (87.50)	76/528 (14.39)	452/528 (85.61)
Cefotaxime	133/216 (61.57)	83/216 (38.43)	162/224 (72.32)	62/224 (27.68)	295/440 (67.05)	145/440 (32.95)
Ceftazidime	134/240 (55.83)	106/240 (44.17)	130/288 (45.14)	158/288 (54.86)	264/528 (50.00)	264/528 (50.00)
Ciprofloxacin	94/240 (39.17)	146/240 (60.83)	65/288 (22.57)	223/288 (77.43)	159/528 (30.11)	369/528 (69.89)
Co-trimoxazole	160/216 (74.07)	56/216 (25.93)	136/224 (60.71)	88/224 (39.29)	296/440 (67.27)	144/440 (32.73)
Fosfomycin	48/240 (20.00)	192/240 (80.00)	64/288 (22.22)	224/288 (77.78)	112/528 (21.21)	406/528 (76.89)
Gentamicin	120/240 (50.00)	120/240 (50.00)	130/288 (45.14)	158/288 (54.86)	250/528 (47.35)	278/528 (52.65)
Imipenem	12/240 (5.00)	228/240 (95)	24/288 (8.33)	264/288 (91.67)	36/528 (6.82)	492/528 (93.18)

Table III: Antimicrobial susceptibility of Gram-positive cocci during the two study periods.

Antimicrobial agent	No. (%) of Isolates during Period-1		No. (%) of Isolates during Period-2		Total No. (%) of isolates in both periods	
	Resistant	Sensitive	Resistant	Sensitive	Resistant	Sensitive
Cefoxitin (for detection of MRSA/MRSE)	95/182 (52.20)	87/182 (47.80)	80/240 (33.33)	160/240 (66.67)	175/422 (41.47)	247/422 (58.53)
Ciprofloxacin	97/213 (45.54)	116/213 (54.46)	69/261 (26.44)	192/261 (73.56)	166/474 (35.02)	308/474 (64.98)
Co-trimoxazole	158/213 (74.18)	55/213 (25.82)	181/261 (69.35)	80/261 (30.65)	339/474 (71.52)	135/474 (28.48)
Erythromycin	125/213 (58.69)	88/213 (41.31)	165/261 (63.22)	61/261 (36.78)	290/474 (61.18)	184/474 (38.82)
Fosfomycin	50/213 (23.47)	163/213 (76.53)	54/261 (20.69)	207/261 (79.31)	104/474 (21.94)	370/474 (78.06)
Fusidic acid	40/213 (18.78)	173/213 (81.22)	52/261 (19.92)	209/261 (80.08)	92/474 (19.41)	382/474 (80.59)
Gentamicin	95/213 (44.60)	118/213 (55.40)	98/261 (37.55)	163/261 (62.45)	193/474 (40.72)	281/474 (59.28)
Penicillin	199/213 (93.43)	14/213 (6.57)	249/261 (95.40)	12/261 (4.60)	448/474 (94.51)	26/474 (5.49)
Pristinamycin	27/213 (12.68)	186/213 (87.32)	38/261 (14.56)	223/261 (85.44)	65/474 (13.71)	409/474 (86.29)
Rifampicin	45/213 (21.13)	168/213 (78.87)	48/261 (18.39)	213/261 (81.61)	93/474 (19.62)	381/474 (80.38)
Vancomycin	1/213 (0.47)	212/213 (99.53)	0/261 (0)	261/261 (100)	1/474 (0.21)	472/474 (99.79)

BSI could limit therapeutic options and obfuscate patient management.[11] Rational and appropriate use of antibiotics requires understanding of prevalent pathogens and drug resistance patterns in a healthcare setting. The frequency of 12.48% of pathogens isolation from the blood culture of children in this study was in accordance with many previous studies done in other countries.[12,13] However, some studies done in nearby countries have reported higher isolation rate.[14,15] Many studies have shown that sepsis particularly affects premature neonates and infants. This study also confirmed this statistic because the frequency of infection was the highest in neonates and infants. Similarly, many studies have identified male gender as a factor associated with increased risk of central line-associated bloodstream infection. This study also revealed a considerable predominance of male patients (69.71%). The definite mechanisms by which gender could affect infection risk are uncertain, but may possibly be associated with unidentified structural or functional dissimilarities between male and female.[16]

The more frequently isolated bacteria from blood culture in this study were *S. epidermidis* (31.44%), followed by *Klebsiellae* (16.15%) and non-fermenting GNB (14.9%). The isolation rate of *Candida* species was 3.65%. These findings are consistent with those of the previous studies and suggest that infections by these agents constitute a significant threat to child survival in our setting.[17,18] Vibrant changes have been observed in the epidemiology of such organisms which have also carried an upsurge in the resistance to several antimicrobial agents, resulting in limitations for therapeutic choices. The incidence of bloodstream infections, especially by non-fermenting GNB, in paediatric patients has increased over the years due to several factors, such as increased use of central venous catheters, more use of parenteral nutrition, mucosal alteration or more aggressive surgery and instrumentation procedures, and the extensive use of broad-spectrum antibiotics. An important observation during this study was the increased occurrence of *Pseudomonas aeruginosa* and of *Burkholderia cepacia*. Several studies have shown that the main risk factors for infection or colonization with multidrug-resistant organisms are lengthy stay in the ICU, previous and extensive use of imipenem, and mechanical ventilation.[19] Transmission of *B. cepacia* from contaminated medicines and devices has been reported. It can also be spread to susceptible individuals by person-to-person contact, contact with contaminated surfaces and exposure to *B. cepacia* in the environment. Infections caused by *B. cepacia* should be taken into consideration because of their high mortality due to multidrug-resistance in ICU settings. Another key observation was the emergence of *S. maltophilia*, which is a worldwide emerging pathogen that causes serious complications, especially in immunocompromised patients.[20]

Candidemia is an important cause of morbidity and mortality in the healthcare setting. The risk factors for getting this infection include a long-term hospitalization in an ICU, previous bacterial infection, central venous catheter use, parenteral nutrition and immuno-suppression. In this study, *Candida* species were isolated in 38 patients and among those 71% were *C. albicans*. Although *C. albicans* remains the most frequent species of yeast isolated from bloodstream infections, yet some studies have described that 40% to 50% of the infections are caused by non-*albicans* species.[21] Therefore, species-level identification is necessary since invasive infections caused by non-*albicans* species are more difficult to treat due to their inherent or acquired resistance to antifungal agents.

For the management of sepsis in paediatric age group, empirical antibiotic therapy must be precise according to the specific environment and determined by the prevalent spectrum of etiological agents and their antibiogram pattern. In this study, the Gram-negative isolates demonstrated a high level of resistance to commonly used antimicrobials, such as ampicillin, third generation cephalosporins, co-trimoxazole and gentamicin. For GNB, a combination of a third generation cephalosporin with an aminoglycoside is usually considered appropriate. However, this study and other reports suggest that 50-70% of the Gram-negative organisms are resistant to these antibiotics and routine use of these agents might increase the risk of infections with ESBL producing organisms.[22,23] Gram-negatives that produced ESBLs were found to be multidrug-resistant and showed high resistance to commonly used antimicrobials. ESBL producing Enterobacteriaceae have spread quickly worldwide and became well recognised in many hospitals.[24,25] Thus, phenotypic screening of beta-lactamases should be performed and identified. In this study, a high percentage of strains were ESBL producers. However, the frequency of such strains decreased by 11.22% during Period-2 of this study. The reduction in the rate of such infections could be due to effective infection control measures. The encouraging findings were that majority of GNB were susceptible to imipenem and amikacin. Such antibiotics proved to be the most effective drugs for all the Gram-negative bacterial isolates, including non-fermenters.

Despite the higher frequency of antimicrobial resistance in Enterobacteriaceae, there were some similarities in patterns; amikacin had the best activity and most of the GNB were susceptible to imipenem except *S. maltophilia*. Trimethoprim-sulfamethoxazole, with a 78% resistance rate, is no more an option for the treatment of infections by GNB except *S. maltophilia* and *Burkholderia cepacia* for which it is one of the main therapeutic options. The percentage of susceptibility of Gram-negative blood-stream isolates to ciprofloxacin increased by 19.1% during Period-2 of the study. This was most likely due to the judicious use of this agent.

In this study, all strains of *Staphylococci* showed resistance to penicillin. Penicillin resistant *Staphylococci* are usually treated with cloxacillin or nafcillin, but the upsetting reality is the emergence of methicillin-resistance. In this study, the rate of methicillin-resistant strains (MRSA, MRSE) was 52.2% during Period-1, but the frequency reduced to 33.33% during Period-2. The reduction of 18.87% in the rate of such infections could credibly be the result of effective infection control measures. Though the majority of the Gram-positive isolates showed sensitivity to fosfomycin, fusidic acid, pristinamycin and rifampicin, but vancomycin remained the drug of choice for our physicians.

There were two limitations of this study. First, though the fungaemia was seen due to *Candida* species, antifungal susceptibility was not carried out during the first period of the study and, therefore, no comparison was done in that context. Secondly, carbapenemase detection was not done during first period of the study and, therefore, no evaluation was done in that regard.

CONCLUSION

Staphylococci and *Klebsiellae* remained the most important bacteria responsible for bloodstream infections in a tertiary healthcare facility in Kabul in the earlier half of this decade. There was an increase in the prevalence of *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Stenotrophomonas maltophilia* emerged as a new hospital acquired pathogen. Presently, the selection of antibiotics for treating sepsis by Gram-negative organism in paediatric age group infections in Kabul is amikacin and imipenem. For Gam-positive organisms, the options are fusidic acid, pristinamycin and rifampicin, besides vancomycin.

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