ORIGINAL ARTICLE

Bacteriologic Profile and Antibiogram of Blood Culture Isolates from a Children's Hospital in Kabul

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ABSTRACT

Objective: To identify the bacterial pathogens causing paediatric septicaemia in Kabul and to determine their antibiogram to improve empirical antibiotic therapy.

Study Design: Cross-sectional study.

Place and Duration of Study: Microbiology Laboratory of FMIC, Kabul, Afghanistan, from January 2010 to June 2012.

Methodology: Blood cultures from suspected cases of sepsis were processed in BD (Becton Dickinson, USA) for culture BACTEC™ 9240 Blood Culture System. Positive growths were examined and isolates were identified by conventional biochemical tests. Bacteria were identified to the species level using various Analytical Profile Index (API) identification strips. Antibiotic susceptibility testing was performed by modified Kirby-Bauer disk diffusion method. Drug resistant strains were studied for extended spectrum beta lactamase (ESBL) production by combination disk method and for methicillin resistant *Staphylococcus aureus* (MRSA) by Cefoxitin disk diffusion method.

Results: Out of a total 3360 blood cultures received from in-patients, 410 yielded monomicrobial growth; hence the frequency of positive blood culture was 12.2%. Out of a total 410 isolates, 212 (51.71%) were gram-negative bacilli and 184 (44.88%) were gram-positive cocci. In addition, 14 (3.41%) *Candida* species were also isolated. The frequently isolated species of gram-negative bacteria belonged to Enterobacteriaceae and included 66 *Klebsiella* (16.1%), 42 *Enterobacter* (10.2%), 35 *Escherichia (E.) coli* (8.5%) and 16 *Serratia* (3.9%) species. In addition, 21 (5.12%) *Pseudomonas* species were also isolated. Correspondingly, amongst gram-positive cocci, the most frequently isolated species were 108 coagulase-negative *Staphylococci* (26.34%) followed by 49 *Staphylococcus aureus* (11.95%) and 21 *Streptococcus* species (5.12%). Among gram-negative isolates, those that produced ESBL i.e., 110 out of 212 (51.9%) were found to be multidrug-resistant and showed high resistance to commonly used antibiotics namely Ampicillin, Gentamicin, 3rd generation Cephalosporins, Fluoroquinolones and Co-trimoxazole. Most of the isolates were susceptible to Imipenem (200/212, 94.3%), Amikacin (172/212, 81.1%) and Fosfomycin (166/212, 78.3%). Amongst gram-positive cocci, majority were resistant to Penicillin, Gentamicin, 3rd generation Cephalosporins, Fluoroquinolones and Co-trimoxazole. However, most were sensitive to *Vancomycin* (183/184) Pristinamycin (161/184, 87.5%) and Fosfomycin (134/184, 72.8%). All *Staphylococci* were resistant to Penicillin and 80/157 (51%) were MRSA.

Conclusion: *Klebsiella, E. coli, Enterobacter* and *Staphylococci* remain the principal organisms responsible for blood stream infection in a paediatrics tertiary care setting in Kabul. The most sensitive among the tested antibiotics for gram-negative organisms were Imipenem, Amikacin, and Fosfomycin and for gram-positive organisms were Vancomycin, Pristinamycin and Fosfomycin.

Key Words: *Blood culture. Bacterial profile. Antibiogram. Paediatric. Septicaemia.*

INTRODUCTION

One of the major causes of deaths in paediatric age group is septicaemia. Sepsis related mortality is largely preventable with rational antimicrobial therapy. Emergence of multidrug resistant bacterial strains is a main problem in the management of sepsis. The changing bacteriologic profile of septicaemia 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 septicaemia yet it remains a gold standard for the diagnosis of bacterial agents.[3] 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.[4] Since these bacteria play a major role in the causation of septicaemia, an extensive awareness is crucial in selection of appropriate antibiotic therapy. Hence, an updated knowledge of pathogens causing sepsis in neonates and infants, especially in developing countries, is vital to plan management strategies.[5] Early diagnosis and proper management of neonatal septicaemia could drastically cut down the morbidity and mortality.[6-9]

Unfortunately, there is a lack of skilled paediatricians and well-equipped microbiology laboratories in Afghanistan. Little is known about the prevalence and incidence of septicaemia in any population since no organized surveillance exists on antimicrobial resistance patterns for common bacteria. To authors' knowledge

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there has been no study done in this field in any part of the country and thus published data on antibiotic resistance is lacking. Hence, the present study was undertaken to determine the types of bacteria and their antibiogram causing septicaemia in paediatric age group in this region of the world.

METHODOLOGY

Blood cultures from 3360 cases of in-patients, aged 1 day to 18 years, clinically suspected having septicaemia, were evaluated from January 2010 to June 2012 in the Microbiology Laboratory of French Medical Institute for Children, Kabul, Afghanistan. Samples from out-patients were excluded. The standard microbiological methods were followed in this study during blood culture and antibiotic sensitivity testing. Blood samples were collected following thorough cleaning of the venous site with 70% alcohol and subsequently followed by povidone iodine. The rubber cap of each of the culture broths bottles was immediately cleaned with 70% alcohol, the used needle replaced with a newer sterile needle and the venous blood injected into commercially supplied bottles from BD in the ratio mentioned for culture in Bactec Machine. As soon as the machine gave the alarm for positive growth in any bottle, technologist on duty pulled out the same, performed gram stain and reported the result to the requesting physician as per the panic result reporting policy. Sub-cultures were made onto chocolate, blood and Bromcresol purple agar (bioMérieux, France) and incubated at appropriate temperature and atmosphere according to standard procedures.[10] Gram stain was performed and organisms were identified based on their colony characteristics and biochemical properties. Bacteria were identified to the species level using various API identification strips (bioMérieux, France). Antibiotic susceptibility tests were done against locally available antibiotics by using disk diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) criteria.[11] The classes of antibiotics included aminoglycosides (Gentamicin and Amikacin), beta-lactamases including carbapenems (Imipenem), cephalosporins (Cefotaxime and Ceftazidime), penicillins (Penicillin, Ampicillin and Augmentin), fluoroquinolones (Ciprofloxacin), glycopeptides (Vancomycin), macrolides (Erythromycin), phosphonic acid derivative (Fosfomycin), streptogramins (Pristinamycin) and sulfonamides (Co-trimoxazole).

Drug resistant strains belonging to gram-negative bacilli were studied for extended spectrum beta-lactamases (ESBL) by combination disk method. Methicillin-resistant *Staphylococcus aureus* (MRSA) was detected by cefoxitin disk diffusion method. For quality control ATTC strains were used including *E. coli* 25922, *Klebsiella oxytoca* 700324, *Pseudomonas aeruginosa* 27853, *Staphylococcus aureus* 29213 and 25923 and *Enterococcus faecalis* 29212. Statistical analysis was done using Microsoft Office Excel. The data analysis involved transcription, preliminary data inspection, content analysis, and interpretation. Qualitative variables were expressed as frequencies and percentages. Institutional ethics committee approved the study.

RESULTS

During the study period, 3360 requests for blood cultures were received from intensive care unit (ICU) and in-patient wards. Out of all samples submitted for blood cultures, 410 (12.2%) yielded monomicrobial growth. Out of total isolates, 212 were gram-negative bacilli (51.71%) and 184 were gram-positive cocci (44.88%). In addition, 14 *Candida* species (3.41%) were also isolated (Table I). The frequently isolated species of gram-negative bacteria belonged to Enterobacteriaceae and included Klebsiella (16.1%), Enterobacter (10.2%), *Escherichia coli* (8.5%) and *Serratia* (3.9%). Other important gram-negative bacilli included *Pseudomonas* species (5.12%) and *Burkholderia cepacia* (1.95%). Amongst gram-positive cocci, the most frequently isolated species were coagulase-negative *Staphylococci* (26.3%) followed by *Staphylococcus aureus* (11.9%). Other important species of gram-positive cocci were *Streptococcus* (5.1%) and *Enterococcus* (1.5%). Generally, majority of isolates showed high resistance to commonly used antimicrobials belonging to aminoglycosides (Gentamicin), cephalosporins (Cefotaxime, Ceftazidime), penicillins (Penicillin, Ampicillin and Augmentin), fluoroquinolones (Ciprofloxacin), macrolides (Erythromycin) and sulfonamides (Co-trimoxazole) (Tables III and IV). A large number of gram-negative isolates i.e., 110/212 (51.9%) were found to be ESBL producers (Table II). ESBL positivity was highest in

Table I: Type, number and percentage of blood culture isolates (n=410).

Organism	Number (%) of total no. of isolates
Gram-negative bacilli (n=212)	
Klebsiella spp.	66 (16.10%)
Enterobacter spp.	42 (10.24%)
Escherichia coli	35 (8.54%)
Pseudomonas spp.	21 (5.12%)
Serratia spp.	16 (3.90%)
Burkholderia cepacia	8 (1.95%)
Proteus spp.	7 (1.70%)
Citrobacter spp.	5 (1.22%)
Acinetobacter spp.	4 (0.98%)
Other species of Enterobacteriaceae	8 (1.95%)
Total Gram-negative bacilli	212 (51.71%)
Gram-positive cocci (n=184)	
Staphylococcus non-*aureus*	108 (26.34%)
Staphylococcus aureus	49 (11.95%)
Streptococcus spp.	21 (5.12%)
Enterococcus spp.	6 (1.46%)
Total gram-positive cocci	184 (44.88%)
Candida species (n=14)	
Candida spp.	14 (3.41%)
Grand Total	410 (100%)

Klebsiella species (72.7%), followed by *E. coli* (62.9%). Yet, most of the gram-negative isolates were susceptible to Imipenem (94.3%), Amikacin (81.1%) and Fosfomycin (78.3%) (Table III). All *Staphylococci* were sensitive to Vancomycin but resistant to Penicillin and 80/157 (51%) were MRSA (Table IV). There was only one strain of *Enterococcus faecalis* which showed resistance to Vancomycin. However, the majority of gram-positive isolates were sensitive to Pristinamycin (88.8%) and Fosfomycin (72.8%) (Table IV).

Table II: Frequency of ESBL producers among gram-negative bacilli (n=212).

Organism	Number of isolates	ESBL producers	Percentage of ESBL producers
Klebsiella spp.	66	48	72.7
Enterobacter spp.	42	19	45.2
E. coli	35	22	62.9
Serratia spp.	16	9	56.2
Others	53	12	22.6
Total	212	110	51.90%

Table III: Antibiotic susceptibility of all gram-negative bacilli (n=212).

Antibiotic	Number and percentage of gram-negative bacilli showing Susceptibility to commonly used antimicrobials	
	Number (%) of resistant	Number (%) of sensitive
Ampicillin (n=183)	129 (70.5%)	54 (29.5%)
Augmentin (n=183)	118 (64.5%)	65 (35.5%)
Amikacin (n=212)	40 (18.9%)	172 (81.1%)
Cefotaxime (n=191)	112 (58.6%)	79 (41.4%)
Ceftazidime (n=212)	120 (56.6%)	92 (43.4%)
Co-trimoxazole (n=191)	138 (72.3%)	53 (27.7%)
Ciprofloxacin (n=212)	82 (38.7%)	130 (61.3%)
Fosfomycin (n=212)	46 (21.7%)	166 (78.3%)
Gentamicin (n=212)	112 (52.8%)	100 (47.2%)
Imipenem (n=212)	12 (5.7%)	200 (94.3%)

Table IV: Antibiotic susceptibility of all gram-positive cocci (n=184) .

Antibiotic	Number and percentage of gram-positive cocci showing susceptibility to commonly used antimicrobials	
	Number (%) of resistant	Number (%) of sensitive
Cefoxitin (for MRSA detection; n=157)	80 (51%)	77 (49%)
Ciprofloxacin (n=184)	84 (45.7%)	100 (54.3%)
Co-trimoxazole (n=184)	140 (76.1%)	44 (23.9%)
Erythromycin (n=184)	108 (58.7%)	76 (41.3%)
Fosfomycin (n=184)	50 (27.2%)	134 (72.8%)
Gentamicin (n=184)	82 (44.6%)	102 (55.4%)
Penicillin (n=184)	172 (93.5%)	12 (6.5%)
Pristinamycin (n=184)	23 (12.5%)	161 (87.5%)
Vancomycin (n=184)	1 (0.5%)	183 (99.5%)

DISCUSSION

Septicaemia in children has a high morbidity and mortality worldwide.[12] Physical signs and symptoms, though useful in identifying possible cases, have limited specificity. Definitive diagnosis is by bacteriologic culture of blood samples to identify organisms and establish antibiotic susceptibility. Rational and appropriate use of antibiotics requires understanding of common pathogens and drug resistance patterns in a community.

We studied 410 positive blood samples from clinically suspected septicaemia cases. The frequency (12.2%) of bacterial isolation from the blood culture of children in this study was in accordance with many previous studies done in other countries.[13-16] The common bacterial species isolated from blood culture in this study belonged to coagulase-negative *Staphylococcus, Klebsiella, Staphylococcus aureus, Enterobacter, E. coli, Pseudomonas, Streptococcus* and *Serratia*. *Candida* species were also isolated from 14 blood samples. These findings are consistent with many those of the previous studies,[17-19] and suggest that infections by these agents constitute a significant threat to child survival in the study setting.

In 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 antibiotic sensitivity pattern. For gram-negative bacilli, a combination of a third generation cephalosporin (Cefotaxime, Ceftriaxone or Ceftazidime) with an aminoglycoside (Gentamicin or Amikacin) is usually considered appropriate. However, recent reports suggest that at least 60 - 70% of the gram-negative organisms are resistant to most of these antibiotics,[20,21] and routine use of these agents might increase the risk of infections with ESBL positive organisms. ESBL producing Enterobacteriaceae have spread quickly worldwide and became well recognized in many hospitals.[22-24] Thus, there is a need for continuous screening for ESBL producers in paediatric unit. In this study, out of all gram-negative isolates, 51.9% strains were ESBL producers. Moreover, gram-negatives that produced ESBLs were found to be multidrug-resistant and showed high resistance to commonly used antimicrobials namely Ampicillin, Gentamicin, third generation Cephalosporins, Fluoroquinolones and Co-trimoxazole. Nevertheless, majority of such isolates were susceptible to Imipenem and many to Amikacin. Such antibiotics proved to be the most effective antimicrobial agents for all the gram-negative bacterial isolates, including non-fermenters, namely *Pseudomonas* and *Acinetobacter* species.

In this study, all strains of *Staphylococci* showed resistance to Penicillin. Penicillin resistant *S. aureus* are usually treated with cloxacillin or nafcillin, but the upsetting reality is the emergence of MRSA. In this study, the rate of MRSA strains was 51%. The occurrence of MRSA is more common because of indiscriminate use of superior antibiotics as an emergency empirical therapy. Even though the majority of the gram-positive isolates showed sensitivity to Pristinamycin and Fosfomycin but Vancomycin remained the agent of choice.

The main factors causing the increase in antimicrobial resistant bacteria are poor infection control practices and inappropriate use of antibiotics. Specific antibiotic use strategies like restriction in superfluous antibiotic use, ensuring more rational use and combination antibiotic therapy may help to check the emergence of resistance.

CONCLUSION

Klebsiella, E. coli, Enterobacter and *Staphylococci* remain the principal bacteria responsible for blood stream infections in a paediatric tertiary care setting in Kabul. Local microbiological data suggesting the best choice of antibiotics for paediatric age group is important for the home physicians when treatment of the septic infant has to be initiated before the result of the blood culture is known. The most sensitive among the tested antibiotics for gram-negatives are Amikacin and Fosfomycin in addition to Imipenem. For gram-positives, the options are Pristinamycin and Fosfomycin besides Vancomycin.

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