ORIGINAL ARTICLE

Bacterial Spectrum and Antimicrobial Susceptibility Pattern in Septic Paediatric Patients

SAMMAR NAVEED[1], AIZZA ZAFAR[2], HUMERA JAVED[3], *MUHAMMAD ATIF[4], KHALID OMER ABD ALLAH ABOSALIF[5], HASAN EJAZ[6]*

ABSTRACT

Aim: To determine the bacterial spectrum and antibiotic susceptibility pattern of sepsis in paediatric patients
Method: A total number of 18,037 samples were collected from clinically suspected septic patients during May to October 2015 and were processed for microbiological analysis. The organisms were identified using standard microbiological techniques, and antimicrobial susceptibility pattern was determined byKirby Bauer disc diffusion method and reported according to CLSI guidelines.
Results: The frequency of positive cultures was 1711(9.48%) which include 1145(66.9%) male patients and 566 (33.0%) females. There were 918(53.6%) cases of Gram-negative, and 793(46.3%) cases of Gram-positiveinfections were diagnosed. Gram-negative bacteria mainly comprised of Klebsiella species 317(34.5%) and Pseudomonas species 181(19.7%), whereas 635(80.1%) Coagulase-negative Staphylococci were predominant among Gram-positive bacteria followed by 97(11.9%) Staphylococcus aureus. Majority of the Gram-negative bacteria were susceptible to imipenem 710(77.3%), meropenem 619 (67.5%), piperacillin-tazobactam 554(60.4%), sulbactam-cefoperazone537 (58.5%) and amikacin 459(50%) while Gram-positive bacteria were sensitive to vancomycin 761(96%), linezolid 737(93%), amikacin 693(87.3%), teicoplanin 574(72.3%) and gentamicin 435(54%).
Conclusion: Sepsis in paediatric patients was commonly caused by Klebsiella species and Coagulase negative Staphylococci. Imipenem and meropenem showed better sensitivity against Gram-negative bacteria while most of the Gram-positive bacteria were susceptible to vancomycin and linezolid.
Keywords: Sepsis, paediatric patients, antibiotic susceptibility, bloodstream infections

INTRODUCTION

Sepsis is a life-threatening condition resulting from the release of toxins produced by bacteria multiplying in the bloodstream. These toxins trigger the release of cytokines causing fever, chills, malaise and lethargy with breathing difficulty, especially in children[1]. According to World Health Organization (WHO) estimation, sepsis accounts for 60-80% of deaths per year in the childhood[2]. The incidence of neonatal sepsis has been reported as 1-2/1000 live births at term and 4-8/1000 live births at preterm neonates[3]. Early onset sepsis can arise from the transfer of pathogens colonising in the maternal genital tract during delivery, and late-onset sepsis can be community, or hospital-acquired[4]. Late-onset sepsis is associated with low gestational age (less than 34 weeks) and low birth weight (less than 1500g in neonates) which contribute to high mortality rate in neonates[5].

Early onset sepsis is usually acquired from the resident flora of maternal genital tracts like Escherichia coli and Streptococcus aglactatiae while late-onset sepsis can be either acquired from community or hospital. The organisms commonly associated with late-onset sepsis are Coagulase-negative Staphylococcus, and Staphylococcus aureus belongs to Gram-positive group whereas Klebsiella pneumoniae and Pseudomonas species from Gram-negative group of bacteria[4].

In children above neonatal age group, Gram-positive bacteria causing sepsis are Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus pyogenes, Streptococcus viridans, Streptococcus aglactatiae, Enterococcus faecalis and Streptococcus pneumoniae. Among the Gram-negative group, the most common bacteria causing sepsis are Enterobacter cloacae, Klebsiella pneumoniae, Acinetobacter spp., Pseudomonas spp. and Escherichia coli[6].

The pathogens present in the bloodstream are a potential threat to every organ of the body. Bacteriological culture for isolation of causative agent and information regarding antimicrobial sensitivity pattern is the gold standard technique in the diagnosis and treatment of sepsis[7]. Sepsis is a life-threatening medical emergency requiring urgent diagnosis and treatment.

The purpose of this study was to determine the causative agents of sepsis and their antibiotic sensitivity pattern among the paediatric patients of a tertiary care hospital.

METHODS

This hospital-based descriptive study was conducted from May 2015 to October 2015 after the approval of the ethical committee of the Institutional Review Board. In this study, 18,037 blood samples were collected from suspected septic children under 16 years of age admitted to The Children's Hospital & The Institute of Child Health, Lahore.

[1-3]Department of Microbiology, The Children's Hospital and The Institute of Child Health, Lahore, Pakistan.
[4-6]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Kingdom of Saudi Arabia.
[5]Faculty of Medical Laboratory Sciences, Omdurman Islamic University, Sudan.
Correspondence: Dr. Hasan Ejaz, Email: hetariq@ju.edu.sa Mobile No. +966 55 762 5174

Blood samples were collected aseptically in the brain heart infusion broth and incubated at 37ºC for up to 7 days. They were processed according to standard microbiological techniques in the Microbiology Department of The Children's Hospital, Lahore. Following the indicator of growth, the samples were sub-cultured on MacConkey agar and Blood agar and incubated for 24-48 hours at 37ºC[8]. Bacterial growth was identified by Gram's staining, colony morphology, biochemical tests and API 20E (bioMerieux). Antibiotic susceptibility testing was performed by Kirby-Bauer Disk Diffusion method. Different antibiotics including carbapenems, cephalosporins, fluoroquinolones, aminoglycosides, penicillins and glycopeptides were applied. The antimicrobial susceptibility results were interpreted according to the CLSI guidelines (CLSI, 2015)[9]. Data analysis was performed using SPPS V. 20.

RESULTS

Out of 18,037 blood samples, 1711(9.48%) had microbial growth mainly comprising of Gram-negative bacteria 918/1711(53.6%) followed by Gram-positive bacteria 793/1711 (46.3%). The majority of positive blood cultures were of male patients 1145(66.9%) followed by female patients 566(33.0%). Out of 1711 patients, 1332 (78.0%) were discharged while 200 (11.6%) leftagainst medical advice whereas, mortality was observed in 179 (10.4%) patients (Table-I).There were 1070(62.5%) isolates recovered from theneonates,298(17.4%) from infants, 183 (10.6%) from 1-5 years of age, 110 (6.4%) from 5-10 years of ageand 50 (2.9%) from 10-18 years of age (Table-I).

The most commonly isolated Gram negative bacteria were Klebsiella spp. 317/918 (34.5%) followed by Pseudomonas spp. 181/918 (19.7%), Escherichia coli 152/918 (16.6%), Enterobacter spp. 105/918 (11.4%), Acinetobacter spp. 45/918 (4.9%), Citrobacter spp. 44/918 (4.8%) and Serratia spp. 30/918 (3.3%). The less commonly isolated bacteria were Stenotrophomonas maltophilia 11/918 (1.2%), Burkholderia spp. 10/918 (1.1%), Salmonella spp. 9/918 (1.0%), Pantoea spp. and Proteus spp. 4/918 (0.4%), Morganella morganii 3/918 (0.3%), Aeromonas hydrophila 2/918 (0.2%) and Shigella spp. 1/918 (0.1%). Among Gram positive bacteria, most common isolates were Coagulase negative Staphylococci 635/793 (80.1%) followed by Staphylococcus aureus 94/793 (11.9%), Streptococcus viridans 41/793 (5.2%), Enterococcus faecalis 15/793 (1.9%), Streptococcus pneumoniae 5/793 (0.6%), Streptococcus pyogenes 2/793 (0.3%) and Streptococcus group G 1/793 (0.1%).

Table-I: General characteristics of patients with positive culture

Gender	**Frequency**	**%age**
Male	1145	66.97
Female	566	33.03
Age		
Neonates (< 28 days)	1070	62.5
Infants (>28 days -1 year)	298	17.4
1-5 year	183	10.6
5-10 year	110	6.4
10-18 year	50	2.9
Outcome		
Discharge	1332	78.0
LAMA (Left against medical advice)	200	11.6
Death	179	10.4

Majority of the Gram negative bacteria were sensitive to imipenem 710(77.3%) followed by meropenem 619 (67.5%), piperacillin-tazobactam 554(60.4%), sulbactum-cefoperazone 537(58.5%), amikacin 459(50%). Gram-negative rods were less sensitive to moxifloxacin 388 (42.3%), ciprofloxacin 323(35.1%), levofloxacin 278 (30.2%), ceftazidime 171(18.7%), ceftriaxone 127(13.8%), cefotaxime 118(12.8%), amoxicillin/clavulanic acid 102 (11.2%), cefixime 83(9.06%) and cefuroxime 76(8.3%) (Table- II).

Gram positive bacteria were mostly sensitive to vancomycin 761 (96%) (100% sensitivity with Staphylococcus aureus and Coagulase negative Staphylococci) followed by linezolid 737 (93%), amikacin 693 (87.3%), teicoplanin 574 (72.3%), gentamicin 435 (54.8%), ciprofloxacin 379 (47.7%) and amoxicillin/clavulanic acid 349 (43.9%) while Staphylococcus spp. were 362 (45.6%) sensitive to oxacillin including 61.7% Staphylococcus aureus and 43.3% Coagulase negative Staphylococci. Gram positive bacteria were less sensitive to cefuroxime 250 (31.6%), ceftriaxone 242 (30.5%), cefradine 188 (23.7%), ampicillin 77 (9.7%) and penicillin 75 (9.4%) (Table-III).

Table-II: Antibiotic susceptibility pattern of Gram negative bacteria (n=918)

Antibiotics	**Frequency**	**%age**
Imipenem	710	77.3
Meropenem	619	67.5
Colistin	621	67.6
Tazobactam/Piperacillin	554	60.4
Sulbactam/Cefoperazone	537	58.5
Amikacin	459	50
Moxifloxacin	388	42.3
Ciprofloxacin	323	35.1
Levofloxacin	278	30.2
Ceftazidime	171	18.7
Ceftrioxone	127	13.8
Cefotaxime	118	12.8
Amoxicillin/Clavulanic acid	102	11.2
Cefixime	83	9.06
Cefuroxime	76	8.3

Table-III: Antibiotic susceptibility pattern of Gram positive bacteria (n=793)

Antibiotics	**Frequency**	**%age**
Vancomycin	761	96
Linezolid	737	93
Amikacin	693	87.3
Teicoplanin	574	72.3
Gentamicin	435	54.8
Ciprofloxacin	379	47.7
Oxacillin	362	45.6
Amoxicillin/Clavulanic acid	349	43.9
Cefuroxime	250	31.6
Ceftriaxone	242	30.5
Cefradine	188	23.7
Ampicillin	77	9.7
Penicillin	75	9.4

DISCUSSION

In the present study, frequency of positive samples was 1711/18037 (9.48%). This was comparable to the frequency of 12% positive cultures reported in a study by

Tariq[10]. In the present study, patients were predominantly males 1145(66.9%) followed by female patients 566(33%) which is comparable to the results of study conducted in Peshawar where the male to female ratio was 1.6:1[11]. In this study, the highest rate of bacterial growth was from neonates 1070(62.5%) followed by the infants 298(17.4%). These results are comparable to the study of Karki that also showed a decreasing trend in the frequency of positive cultures with increasing age[12].

In our study, Gram-negative bacteria 918(53.6%) were the most common isolates with the predominance of Klebsiella spp. 317(34.5%) followed by Gram-positive bacteria 793 (46.3%) with majority of Coagulase negative Staphylococci 635(80.1%). Similar results were obtained in a research from Kabul where Klebsiella spp. 66(16.10%) were the predominant organism among Gram-negative bacilli 212 (51.7%) followed by Coagulase negative Staphylococci 108(26.34%) among 184(44.8%) Gram-positive cocci.[10] In contrast to this, a study conducted on Iranian children observed a high frequency of Gram-positive yield with the predominance of Coagulase negative Staphylococci 198(65.8%) followed by Gram-negative with Escherichia coli 60(42.9%) being the most common isolate[6]. The variation observed in our study was due to reasons that the pattern of sepsis may vary in different regions of the world.

In the current study, Gram-negative bacteria showed good sensitivity 710(77.3%) with imipenem and 619 (67.5%) sensitivity with meropenem among Carbapenems. Khan et al (2012) also reported corresponding results with imipenem[13]while Hamid et al. also observed maximum sensitivity with Carbapenems[14]. Gram-negative bacteria were 554 (60.4%) sensitive to piperacillin-tazobactam and 537(58.5%) sensitive to sulbactam/cefoperazone. A study by Vanitha et al (2012) reported it to be 56.1% to piperacillin-tazobactam and 43.8% to sulbactam-cefoperazone[15]. In contrast to this, (98%) and (97%) sensitivity respectively was reported by Desai et al. (2011)[16]. The difference in the antimicrobial susceptibility pattern may have resulted from the frequent use of these specific groups of antibiotics in our hospital over the years leading towards increased resistance.

Gram-negative bacteria were less sensitive to cephalosporins as these bacteria were 171(18.7%) sensitive to ceftazidime followed by 127(13.8%) to ceftriaxone, 118(12.8%) to cefotaxime and 83(9.06%) to cefixime. Khan et al (2012) also observed Gram-negative bacteria to be less sensitive[11] while Bindu et al (2013) found that bacteria were 93%, 71%, 64% and 57% susceptible to this cephalosporins[17]. Among the fluoroquinolones, Gram-negative bacteria showed 388(42.3%), 323(35.1%) and 278(30.2%) sensitivity to moxifloxacin, ciprofloxacin and levofloxacin respectively. Bhatt et al. (2012) reported ciprofloxacin was 30.0% while levofloxacin was 82.1% sensitive against these bacteria.[18] Gram-negative bacteria were 459(50%) susceptible to amikacin and 102(11.2%) to amoxicillin/clavulanic acid. Al-Rawazaq et al (2012) observed 60% and 26.6% sensitivity[19]. A study in Lagos reported that Gram-negative bacteria were 50% sensitive to amoxicillin/clavulanic acid2[20]. Reduced sensitivity of Gram-negative bacteria in our study might have resulted from overuse of these antibiotics and transfer of resistant genes among bacteria in our overcrowded setup.

Gram-positive bacteria showed good sensitivity with glycopeptides like vancomycin 761(96%), teicoplanin 574(72.3%) and aminoglycosides like amikacin 693(87.3%), gentamicin 435(54.8%) with no VRSA and VRSE strains isolated. A study in Lahore reported 100% sensitivity of vancomycin[14]. Tariq (2014) in Kabul reported 54.3% sensitivity[10] and Uzodimma et al (2013) reported 77.4% sensitivity of ciprofloxacin against Gram-positive bacteria[20].

The frequency of oxacillin-resistant Staphylococcus aureus was 38.3%. A study in Libya reported 28.5% oxacillin resistant and 40.2% borderline oxacillin-resistant Staphylococcus strains[21]. The second most sensitive antibiotic against Gram-positive bacteria was Linezolid showing 737(93%) sensitivity which is comparable to 100% sensitivity reported by Mustafa et al. (2014)[22].

This study presents the current bacterial profile and antibiotic susceptibility pattern in paediatric population which varies time to time and according to geographical distribution. Klebsiella spp. and Coagulase negative Staphylococci are most common causative bacteria of sepsis in hospitalised cases. Most commonly affected population is neonates while imipenem and meropenem were comparatively effective in vitro for Gram-negative bacteria while vancomycin and linezolid showed relatively better in vitro susceptibility against Gram-positive bacteria.

Grant Support & Financial Disclosures: Nothing to declare.

REFERENCES

1. Ayobola ED, Egbule OS, and Omonigh O. Study of prevalence and antimicrobial susceptibility of blood culture bacterial isolates. Mal J Microbiol. 2011;7:78-82.
2. Kisson N, Carcillo J, Choueiry JA, Espinosa V, Argent A, Devictor D, et al.World Federation of Pediatric Intensive Care and Critical Care Societies: Global Sepsis Initiative. Pediatr Crit Care Med. 2011;5:494-503.
3. Daniela ML. Paediatric Sepsis diagnosis, etiology, evolution. University of medicine and pharmacy Craiova faculty of General Medicine.2010. 1-11.
4. Zaidi AK, Thaver D, Ali SA, Khan TA. Pathogens associated with sepsis in newborns and young infants. Pediatr Infect Dis J. 2009; 28:S10-8.
5. Makhoul IR, Sujov P, Smolkin T, Lusky A, Reichman B. Epidemiological, clinical, and microbiological characteristics of Late-Onset Sepsis among Very Low Birth Weight Infants in Israel: A National SurveyPediatrics. 2002;109: 34-39.
6. Kalantar E, Motlagh M, Lordnejad H, Beiranvand, S. The prevalence of bacteria isolated from blood cultures of Iranian children and study of their antimicrobial susceptibilities. Jundishapur J Nat Pharm Prod. 2008; 3: 1-7.
7. Qureshi M, Aziz F. Prevalence of microbial Isolates in blood cultures and their antimicrobial susceptibility profiles. Bio Medica. 2011; 27:136-139.
8. Cheesbrough M. District Laboratory Practice in Tropical countries. Part-2. Cambridge University Press. 2002;135-162.
9. Tariq TM. Bacterial profile and antibiogram of blood culture isolates from a Children's Hospital in Kabul. J Coll Physicians Surg Pak. 2014;24:396-399. doi:06.2014/JCPSP.396399.
10. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; CLSI document M100-S25: CLSI, 2015.

11. Khan QA, Iqbal H, Rehaman H. Trends in patterns of resistance among microorganisms causing neonatal sepsis in Peshawar. JPMI. 2012;26:165-169.
12. Karki S, Rai GK, Manandhar R. Bacteriological analysis and antibiotic sensitivity pattern of blood culture isolates in Kanti Children Hospital. J Nepal Paediatr Soc.2010;30(2): 94-97.
13. Khan MR, Maheshwari PK, Masood K, Qamar FN, Haq A. Epidemiology and outcome of sepsis in tertiary care PICU of Pakistan. Indian J Pediatr.2012;79:1454-1458.
14. Hamid MH, Zafar A, Maqbool S. Nosocomial bloodstream infection in a tertiary care paediatric intensive care unit. J Coll Physician Surg Pak.2007; 17: 416-419.
15. Vanitha RN, Kannan G, Venkata NM, Vishwakanth D, Nagesh VD, Yogitha M, et al. A retrospective study on bloodstream infections and antibiotic susceptibility patterns in a tertiary care teaching hospital. Int J Pharm Pharm Sci.2012;4: 543-548.
16. Desai KJ, Malek SS, Parikh A. Neonatal septicemia: Bacterial isolates and their antibiotics susceptibility patterns. Natl J Integr Res Med. 2010;1(3): 12-15.
17. Bindu D, Chitralekha S, Menezes GA,Illamani V. Bacterial profile and antibiotic susceptibility pattern of blood culture Isolates from pediatric age group attending a tertiary care centre. Res J Pharm Biol Chem Sci.2013;4:299-303.
18. Bhatt KS, Patel AD, Gupta P, Patel K, Joshi G. Bacteriological profile and antibiogram of neonatal septicemia. National Journal of Community Medicine. 2012; 3: 238-241.
19. Al-Rawazq HS, Mohammad AK, Al-Zubaiday RH. Bacterial isolates in blood culture of children with septicemia. J Fac Med Baghdad. 2012;54: 96-99.
20. Uzodimma CC, Njokanma F, Ojo O, Falase M, Ojo, T. Bacterial isolates from blood cultures of children with suspected sepsis in an urban Hospital in Lagos: A Prospective Study Using BACTEC Blood Culture System. Internet J Pediatr Neonatol. 2013;16()1:. 1-8.
21. Ellabib MS, Ordonez A, Ramali A, Walli A, Benyayad T, Shebrlo, H. Changing pattern of neonatal bacteremia. Microbiology and antibiotic resistance. Saudi Med J. 2004;25(12):1951-1956.
22. Mustafa M, Ahmed, SL. Bacteriological profile and antibiotic susceptibility patterns in neonatal septicemia in view of emerging drug resistance. J Med Allied Sci. 2014;4: 2-8.