Indian Journal of Microbiology Research 6 (2019) 261–265

Content available at: iponlinejournal.com

Indian Journal of Microbiology Research

Journal homepage: www.innovativepublication.com

Oroginal Research Article

Bacteriological profile and antibiogram of blood culture isolates from paediatric patients with special reference to ESBL and MRSA in a tertiary care centre

Hetal G Vaghela[1], Bithika Duttaroy[1,*], Khyati C Prajapati[1]

[1] *Dept. of Microbiology, GMERS Medical College and Hospital Gotri, Vadodara, Guajarat, India*

ARTICLE INFO

Article history:
Received 24-07-2019
Accepted 03-09-2019
Available online 09-09-2019

Keywords:
Antibiotic susceptibility tests
Blood culture
Bacterial profile
ESBL
MRSA
Paediatric
Septicaemia

ABSTRACT

Introduction and Objective: Blood stream infections are important causes of morbidity and mortality in neonates and children. Blood culture remains the gold standard for their diagnosis. Emergence of multi drug resistant bacterial strains is a major problem in the management of sepsis. The antimicrobial susceptibility patterns help to guide the choice of empiric antimicrobial regimen for the patients with bacteremia and septicaemia. The present study was undertaken to identify the common bacterial pathogens associated with paediatric sepsis and to determine their antibiotic susceptibility pattern.

Material and Methods: A retrospective observational study was carried out by reviewing the records of blood cultures received from clinically suspected paediatric patients of septicaemia between January to December 2017 in the Department of Microbiology, G.M.E.R.S Medical College and Hospital Gotri, Vadodara, Gujarat. 713 samples of blood cultures were received and processed during that period. The isolates were identified by conventional biochemical tests. Antibiotic susceptibility testing was performed by Modified Kirby-Bauer disc diffusion method and the screened strains were further processed for detection of Extended Spectrum Beta Lactamases (ESBL) and Methicillin Resistant *Staphylococcus aureus* (MRSA) according to CLSI guidelines.

Results: Out of the 713 Blood cultures, 161 (22.58%) were culture positive, of which 120 (74.54%) were Gram negative isolates and 41 (25.46%) were Gram positive. MRSA was detected in 38.46 % of the *Staphylococcus aureus (S.aureus*) isolates. 54% of *Klebsiella spp* and 52.63 % of *Escherichia coli* were found to be ESBL producers.

Conclusion: High rates of isolation of MRSA and ESBL stresses on the need for a continued screening and surveillance for antibiotic resistance in Paediatric Care Units, which will influence the appropriate empiric treatment and infection control strategies for prevention of septicaemia in paediatric patients.

1. Introduction

Blood stream infections present a serious challenge to the clinicians as a major cause of death in the paediatric patients.[1,2] They constitute a medical emergency that requires timely detection and identification of the blood borne pathogens and their antibiotic sensitivity pattern.[3] The rate of blood stream infections in children ranges between 20-25% in developing countries.[4] Blood culture remains the gold standard for laboratory diagnosis of bloodstream infections (BSIs) in infants and children.[5,6]

Although both Gram negative and Gram positive bacteria are associated with these infections, Gram negative bacterial infections are more fatal and cause more serious therapeutic problems as multi drug resistant strains are more common among them.[7–9] In almost all cases, empiric antimicrobial therapy is initiated before the results of blood culture are available.[10] This needs to be done carefully as injudicious use of higher antibiotics leads to the development of multi drug resistant (MDR) organisms, specifically MRSA and ESBL producing bacterial isolates. With emergence of MDR organisms and wide variation in bacterial resistance pattern based on the geographical and regional location

* *Corresponding author*.
E-mail address: drbithika@yahoo.com (B. Duttaroy).

https://doi.org/10.18231/j.ijmr.2019.057
2394-546X/© 2019 Published by Innovative Publication.

and in different periods of time, there is a need for an ongoing review of the causative pathogens and their drug susceptibility patterns.[7,8,11] These data are used to determine empiric antibiotic therapy and also to alert the clinicians to emerging pathogens that may pose a threat to the community.[7,12]

Keeping in mind the high mortality and morbidity associated with BSIs, a right choice of empiric therapy is of utmost importance. Therefore, present study was under taken to find out the etiological profile and antibiotic sensitivity pattern of the pathogens causing bacteremia to serve as a useful guide for the clinicians to initiate empiric antibiotic therapy.

2. Materials and Methods

This was a retrospective observational study carried out by reviewing the records of the blood cultures received from the paediatric wards between January to December 2017 in the Department of Microbiology, G.M.E.R.S Medical College and General Hospital Gotri, Vadodara, Gujarat. Data collection included age and sex of the patients, the reports of the blood culture and antibiotic sensitivity testing. Blood samples were collected and processed following standard methods.[13,14] The isolation and identification of organisms was done as per standard guidelines.[14] Antibiotic susceptibility tests (AST) were done using disk diffusion method and zone sizes were measured and interpreted in accordance with Clinical and Laboratory Standards Institute (CLSI) criteria.[15] Separate panels of antibiotics were used for Gram positive isolates, *Enterococci*, Gram negative isolates (*Enterobacteriacea*e), *Pseudomonas* and *Acinetobacter* as per CLSI guidelines.[15] Vancomycin susceptibility was detected using Etest strip. ATCC strains of *Escherichia coli* 25922, *Klebsiella* o*xytoca* 700324, *Pseudomonas aeruginos*a 27853, *Staphylococcus aureu*s 29213 and 25923 and *Enterococcus faecali*s 29212 were used as quality control for culture and AST. The screened strains were further processed for detection of ESBL production and Methicillin resistance according to CLSI guidelines.[15]

Detection of Methicillin resistance in *Staphylococcus aureu*s was done using 30 microgram Cefoxitin disc as per CLSI guidelines. Reference strains used were *Staphylococcus aureu*s ATCC 43300 and ATCC 25923. ESBL production was detected by combined disc method using both Cefotaxime (30μg) and Cefotaxime-clavulanate (30μg/10μg) as well as Ceftazidime (30μg) and Ceftazidime-clavulanate (30/10μg) (HiMedia, Mumbai, India). An increase $\geq$ 5mm in the zone of inhibition in the disc containing Clavulanate compared to drug alone was considered as positive for ESBL producer. For ESBL standardization, *Escherichia coli* ATCC 25922 and *Klebsiella pneumonia*e ATCC 700603 were used as negative and positive controls. Multi drug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial categories.[16]

2.1. Statistical analysis

Data analysis was done with MS Office Excel spreadsheet, involving transcription, preliminary data inspection, content analysis, and interpretation. The results were expressed as percentages for the analysis of various epidemiological details as well as for analysing the distribution of the bacterial isolates and their sensitivity pattern. Prior approval was obtained from the Institutional Human Ethical committee.

3. Results

713 blood cultures of paediatric patients suspected of septicaemia were received and processed.161 samples (22.58%) were culture positive, with all of them showing a monobacterial growth, yielding a total of 161 bacterial isolates. Candida was not isolated from any of the samples.

Although a majority of the blood cultures were received from the neonates (48.66%) the rate of culture positivity was similar across the different age groups except for infants where it was low. A higher number of blood cultures and a higher positivity rate was seen in the male children as compared to the females. Table 1

Gram negative bacilli formed the major proportion of the total isolates (74.54%). *Klebsiella Spp.* (37.89%) was the predominant isolate followed by *Staphylococcu*s *aureu*s (16.15%), *Acinetobactor spp*. (14.91%), *Escherichi*a *col*i (11.80%), *Enterococcus spp*. (09.32%), *Pseudomonas spp*. (06.83%), *Salmonella typh*i (01.80%), *Salmonella paratyph*i A (0.62%) and *Enterobacter spp*. (0.62%). (Figure 1)

Antibiotic susceptibility patterns of the isolates have been depicted in Figures 2, 3 and 4. Among the *Enterobacteriaceae*, 38% of *Klebsiella spp*. and 36% of *Escherichia coli* were MDR but were highly susceptible to Levofloxacin, Carbapenems and Piperacillin-Tazobactum. A high degree of resistance was observed to most of the Cephalosporins except for Cefepime. 54% of *Klebsiella sp*p and 52.63 % of *Escherichia col*i were found to be ESBL producers. The other *Enterobacteriaceae* isolates comprising a very small proportion were sensitive to all the drugs. Among the Gram negative non fermentors, *Acinetobacter spp.* were highly sensitive to Carbapenems and Fluoroquinolones, but lower sensitivity was noted against Cephalosporins. *Pseudomonas sp*p were sensitive to most of the drugs with all of them showing sensitivity to Carbapenems. (Figure 3) MDR was seen in about 20% of the Gram negative non fermentors.

*Staphylococcus aureu*s was the predominant Gram positive isolate (16.5%) followed by *Enterococcus spp*. (9.32%). A majority of *Staphylococcus aureu*s isolates

(65.38%) were resistant to Penicillin but showed high sensitivity to Gentamicin and Levofloxacin. 38.46% of them were found to be MRSA. All the isolates were sensitive to Vancomycin and Linezolid. All the *Enterococcus spp*. were sensitive to Vancomycin and Linezolid but were resistant to most of the other drugs. (Figure 4) Around 36% of these Gram positive isolates were MDR.

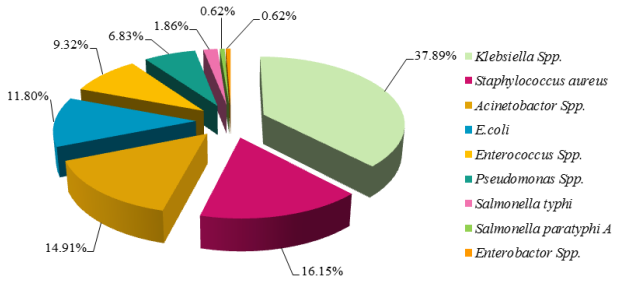

Fig. 1: Frequency distribution of the various bacterial isolates from the Positive blood cultures.

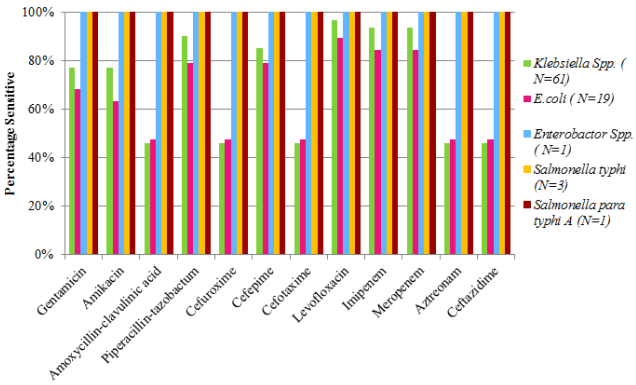

Fig. 2: Antibiotic sensitivity pattern of *Enterobacteriaceae*

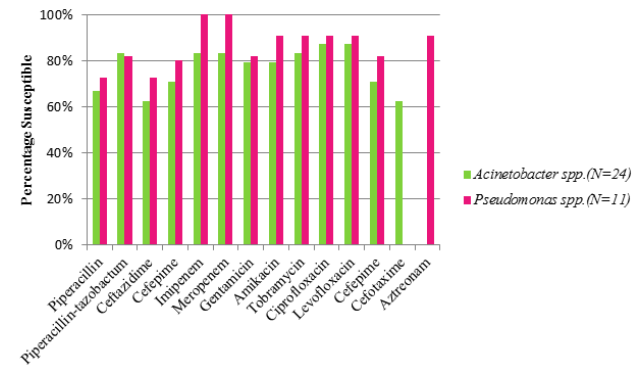

Fig. 3: Antibiotic sensitivity pattern of *Gram negative Non-Fermenters*

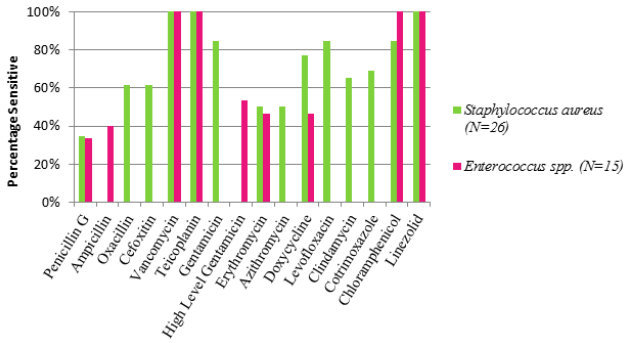

Fig. 4: Antibiotic Sensitivity Pattern of *Gram positive* isolates

4. Discussion

Septicaemia in children has a high morbidity and mortality worldwide. Physical signs and symptoms, though useful in identifying possible cases, have limited specificity in diagnosis.[6,7] Growing resistance to conventional and even newer antibiotics is also a matter of serious concern.[17] Blood culture plays an important role by timely and correctly identifying the underlying pathogens and based on their drug resistance patterns guide the clinicians regarding the empirical as well as the definitive treatment.

The rate of blood stream infections in children is about 20-25% in developing countries.[3,4] In the present study, the rate of Blood culture positivity was 22.58% which was comparable to studies carried out in India and abroad.[3,10,12,17–19] A higher rate of blood culture positivity (44.0%) in children was reported by Prabhu K et al which was attributed to the weaker /immature immune system in children.[4,19] Conversely a lower rate of culture positivity was reported by some other studies.[2,6–8,11] Variation in BSI rates could be due to various factors like sampling volume, timing of blood collection and the common practice of prescribing antibiotics by the local health practitioners before the patients reach the tertiary care hospital.

Although a majority of the samples were received from the neonates, we found the culture positivity rate to be marginally higher in this group. Karki et al[11] have reported a higher isolation rate in the neonates, while Tiwari et al[17] found a higher positivity rate in the age group of 5 to 10 years. The culture positivity rate was relatively higher in the male children as compared to the female children in our study. Similar findings have been reported by other studies.[11,17] We could not find the exact reason for this male preponderance, although in our case it could be partly attributed to the higher number of samples received from the male children.

In the current study, Gram negative isolates (74.54%) outnumbered the Gram positive (25.46%), with *Klebsiella spp* and *Staphylococcus aureus* as the most frequent organisms. Similar findings have been reported by Tiwari et

Table 1: Association between the rate of culture positivity and demographic profile of the study group.

Age Group	No. and % of Samples received.	Culture Positive	
		No.	%
0-1 month	347 (48.66%)	84	24.20%
1 month – 1 year	90 (12.62%)	15	16.66%
>1 year – 14 years	276 (38.70%)	62	22.46%
Total	713 (100%)	161	22.58%
Gender			
Male	393 (55.11%)	105 (26.71%)	
Female	320 (44.88%)	56 (17.5%)	
Total	713(100%)	161 (22.58%)	

al.[17] The predominance of Gram negative bacteria has been reported by many authors across India and elsewhere and reflects the current trends in bacteriology of blood stream infections in children.[3,7,8,10,12,17,19] Conversely, studies from India and abroad have found a higher rate of Gram positive isolates.[4,6,11,20] Geographical location, endemicity of the etiological agents and seasonal variation are the major factors determining the type of isolates.[8,19]

The antibiotic sensitivity patterns revealed a high degree of resistance to the Cephalosporins among the Gram negative isolates with minimal resistance to Carbapenems and Fluoroquinolones. This high level of resistance to the second and third generation Cephalosporins could be attributed to their large scale use for management of febrile illness in both inpatients and outpatients. 54% of *Klebsiella spp* and 52.63 % of *Escherichia coli* were found to be ESBL producers, which is a matter of great concern given the fact that the Cephalosporins still continue to be the mainstay for therapy of most childhood infections. Other previous studies[4,7,9,20] corroborate the same.

Methicillin resistance was detected in 38.46% of the *Staphylococcus aureus* (MRSA), which is consistent with many of the studies in India and elsewhere.[3,4,7,8,17] Vancomycin remains the drug of choice for the MRSA isolates as all the Gram positive isolates were sensitive to it. This fact has also been reported by several previous studies.[3,4,7,9,10,20]

A high proportion of both Gram positive and Gram negative isolates were found to be MDR in this study. 36% of Gram positive isolates, 37% of Enterobacteriaceae and 20% of Gram negative non fermentors were MDR. Many of the previous studies[3,7–10,21] have also reported high frequency of MDR Gram positive and Gram negative isolates to the tune of 58.2% in Gram positive and 67.1% in Gram negative. This suggests a high resistance gene pool attributed to gross misuse and inappropriate usage of the antibacterial agents combined with poor clinical practices.[21] Establishment of an appropriate and rational antibiotic policy is therefore essential to control this growing problem.

5. Conclusion

Blood culture still remains as one of the most important diagnostic tool available to the physician for early diagnosis of bacteremia and septicemia. Instituting an appropriate empirical therapy based on the prevalence of the bacterial isolates and their antibiotic profiles is integral to the successful treatment of sepsis. The present study provided much needed information on the prevalence of bacterial pathogens in blood stream infections and their antibiotic sensitivity patterns in the paediatric age group. High rates of isolation of MRSA and ESBL in addition to resistance to the commonly used antibiotics, warrants continuous monitoring of etiology and AST patterns of the blood culture isolates.

Poor infection control practices coupled with inappropriate and irrational use of antibiotics are the main culprits for the widespread antimicrobial resistance. Strict adherence to infection control practices and following the core principles of antibiotic stewardship would definitely go a long way in helping decrease or prevent emergence of resistance.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. Meremikwu MM, Nwachukwu CE, Asuquo AE, Okebe JU, Utsalo SJ. Bacterial isolates from blood cultures of children with suspected septicaemia in Calabar. *BMC Infect Dis*. 2005;(5):110.
2. Berkley JA, Lowe BS, Mwangi I, Williams T. Bacteremia among children admitted to a rural hospital in Kenya. *N Engl J Med*. 2005;352:39–47.
3. Negussie A, Mulugeta G, Bedruba AI, Shimeles D, Lema T, Aseffa A. Bacteriological Profile and antimicrobial Susceptibility Pattern of Blood Culture Isolates among Septicemia Suspected Children in Selected Hospitals of Addis Ababa, Ethiopia. *Int J Biol Med Res*. 2015;6(1):4709–17.
4. Prabhu K, Bhat S, Rao S. Bacteriological Profile and Antibiogram of Blood Culture Isolates in a Paediatric Care Unit. *J Lab Physicians*. 2010;2(2):85–88.
5. Bard JD, Tekippe EM. Diagnosis of Bloodstream Infections in Children. *J Clin Microbiol*. 2016;54(6):1418–24.
6. Pan F, Zhao W, Zhang H. Value of Time to Positivity of Blood Culture in Children with Bloodstream Infections. *Can J Infect Dis Med*

Microbiol. 2019;p. 5975837. Available from: 10.1155/2019/5975837.
7. Tariq M. Bacteriological profile and antibiogram of blood culture isolates from a childrens Hospital in Kabul. *J Coll Physicians Surg Pak*. 2014;24(6):396–9.
8. Parajuli NP, Parajuli H, Pandit R, Shakya J, Khanal PR. Evaluating the Trends of Bloodstream Infections among Pediatric and Adult Patients at a Teaching Hospital of Kathmandu, Nepal: Role of Drug Resistant Pathogens. *Can J Infect Dis Med Microbiol*. 2017;p. 8763135. Available from: 10.1155/2017/8763135.
9. Gupta S, Kashyap B. Bacteriological profile and antibiogram of blood culture isolates from a tertiary care hospital of North India. *Trop J Med Res*. 2016;19:94–9.
10. Maham S, Fallah F, Gholinejad Z, Seifi A, Hoseini-Alfatemi SM. Bacterial etiology and antibiotic resistance pattern of pediatric bloodstream infections: A multicenter based study in Tehran. *Ann Ig*. 2018;30(4):337–45.
11. 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):94–7.
12. Murty DS, Gyaneshwari M. Blood cultures in pediatric patients: A study of clinical impact. *Indian J Med Microbiol*. 2007;25(3):220–24.
13. Isenberg HD. Specimen collection, Transport, and Acceptability. In Clinical Microbiology Procedures Handbook, Vol 1, 2nd edn update, ASM PRESS, 2007: Section 2. Garcia LS, Isenberg HD, Editors in Chief. ;. .
14. York MK. Aerobic Bacteriology. In Clinical Microbiology Procedures Handbook, Vol 1, 2nd edn update. ASM PRESS, 2007: Section 3. Garcia LS, Isenberg HD, Editors in Chief. ;. .
15. CLSI. In: Performance Standards For Antimicrobial Susceptibility Testing. 27th ed. CLSI supplement M100. Wayne, PA ; 2017,. .
16. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, et al. Multidrug resistant, extensively drug-resistant and pandrug-resistant bacteria:an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect*. 2012;18(3):268–81.
17. Tiwari DK, Golia S, Sangeetha KT, Vasudha CL. A study on the bacteriological profile and antibiogram of bacteria in children below 10 years in a tertiary care hospital in Bangalore, India. *J Clin Diagn Res*. 2013;7(12):2732–35.
18. Sharma M, Goel N, Chaudhary U, Aggarwal R, Arora DR. Bacteremia in children. *Indian J Pediatr*. 2002;69:1029–32.
19. Nwadioha SI, Nwokedi E, Kashibu E, Odimayo MS, Okwori EE. A review of bacterial isolates in blood cultures of children with suspected septicemia in a Nigerian tertiary Hospital. *Afr J Microbiol Res*. 2010;4(4):222–5.
20. Thakur S, Thakur K, Sood A, Chaudhary S. Bacteriological profile and antibiotic sensitivity pattern of neonatal septicaemia in a rural tertiary care hospital in North India. *Indian J Med Microbiol*. 2016;34(1):67–71.
21. Wasihun AG, Wlekidan LN, Gebremariam SA, Dejene TA, Welderufael AL. Bacteriological profile and antimicrobial susceptibility patterns of blood culture isolates among febrile patients in Mekelle Hospital, Northern Ethiopia. *Springer Plus*. 2015;4:314.

Author biography

Hetal G Vaghela Ex. Tutor

Bithika Duttaroy Professor

Khyati C Prajapati Tutor

Cite this article: Vaghela HG, Duttaroy B, Prajapati KC. Bacteriological profile and antibiogram of blood culture isolates from paediatric patients with special reference to ESBL and MRSA in a tertiary care centre. *Indian J Microbiol Res* 2019;6(3):261-265.