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Original Research Article

Bacteriological profile of bloodstream infections in pediatric age group in a tertiary care centre, Solapur

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ABSTRACT

Background: Bloodstream infections (BSI) due to multidrug-resistant organisms from paediatric setup have become increasingly common. BSI can be transient and be rapidly cleared out by the host defence mechanism or be associated with high morbidity and mortality if it becomes persistent.

Materials and Methods: Blood samples were collected from 1112 children within a 1-year study period (July 2022 to June 2023) which included all children admitted with complaint of fever and suspected of having sepsis in Paediatric department of Shri Chhatrapati Shivaji Maharaj Sarvopchar Rugnalaya (SCMSR), Solapur. Blood volume was collected depending upon age and body weight of the patient with aseptic precautions and inoculated in Brain Heart Infusion broth (BHI) which was incubated at 37°C for 7 days. Subcultures were made on blood agar and MacConkey agar plates. Organisms were identified by biochemical reactions and antibiotic susceptibility test of the isolates were performed by disk diffusion.

Results: Out of 1112 suspected cases, 175 (15.74%) were culture positive. *Klebsiella species* (27.43%) was the most common isolate followed by *Acinetobacter species* (16.57%). Most common Gram positive isolate was *Staphylococcus aureus* (11.43%) followed by *Enterococcus spp.* (6.86%). Total Multidrug resistant (MDR) isolates in Gram negative and Gram positive were 50.35% and 31.25% respectively.

Conclusion: High rates of antibiotic resistance are observed in both gram positive and gram negative organisms. Thus, there is an urgent need for proper implementation of antibiotic stewardship programme and infection prevention and control measures.

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1. Introduction

Bacteraemia is the presence of viable bacteria in blood, but without any clinical signs or symptoms.^{1,2} Bacteraemia may be transient, intermittent or continuous. Transient bacteraemia may occur spontaneously or else minor events like vigorous chewing or dental surgery. Intermittent bacteraemia can occur when bacteria from an infected site are sporadically released into the blood from a diffuse infection, an extravascular abscess or empyema cavity.^{3,4}

Bacteraemia may be a phase in the natural course of some infections like typhoid or brucellosis or can occur as a spill over effect in serious infections when patient's defence become inadequate.³ Continuous bacteraemia usually occurs in cases when organisms have direct access to blood, such as infective endocarditis or intravenous catheters.⁴ Bloodstream infections (BSI) or septicaemia denotes an overwhelming invasion of bloodstream with or without a known focus of infection, when circulating bacteria multiply at a rate that exceeds their removal with phagocytes.^{3,5} It is a life-threatening organ dysfunction caused by a dysregulated host response to infection.¹

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Several risk factors have been identified both in the neonates and children which makes them susceptible to BSI.⁴ The patient age, vaccination coverage and exposure to invasive procedures are some of the important predisposing factors of BSI occurrence. The reported rates are higher in younger children particularly neonates due to the relative immaturity of their immune system, as well as their degree of contact with siblings and associated exposure to pathogens.^{4,5} While there has been a decrease in infections from vaccine preventable pathogens, incidence of nosocomial infections have risen. This may have been as a result of the increased use of invasive procedures like intravenous catheters and intubation in hospitals and intensive care units.⁵

Blood cultures remain the 'gold standard' for the detection of BSI.⁵ However, due to recent exposure to antibiotics, the nature of the infection or volume of blood obtained, the yield from blood culture may vary.⁵ The optimum volume to be collected depends on body weight and total body volume, 1–5 ml is recommended from infants and children.^{2,5}

Current under 5 mortality rate of India is 29.1 deaths per 1000 live births.⁶ Estimated 41.5% (20.3 million) of incident sepsis cases and 26.4% (2.9 million) deaths related to sepsis worldwide were among children younger than five years.⁷ It is estimated that India has the highest neonatal mortality due to neonatal sepsis caused by bacteria resistant to first-line antibiotics. Approximately one fifth of neonates with sepsis die in the hospital, and the mortality rises to 50% for those with culture-proven sepsis.⁸ Blood culture positivity rate in paediatric population was 20.25% in a study conducted by Dave Mukti et.al. in Mumbai City (2022).⁹ BSI due to multidrug-resistant organisms, especially from paediatric intensive care units (PICU), are being increasingly reported across the world.¹⁰ The majority of the BSI are reported from tertiary NICU's who show very high rates of MRSA BSI (53.6%).⁸

2. Materials and Methods

2.1. Study design

Cross-sectional study conducted on a total of 1112 samples collected from the admitted children within a 1-year study period (July 2022 to June 2023) which included all children up to 14 years of age.

2.2. Sample

Blood samples were collected from children admitted with fever and suspected of having sepsis in Pediatric ward, PICU and NICU under Pediatric department, Shri Chhatrapati Shivaji Maharaj Sarvopchar Rughalaya (SCSMSR), Solapur, Maharashtra. Patient with known clinical condition such as malignancies, tuberculosis etc. were excluded from the study. Single sample from one

patient was considered. If paired samples of one patient were sent it was considered as single specimen.

The cases were categorized into 5 clinical groups according to their age: 0-1 month–neonates, 1 month-1 year old, 1year-5 years old, 5 years -10 years and 10 years -14 years.

Blood for culture was collected following strict aseptic precautions. 1 ml (neonates) and 5 ml (children) blood was collected and inoculated into 10 ml and 50 ml of brain heart infusion broth (1:10 dilution), respectively.

2.3. Processing

The culture bottles were incubated at 37°C aerobically and periodic subcultures were done onto Mac Conkey's agar, blood agar and chocolate agar after overnight incubation on day 2, day 4 and finally on day 6. The growth obtained was identified by conventional biochemical tests and the antibiotic sensitivity testing was performed on Mueller–Hinton agar plates by Kirby–Bauer disc diffusion method. Zone diameter was measured and interpreted as per the Clinical and Laboratory Standards Institute (CLSI) guidelines, of respective year.¹¹

Methicillin resistance in *Staphylococcus aureus* (MRSA) was tested using Mueller-Hinton agar with Cefoxitin disc (30 µg) by Kirby-Bauer disc diffusion method. A zone size of ≥ 22 mm was considered sensitive and ≤ 21 was considered resistant. High level Aminoglycoside resistance in *Enterococcus species* was tested using High level Gentamicin disc (120 µg). Vancomycin susceptibility in MRSA isolates were measured with Vancomycin Ezy MICTM strip (VAN) (0.016-256 µg/ml) by Epsilometer test.

Multidrug resistant (MDR) organism is defined as an isolate that is tested not-susceptible to at least one agent in ≥ 3 antimicrobial classes like β-lactam or β-lactam β-lactamase Inhibitor (BLBLI) combination agents, quinolones, aminoglycosides, macrolides.²

2.4. Statistical analysis

Analysing the distribution of different bacterial isolates and their sensitivity pattern and the interpretation of results were done using Microsoft Excel.

3. Results

The total number of patients enrolled in the study were 1112. Out of 1112 specimens, 175 were culture positive i.e. 15.74% of the total number of specimens tested.

In the present study, highest number of culture positive blood specimen in suspected BSI patients was observed in the age group of 1 month to 1 year (32.87%) followed by neonates (26.38%) (Table 1).

From 1112 suspected BSI children, female children were 57.91% and male 42.09%. Also, the culture positive

Table 1: BSI in children according to age group

Age	Total specimens tested	Blood culture positive	Percentage of positive
0-1 month	307	81	26.38%
1 month- 1 year	146	48	32.87%
1 year- 5 years	191	16	8.37%
5 years- 10 years	219	14	6.39%
10 years- 14 years	249	16	6.42%
Total	1112	175	15.74%

Table 2: BSI in children according to gender

Gender	Total specimens tested	Blood culture positive
Male	468 (42.09%)	52 (29.71%)
Female	644 (57.91%)	123 (70.29%)
Total	1112	175

percentage was highest in female group 70.29%. (Table 2) Blood culture positivity ratio in Male : Female was 1: 2.37.

Table 3: Distribution of the isolates according to Gram reaction in the present study

Distribution of Gram positive cocci in organisms isolated from Blood Culture (n=175)		
Isolate	Total	Percentage
<i>Staphylococcus aureus</i>	20	11.43%
<i>Enterococcus</i> spp	12	06.86%
Total	32	18.29%
Distribution of Gram negative bacilli in organisms isolated from Blood Culture (n=175)		
Isolate	Total	Percentage
<i>Klebsiella</i> spp.	48	27.43%
<i>Acinetobacter</i> spp.	29	16.57%
<i>Escherichia coli</i>	28	16.00%
<i>Citrobacter</i> spp.	20	11.43%
<i>Pseudomonas</i> spp.	17	09.71%
<i>Enterobacter</i> spp.	1	00.57%
Total	143	81.71%

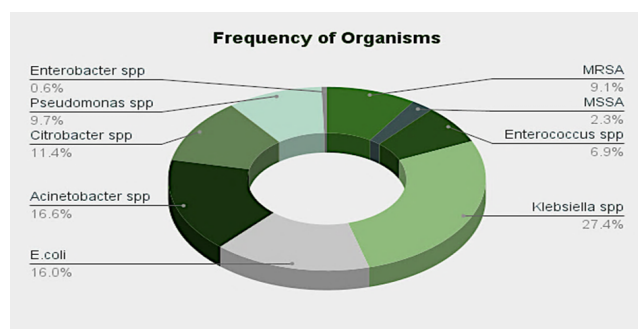


Figure 1: Distribution of the isolates obtained by blood culture in suspected patients of BSI

All of the 175 isolates have shown mono bacterial growth. *Klebsiella* species (27.43%) was the predominant organism isolated, followed by *Acinetobacter* species (18.75%) (Table 3, Figure 1). From total 175 isolates, Gram positive and Gram negative isolates were 18.29% and 81.71% respectively.

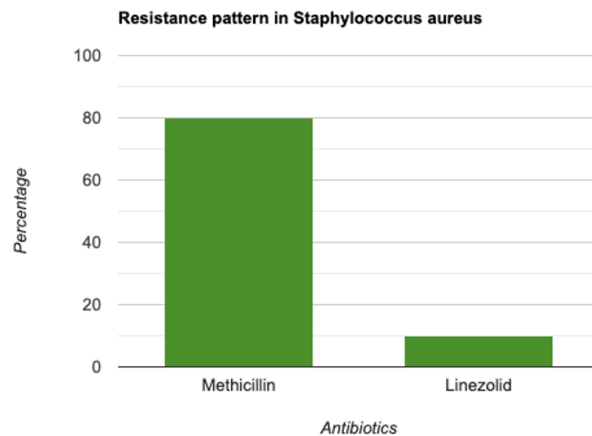


Figure 2: Resistance pattern in *Staphylococcus aureus* isolated in present study

Out of 20 isolates of *Staphylococcus aureus*, 80% (16) were methicillin resistant and 10% (2) were Linezolid resistant (Figure 2).

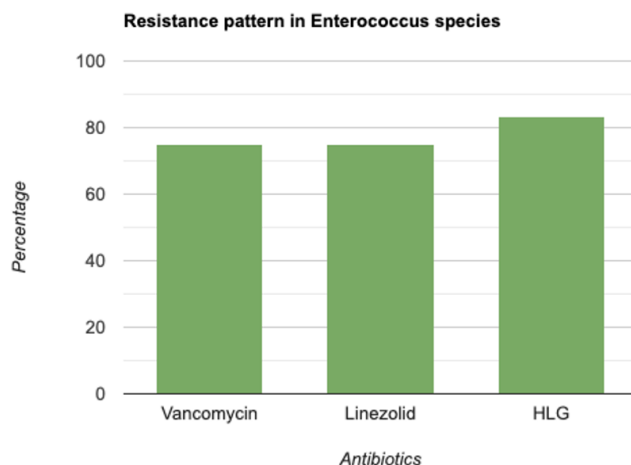


Figure 3: Resistance pattern in *Enterococcus* species isolated in present study

Among 12 isolates of *Enterococcus species* 10 (83.33%) were High level Aminoglycoside resistant, 75% (9) were Linezolid resistant and 75% (9) were Vancomycin resistant (VRE) (Figure 3).

Among the Gram negative isolates resistance to β -lactam and β -lactam+ β -lactamase inhibitor combination antibiotics are very high among the isolates except Piperacillin

Table 4: Different antibiotic resistance pattern in Gram positive isolates

Antibiotics	MRSA (n=16)	MSSA (n=4)	Enterococcus species (n=12)
Ampicillin	NT	NT	11(91.67%)
Azithromycin	13(81.25%)	03(75.00%)	NT
Ciprofloxacin	11(61.75%)	01(25.00%)	11(91.67%)
Cotrimoxazole	06(37.50%)	01(25.00%)	NT
Clindamycin	10(62.50%)	00(00.00%)	NT
Erythromycin	15(93.75%)	02(50.00%)	11(91.67%)
Gentamicin	03(18.75%)	00(00.00%)	NT
High level Gentamicin	NT	NT	10(83.33%)
Linezolid	02(12.50%)	00(00.00%)	09(75.00%)
Levofloxacin	NT	NT	11(91.67%)
Vancomycin	03(18.75%)	NT	09(75.00%)
Tetracycline	04(25.00%)	02(50.00%)	09(75.00%)

NT- Not Tested

Table 5: Different antibiotic resistance pattern in Gram negative isolate

Antibiotics	<i>Klebsiella species</i> (n=48)	<i>Acinetobacter species</i> (n=29)	<i>Escherichia coli</i> (n=28)	<i>Citrobacter species</i> (n=20)	<i>Pseudomonas species</i> (n=17)	<i>Enterobacter species</i> (n=01)
Amikacin	40(83.33%)	24(82.76%)	16(57.14%)	14(70.00%)	10(58.82%)	00(00.00%)
Ampicillin	NT	NT	24(85.71%)	NT	NT	NT
Amoxicillin	42(87.50%)	NT	20(71.43%)	NT	NT	NT
Clavulanate						
Aztreonam	40(83.33%)	NT	24(85.71%)	16(80.00%)	04(23.53%)	01(100.00%)
Ciprofloxacin	39(81.25%)	21(75.00%)	21(91.67%)	13(65.00%)	05(29.41%)	01(100.00%)
Ceftazidime	46(95.83%)	24(82.76%)	25(89.29%)	16(80.00%)	05(29.41%)	00(00.00%)
Cefotaxime	45(93.75%)	25(86.21%)	25(89.29%)	19(95.00%)	NT	01(100.00%)
Ceftriaxone	44(91.67%)	25(86.21%)	23(82.14%)	16(80.00%)	NT	00(00.00%)
Cotrimoxazole	30(62.50%)	21(72.41%)	13(46.43%)	13(65.00%)	NT	00(00.00%)
Gentamicin	31(64.58%)	19(65.52%)	14(50.00%)	15(75.00%)	10(58.82%)	00(00.00%)
Imipenem	10(20.83%)	15(51.72%)	04(14.29%)	02(10.00%)	03(17.65%)	00(00.00%)
Meropenem	NT	NT	NT	NT	03(17.65%)	NT
Levofloxacin	38(79.17%)	25(86.21%)	20(71.43%)	12(60.00%)	06(35.29%)	00(00.00%)
Piperacillin	37(77.08%)	22(75.86%)	18(64.29%)	11(55.00%)	02(11.76%)	00(00.00%)
Tazobactam						
Tetracycline	21(43.75%)	19(65.52%)	10(35.71%)	14(70.00%)	NT	00(00.00%)
Tobramycin	NT	NT	NT	NT	08(47.06%)	NT

NT- Not Tested

Tazobactam (62.94%) and Meropenem (17.65%). Rates of resistance to non-β-lactam antibiotics were as follows: Amikacin- 72.73%; Gentamicin- 69.23%; Ciprofloxacin- 69.93%; Levofloxacin- 70.63%; and Cotrimoxazole- 61.11%.

Antibiotic resistance of Imipenem in Enterobacterales is 16.49% and Meropenem resistance in *Pseudomonas species* was 17.65%. Resistance to third generation cephalosporins were very high among the Gram negative isolates (Overall 85.82%): Ceftazidime (81.12%), Ceftriaxone (85.71%) and Cefotaxime (91.27%).

Out of 175 isolates, 81.72% were Gram negative and 18.29% were Gram positive organisms of them MDR isolates were 50.35% and 31.25% respectively.

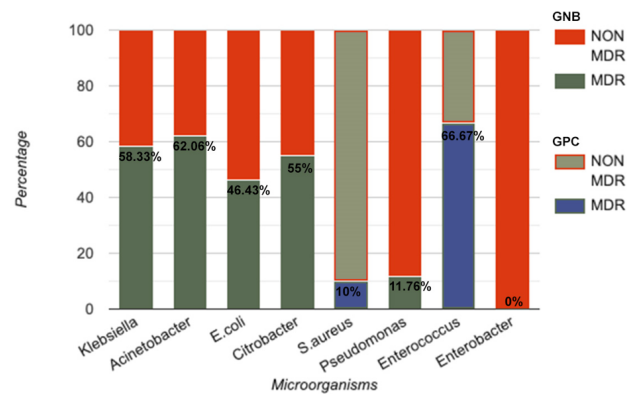


Figure 4: Stacked bar diagram showing MDR and Non MDR percentage in different isolates

4. Discussion

Sepsis, a medical emergency occurs when any infection of the body enters the bloodstream and triggers the cascading inflammatory response leading to widespread tissue injury and organ dysfunction can ultimately lead to death.^{2,9} The timely detection, identification and susceptibility testing of a causative species of bacteria are essential for the proper treatment, and better prognosis of patient.⁴

In this study, out of 1112 blood cultures that were received in department from children with suspicion of septicaemia during the study period, blood culture confirmed septicaemia was 175 (15.74%). These findings are comparable with result from study done in Srinagar (14% positivity) by Hussain M.S. et al.,¹² but is lower than observed in studies done in Kolhapur (24.5%) by V.S. Varkar, S.J. Ghosh,¹³ in Bangalore (25%) by Devendra Kumar Tiwari et al.⁴ and in Chandigarh (30%) by Lakshmi, K. S. et al.¹⁴ The low level of recovery of bacteria (confirmed BSI) may be owing to the fact that the samples were collected at the slightest suspicion of sepsis and a lot of the children had been pre-treated with antibiotics resulting in a higher denominator.

BSI was more common in the age group of 1 month to 1 year (32.87%) which was in contrast to studies done by Devendra Kumar Tiwari et al.⁴ where the maximum incidence of BSI was seen in ages 5 years to 10 years (35.71%), and also Vidhya R et al.¹⁵ who reported that BSI was most frequently encountered in neonates (69.9%). We have observed that blood culture positive percentage in children below 1 year of age was 28.47%. Children below 1 year of age are at an increased risk of developing sepsis because of their weak immune system, particularly if they are born prematurely, with low birth weight or if their mother had an infection during pregnancy.

In our study, it was observed that the blood culture positivity was higher in females (70.29%) compared to males (29.71%). Male blood culture positivity was greater in studies done by Hussain M.S. et al.¹² (65%), Devendra Kumar Tiwari et al.⁴ (26.87%) and Anees, M. et al.¹⁶ (64%). This discrepancy might have occurred due to the higher number of samples obtained from female children (57.91%) for the study.

We had a greater number of Gram negative isolates (81.72%) compared to Gram positive isolates (18.29%). Similar findings were noted by Devendra Kumar Tiwari et al.⁴ (71.87% Gram negative septicaemia), Anees, M. et al.¹⁶ (71.1% Gram negative septicaemia) and contrary to findings of by Hussain M.S. et al.¹² (44% Gram negative septicaemia) where Gram positives isolates were more (56%).

Klebsiella species was the commonest isolate (27.43%) associated with bacteraemia in our study. Devendra Kumar Tiwari et al.,⁴ also reported a high incidence of Klebsiella (43.75%) isolated from blood cultures. *Staphylococcus*

aureus (11.43%) and *Klebsiella pneumoniae* were the commonest reported Gram-positive and Gram-negative pathogens, respectively. Dharmapalan et al.,⁸ Devendra Kumar Tiwari et al.⁴ and Hussain M.S. et al.¹² also found *Klebsiella* species and *Staphylococcus aureus* as the commonest Gram negative and Gram positive isolates in their study.

Our study shows 80% of the *Staphylococcus aureus* isolates as MRSA which is very similar to the results in the study by Kaistha et al. (83.9%),¹⁷ Devendra Kumar Tiwari et al.⁴ had noted 33.33% MRSA while Vidhya R et al.¹⁵ has noted 69.2% MRSA isolates. Third generation cephalosporins and carbapenem resistance in our isolates were high; 85.82% and 80% respectively. Since cephalosporins are the first-line antibiotics recommended in India in pediatrics practice for enteric fever, meningitis, and pneumonia, its high rate of resistance is worrisome on the efficacy of these antibiotics. Resistance to carbapenems too is now commonly reported from different centers in India.¹⁰

In our study 46.86% of isolates were multidrug resistant which is higher than the study conducted by Folgori et al. (39%, 2011)¹⁸ and also Tsai M.H. et al. (18.6%, 2014)¹⁹ who have studied MDR strains on NICU patients. Out of 175 isolates in our study 82 isolates were MDR. Among 82 MDR isolates, Gram negative isolates were 50.35% and MDR Gram positive isolates were 31.25%. In the study conducted by Parajuli et al., he found that MDR Gram negative and MDR Gram positive isolates were 34.8% and 54.4% respectively.²⁰ The highest percentage of MDR isolates were seen in *Enterococcus species* (66.67%) and the lowest in *Enterobacter species* (0%). As there was only one isolate of *Enterobacter species* in this study so determining MDR in the said isolate is insignificant. The higher rate of MDR isolates in this study can be ascribed to the fact that this is a recent study as compared to the other studies. The length of the hospitalization, together with the excessive use of broad-spectrum antibiotics, increases the risk to develop polyclonal outbreaks caused by highly resistant Gram-negative microorganisms, often carrying resistance genes.¹⁸

5. Conclusion

Bacterial infections are the major causes of morbidity and mortality in children. A very high percentage of multidrug resistance (46.86%) was observed in the present study. Growing resistance to conventional and even newer antibiotics is a serious cause of concern.⁴ Antimicrobial resistance (AMR) has emerged as a leading public health threat of the 21st century.⁸ There is a need for continuous screening and surveillance of the antibiotic susceptibility pattern in the paediatric population. Proper antibiotic stewardship programme needs to be implemented with formulation of hospital antibiotic policy as well as a protocol for the effective management and prevention of

drug resistance. This will result in reduction of hospital stays, healthcare expenses, and mortality rates. This will also ensure more rational use of antibiotics and reduce the emergence of drug resistant strains.

6. Sources of Funding

None.


7. Conflict of Interest

There is no conflict of interest among the authors.


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