

A Study on Bacteriological Profile and Antibiotic Sensitivity Pattern for Sepsis in Children Aged 2 Months to 5 Years in a Tertiary Care Hospital

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Abstract

Background: Septicemia is a leading cause of morbidity and death in paediatric patients worldwide. One of the most difficult infections to control is that of drug-resistant pathogens.

Aim: The study is aimed to determine the principal etiological agents and antimicrobial susceptibility pattern of septicemia in paediatric patients at a tertiary care hospital.

Material and Methods: A cross-sectional study was conducted on 2 months to 5 years old sepsis suspected patients. Blood samples were cultured by conventional method and Kirby-Bauer disc diffusion method was done to screen for antimicrobial susceptibility.

Results: Of 300 participants, 103 (34.3%) of them had a positive blood culture. Among the Gram-negative isolates, *Escherichia coli* (67.75%) was the predominant isolate of which 17.3% were Carbapenem resistant. Among the positive isolates, *Staphylococcus aureus* (74.5%) was the predominant one and 35.29% were Methicillin resistant. Gram-negative isolates showed highest susceptibility to Amikacin, Meropenem and Piperacillin-Tazobactam. Gram positive organisms were sensitive to Linezolid, Vancomycin, Teicoplanin, Cotrimoxazole and Azithromycin. 44.6% of isolates showed multidrug resistance and was highest in *Escherichia coli*. The average duration of hospital stay in culture positive cases was 16.9 days and 6.8 days in culture negative cases. Mortality rate observed was 10.6 % (31/300) and was highest in infants <1year and was more associated with *Staphylococcus aureus* infection.

Conclusion: Given the variable nature of antibiotic susceptibility patterns and etiological agents of septicemia, it appears that ongoing assessment of the most common pathogens associated with bloodstream infections and detection of their sensitivity patterns to locally available antibiotics are reasonable measures.

Keywords: Antimicrobial Susceptibility, Multi-Drug Resistance, Paediatric, Septicemia.

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Introduction

Sepsis is one of the leading causes of illness and death in babies and children across the

world, especially in underdeveloped nations [1]. Sepsis results from the release

of inflammatory mediators, an abnormal distribution of intravascular volume, and a decrease in myocardial function, all of which contribute to septic shock. Septic shock is a clinical condition caused by tissue hypoperfusion caused by microbial infection. Septicaemia in children is caused by malnutrition, persistent medical issues, significant injury, chronic antibacterial medication, and immunosuppressant therapy. Polymicrobial sepsis is more common in high-risk individuals and is linked to catheters, gastrointestinal disorders, neutropenia, and cancer [2].

Septicaemia in children is characterised by fever, difficulty breathing, tachycardia, malaise, rejection of feeding, or lethargy [3]. These can cause major complications such as shock, multiple organ failure, disseminated intravascular coagulation, and so on. As a result, blood stream infections are one of the most dangerous circumstances, and rapid detection and identification of blood stream pathogens is critical. For the provisional diagnosis of septicaemia, clinical examination utilising a mix of symptoms and signs is a good guidance. Nonetheless, bacteriologic culture to isolate the offending organism remains the cornerstone of septicaemia diagnosis [4].

Paediatric sepsis may be caused by a number of reasons. That may differ depending on where you live and how your health-care system is set up. The presence of indwelling intravenous devices, the use of steroids and immunomodulators, extended hospitalisation, chronic antibiotic treatment, surgery, burns or bedsores, and significant traumas have all been recognised as risk factors for paediatric sepsis [5-7]. Septicemia can be caused by underlying disorders such as chronic renal disease [8,9], hematologic malignancies [10,11], immune weakened people, and HIV/AIDS

Throughout the last two decades, Multidrug resistance in several bacterial pathogens has reached epidemic proportions [10]. This

pattern of resistance varies according to geographic and regional location, health-care setup, and existing practice [11,12]. The administration of antimicrobial medications at the proper time is critical and the only approach to treat septicemia. Antibiotic resistance, on the other hand, is a rapidly expanding issue in poor nations. [13] Improper sepsis therapy aggravates the condition, resulting in patient mortality and the establishment of novel drug-resistant strains. This has become a significant health issue with widespread economic and societal consequences [12]. Infections produced by multi-drug resistant strains necessitate longer hospital admissions, a greater risk of mortality, and the administration of more expensive drugs [14]. Early diagnosis by blood culture helps in early initiation of effective antimicrobial agents, early recovery and to formulate institutional empirical antibiotic therapy for septicemia in children.

The present study was conducted to identify the etiological agents and antimicrobial susceptibility patterns in septicemia among children attending Government general hospital, a tertiary care hospital.

Materials and Methods

A cross-sectional study was conducted among 300 children of age group 2 months-60 months with suspected septicaemia admitted to Department of Paediatrics, Government General Hospital, Anantapur between December 2020 to June 2022

Data collection procedure: The study included 300 children aged 2 months to 60 months who were hospitalised to the paediatric critical care unit for sepsis. Each patient's biodata, socioeconomic position, length of hospitalisation, outcome, and were all documented using a predesigned data collecting proforma. Following blood collection, the proper therapy was started, and the hospitalised youngsters were watched over until they were recovered or passed away. Parents' informed permission was obtained.

The technique was carried out by a skilled laboratory technician who prepared the venipuncture site with 70% isopropyl alcohol, wearing sterile gloves. Before venipuncture, the skin was given at least one minute to dry. Blood samples from newborns and children older than 1 year were obtained and diluted 1:10 into 10 and 50 milliliters of brain heart infusion broth, respectively.

Samples were processed in the microbiology section by incubating bottles aerobically for 7 days at 37° C. Every day, bottles were examined for growth, turbidity, red cell hemolysis, gas bubbles, and the development of distinct colonies of clots. Subcultures from Brain Heart Infusion (BHI) broth were carried out on Nutrient agar, Mac Conkey agar, Blood agar, and Chocolate agar of Hi Media labs after 24 hours, 3 days, and 7 days. After 7 days of incubation, blood culture bottles that showed no development on the subculture were deemed negative. Following development of growth on culture media, the colonies were examined and processed for pathogen identification and antibiotic susceptibility testing. Repeat blood samples were taken for culture after Coagulase Negative Staphylococci (CONS) were isolated, and the pathogen's

identity was verified by excluding out skin commensals.

Test for antimicrobial susceptibility: A standardised inoculum of an organism is inoculated onto the Muller Hinton agar plate using the agar disc diffusion method (Kirby-Bauer method), which is the most widely used approach. The agar surface is then covered with filter paper discs coated with antibiotics. After 18 to 24 hours of incubation, the zone of inhibition against bacterial growth around each disc is measured and interpreted as per CLSI guidelines for resistance or susceptibility.

After the collection of a blood sample, all children received treatment as directed by the unit protocol, including intravenous fluid therapy, antipyretics, and empirical antibiotics. Upon the receipt of culture results, antibiotics were modified in accordance with sensitivity patterns.

Statistical Analysis

The patients' information was entered into a predesigned structured proforma together with all the data. Subsequent data were placed into a Microsoft Excel sheet 16.0 and analysed using SPSS version 25.0. Using the chi-square test, a statistical comparison between dependent and independent variables was made.

Results

Out of 300 children admitted with suspected septicemia, blood culture positive rate was 34.3% and culture negative rate was 65.7%.

Table 1: Blood culture positivity rate

Blood Culture result	No. of Cases	%
Culture Positive	103	34.3
Culture Negative	197	65.7
Total	300	100.0

Blood culture positivity was highest in 2-6 months age group, and it was 48.5%.

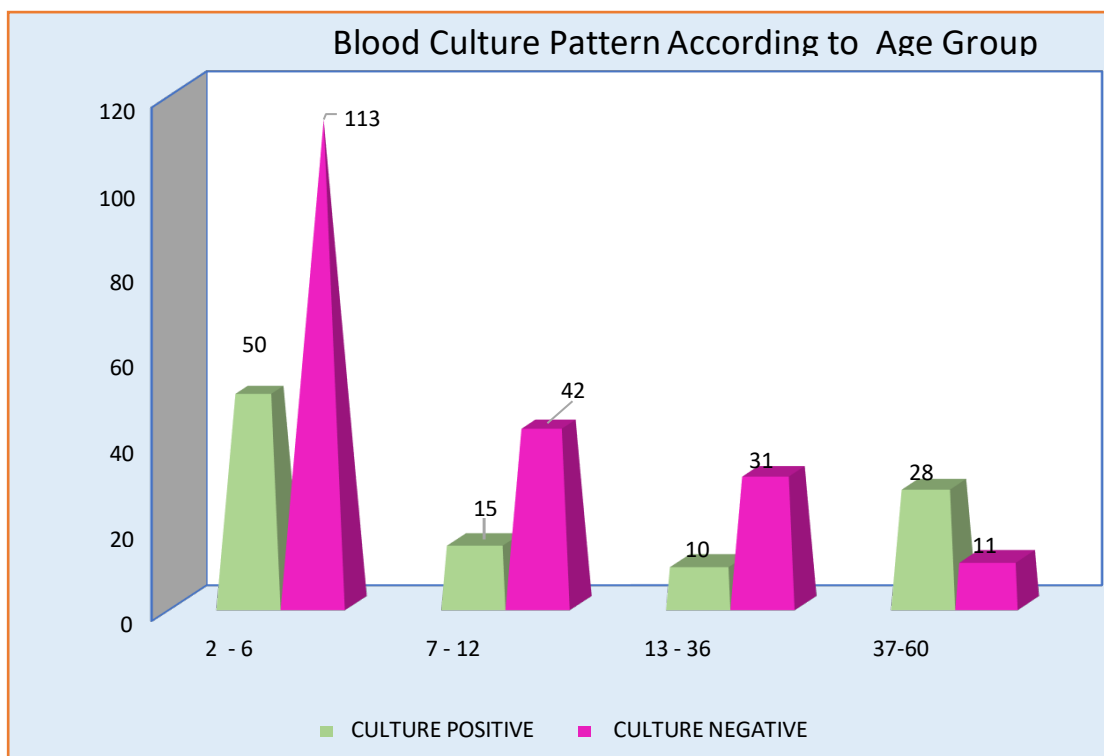


Figure 1: Blood culture positivity according to age group

Total number of Gram-negative isolates were 52 out of 103 contributing to 50.45% whereas total number of Gram-positive organisms isolated were 51 out of 103 contributing to 49.5%.

Table 2: Distribution of isolates from Blood culture

Type of isolate	Number (%)	P value
No. of Gram-negative organisms	52 (50.5%)	>0.05
No. of Gram-positive organisms	51(49.5%)	
Total	103	Not significant

Among the Gram- negative isolates, the most common organism isolated was Escherichia coli (67.75%), followed by Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter and Sphingomonas paucimobilis. Carbapenem resistant Escherichia coli (CRE) were 17.3%.

Table 3: Distribution of Gram-negative organisms in blood culture

Gram-negative organisms	No. of Cases	%	%
Escherichia coli (CSE)	26	50.45	67.75%
Escherichia coli (CRE)	9	17.3	
Klebsiella pneumoniae	9	17.3	
Pseudomonas aeruginosa	5	9.6	
Acinetobacter	2	3.8	
Sphingomonas paucimobilis	1	2	
Total	52	100	

Among Gram-positive organisms, the most predominant organism was *Staphylococcus aureus* 38 (74.5%), followed by *Streptococcus pneumoniae*. Coagulase negative Staphylococci (CONS- *Staphylococcus hominis*, *Staphylococcus haemolyticus*, *Staphylococcus epidermidis*) and *Enterococcus gallinarum* contributed equal occurrence. Methicillin resistant *Staphylococcus aureus* (MRSA) were 35.29%.

Table 4: Distribution of Gram-positive organisms in blood culture

Gram-Positive organisms	No. of Cases	%	
Staphylococcus aureus (MSSA)	20	39.21%	74.5%
Staphylococcus aureus (MRSA)	18	35.29%	
Streptococcus pneumoniae	8	15.77%	
Staphylococcus hominis	2	3.92%	
Staphylococcus haemolyticus	1	1.96%	
Staphylococcus epidermidis	1	1.96%	
Enterococcus gallinarum	1	1.96%	
Total	51	49.5%	

Among the Gram-negative organisms 59.61% were sensitive to Amikacin, followed by Meropenem, Piperacillin- Tazobactam.

Table 5: Antibiotic sensitivity pattern of Gram-negative organisms

Antibiotics	Sensitive N (%)	Intermediate Sensitive N (%)	Resistant N(%)
Amikacin	31(59.61)	2(3.84)	19(36.5)
Meropenem	29(55.76)	1(1.92)	22(42.3)
Piperacillin + Tazobactam	27 (51.92)	4(7.69)	21(40.3)
Ciprofloxacin	20 (38.46)	4(7.69)	28(53.8)
Amoxicillin + Clavulanic acid	20(38.46)	1(1.92)	31(59.6)
Trimethoprim+ Sulfamethoxazole	18(34.61)	3(5.76)	31(59.6)
Ofloxacin	09(17.30)	0	43(82.6)
Ceftriaxone	08(15.38)	0	44(84.6)
Teicoplanin	02(3.84)	0	50(96.1)
Oxacillin	02(3.84)	0	50(96.1)
Linezolid	02(3.84)	0	50(96.1)
Vancomycin	01(1.92)	0	51(98)
Cefazoline	01 (1.92)	0	51(98)
Ampicillin	00	0	52(100)
Amoxicillin	00	0	52(100)

Among 51-gram positive isolates, most of them (78.4%) were sensitive to linezolid followed by vancomycin and Teicoplanin.

Table 6: Antibiotic sensitivity pattern of Gram-positive organisms

Antibiotics	Sensitive N (%)	Intermediate Sensitive N (%)	Resistant N (%)
Linezolid	40(78.4)	0	11(21.5)
Vancomycin	33(64.7)	0	18(35.1)
Teicoplanin	30(58.8)	5(9.8)	16(31.3)
Trimethoprim+sulfamethoxazole	27(52.9)	3(5.8)	21(41.1)
Azithromycin	27(52.9)	13(25.4)	11(21.5)
Ciprofloxacin	12(23.52)	4(7.84)	35(68.6)
Oxacillin	8(15.68)	0	43(84.3)
Piperacillin +tazobactam	6(11.6)	0	45(88.2)
Meropenem	6(11.6)	0	45(88.2)
Amikacin	5(9.8)	0	46(90.0)
Ceftriaxone	3(5.8)	0	48(94.1)
Cefixime	0	23(45.0)	28(54.9)

Cefazoline	0	0	51(100.0)
Cefotaxime	0	32(62.7)	19(37.2)
Ceftazidime	0	35(68.6)	16(31.3)
Cefepime	0	32(62.7)	19(37.2)
Clarithromycin	0	38(74.5)	13(25.4)
Doxycycline	0	32(62.7)	19(37.2)
Gentamycin	0	3(5.8)	48(94.1)
Ofloxacin	2(3.92)	0	49(96.0)
Cefpodoxime	0	0	51(100.0)
Cefoperazone	0	0	51(100.0)
Amoxicillin + clavulanic Acid	0	0	51(100.0)
Amoxicillin	0	0	51(100.0)
Ampicillin	0	0	51(100.0)

Incidence of MDR (Multidrug Resistant) organisms among isolates was 44.6% (46/103). 60% of *E. coli* species, 55.5% of *Klebsiella* species, 47.3% of *Staphylococci* and 40% of *Pseudomonas* species were multi drug resistant.

Table 7: Multidrug resistance patterns among isolates

Organisms (N)	Frequency of multidrug resistant organisms n (%)
Escherichia coli (35)	21(60%)
Klebsiella (9)	5(55.5%)
Staphylococcus aureus (38)	18(47.3%)
Pseudomonas (5)	2(40%)
Total	46

Overall, the average duration of hospital stay was 10.2 days. Those with positive cultures were in the hospital on an average of 16.9 days, whereas those with negative cultures stayed there on an average of 6.8 days.

Table 8: Duration of hospital stay

Age	Average Duration of hospital Stay					
	Culture negative		Culture positive		Total	
	No. of days/ cases	Average	No. of days/ Cases	Average	No. of days/ Cases	Average
2 – 6 Months	757 / 113	6.7	832 / 50	16.6	1589 / 163	9.7
7 – 12 Months	279 / 42	6.6	253 / 15	16.9	532 / 57	9.3
13 – 36 Months	226 / 31	7.3	170 / 10	17.0	396 / 41	9.7
37-60 Months	69 / 11	6.3	484 / 28	17.3	553 / 39	14.2
Total	1331/200	6.8	1739/103	16.9	3070 / 300	10.2

The overall mortality was seen in 31 cases among the 300 (10.3%) of which 23 cases were among culture positive cases. Among all the sepsis cases, 12 (38.7%) deaths were reported in 2 to 6 months age group, 9 (29.0%) deaths in 7 to 12 months age group accounting to 67.7% deaths occurring in infants.

Table 9: Mortality among the sepsis cases

Age	Death			
	Culture Negative	Culture positive	Total	
2 - 6 Months	3	9	12(38.7%)	67.7%
7 - 12 Months	2	7	9(29.0%)	
13 - 36 Months	1	3	4(12.9%)	
37-60 Months	2	4	6(19.4%)	
Total	8	23	31	
Chi-square	$\chi^2 = 0.0847$ p value <0.05 not significant			

In our study, 11 deaths were due to *Staphylococcus aureus* which accounts for majority of the causative agent,

Table 10: Deaths among culture positive isolates

Bacteria	Total no of isolates	Death (n %)
Staph. aureus	38	11(28.94%)
E. coli	35	8(22.85%)
Klebsiella	9	2(22.22%)
Pseudomonas	5	1(20%)
Streptococcus pneumoniae	8	1(12.5%)
Total	95	23

Discussion

In our study, 103 of 300 suspected cases of sepsis were culture positive, with a 34.3% blood culture positivity rate. Prabhu k *et al* observed a blood culture positive rate of 43.78%, [15] 29.8% by Evance Godfrey *et al* [16], 25.9% by Acquah *et al*, [17] 25% by Tiwari *et al* [18], and 4.4% by Sangeetha *et al* [19].

In the current study, the frequency of Gram-negative bacteria in culture was 50.5% (52/103) and Gram-positive bacteria was 49.5% (51/103) correspondingly which was not a significant difference. This agreed with Tariq *et al* (51.7% versus 44.8%) and Sangeetha *et al* [19] (43.05% vs 56.94%). In contrast to our findings, Tiwari *et al* [18] found that Gram-negative organisms predominated with a frequency of 72.7% and 71.87%, respectively.

Staphylococcus aureus was the most common pathogen in this investigation, accounting for 46.4% (46/103) of all isolates, followed by *E. coli* (34%(35/103) and *Klebsiella* species (8.73%(9/103). Similarly, to our investigation, Evance Godfrey *et al* [16], and Adugna Negussie *et*

al [20] identified *Staphylococcus aureus* as the major isolate (39.7%), (23.2%), respectively. In contrast to our findings, Sangeetha *et al*. [19] and Tariq *et al* [18] identified Coagulase negative *Staphylococcus* as the most common isolate.

E. coli (CSE- Carbapenem sensitive *E. coli*), *E. coli* (CRE- Carbapenem resistant *E. coli*), *Pseudomonas*, and *Acinetobacter* were the most prevalent Gram negative species recovered. *E. coli* was the most common Gram-negative organism identified in Evance Godfrey *et al* investigation, as it was in ours [16] In contrast to our investigation, the most prevalent Gram-negative bacterium identified was *Klebsiella* species, as reported by Sangeetha *et al* [19] and Tariq *et al* [18].

In our investigation, Gram negative isolates were most susceptible to Amikacin, Meropenem, and Piperacillin-Tazobactam, followed by Ciprofloxacin, Amoxiclav, and Cotrimoxazole. Linezolid, Vancomycin, Teicoplanin, Cotrimoxazole, and

Azithromycin were all effective against Gram-positive bacteria. According to Sangeetha *et al* [19], Gram negative isolates were 100% responsive to Colistin and Tigecycline, whereas Gram positive isolates were sensitive to linezolid, vancomycin, and teicoplanin.

Gram negative isolates showed more than 90% resistance to Ampicillin, Amoxiclav, Cefoperazone, Cefpodoxime, and 95% resistance to Ofloxacin and Gentamycin in our study, while Gram positive isolates showed 100% resistance to Ampicillin, Amoxiclav, Cefoperazone, Cefpodoxime, and 95% resistance to Ofloxacin and Gentamycin.

Evance Godfrey *et al* [16] discovered enhanced resistance to amoxicillin, ampicillin, amoxiclav, gentamycin, and ciprofloxacin in their investigation. This was consistent with findings from other research in both developed and underdeveloped nations. Sangeetha *et al* [19]. discovered resistance to comparable medicines, which might be attributed to indiscriminate antibiotic usage.

In our study, 44.6% of the isolates tested positive for multidrug resistance. Drug resistance was found in 60% of *E. coli*, 42% of staphylococci, 55% of klebsiella, and 40% of pseudomonas.

Our study found 44.6% MRSA prevalence, which was similar to Yusuf *et al*. study, which found 41.7% MRSA prevalence. Sabouni *et al* [21]. found that MRSA causes 60% of bloodstream infections. According to Sangeetha *et al* [19], the prevalence of MRSA was 27.27%.

The average hospital stay in our research was 10.2 days. A total of 16.9 days were seen in culture positive cases, with the 3–5-year age group having the longest hospital stay. Daniela Nasu *et al* [22]. found that the average hospital stay was 11 days. The typical hospital stays for patients admitted to the critical care unit in a study by Sahiledengle *et al* [23]. was 7 days.

The fatality rate in our study was 10.6% (31/300), with mortality rates higher in culture positive children than in culture negative children, which is consistent with previous research. The case fatality rate from sepsis was 9.4%, according to Evance Godfrey *et al* [13], which is close to our study.

Staphylococcus sepsis was associated with the highest mortality rate in our research, followed by *E. coli*, Klebsiella, Pseudomonas, and Streptococcus pneumoniae. Unlike our findings, Evance Godfrey *et al* [16] discovered that children infected with *E. coli* and CONS bacteria died at a greater rate.

Conclusion

The prevalence of blood culture positive rate was greater in the early infancy age group of 2-6 months. *Staphylococcus aureus* was the most often isolated organism, followed by *Escherichia coli*.

Multidrug resistance was found in *E. coli*, followed by *Staphylococcus aureus* in culture positive isolates in our investigation. Based on the current result, we urge that blood culture and sensitivity investigations be performed on a regular basis in the health care system, as well as the development of a suitable antibiotic policy for the treatment of bloodstream infections in children. Inadvertent antibiotic use should be limited to minimise the formation of multidrug resistant organisms and to reduce childhood mortality.

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