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Original Research Article

Neonatal Septicemia: Analysis of Blood Culture Isolates and Antibiotic Resistance Patterns

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Abstract:

Background: Bloodstream infections (BSIs) pose a major source of morbidity and mortality in neonates and pediatric patients, due to their undeveloped immunity and exposure to invasive interventions. The incidence of antimicrobial resistance makes therapeutic management more difficult, emphasizing the importance of timely identification and appropriate therapy.

Aim: To assess the bacterial infection source causing pediatric BSIs, identify the most significant pathogens, and assess the susceptibility profile of the various pathogens to prescribe antibiotics.

Methodology: An observational study in a hospital setting was performed on 90 neonates suspected of having clinical septicemia in the department of Microbiology, Patna Medical College and Hospital, Patna, Bihar, India. Blood samples were collected under aseptic conditions, cultured and identified for bacterial isolates by standard biochemical methods along with antibiotic susceptibility testing as advised by CLSI.

Results: Staphylococcus aureus was isolated at a higher rate than any of the other organisms investigated (51.1%); coagulase negative staphylococci, and Klebsiella pneumoniae were isolated at the same rate (12.2%); Gram-negative pathogens were noted, including Salmonella typhi (8.9%) and Pseudomonas aeruginosa (6.7%). The isolates demonstrated high resistance to penicillin and the antibiotics most used in therapy; vancomycin and aminoglycosides demonstrated susceptibility to most Gram-positive isolates. Non-fermenters demonstrated considerable resistance, creating a more challenging situation for therapeutic purposes.

Conclusion: Most pediatric BSIs are caused by Gram-positive organisms with considerable antimicrobial resistance, and clinical judgement regarding timely blood culture diagnostics and directed therapy is the best way to optimize outcomes and decrease mortality.

Keywords: Bloodstream Infection, Blood Culture, Pediatric Septicemia, Antimicrobial Resistance, Staphylococcus Aureus and Neonates.

Introduction

Infections of the bloodstream (BSIs) are a serious clinical issue in children, especially neonates and younger children, who often have immature immune systems and are more susceptible to invasive pathogens. In general, BSIs are often associated with extended length of stay, invasive medical management, and other co-morbid diseases, making neonates/infants especially vulnerable to invasive pathogens [1]. The clinical indications or symptoms of BSIs in children are nonspecific, and long delays in diagnosis or treatment can result in rapid disease progression, septicemia, multi-organ impairment, and, if not treated promptly, increased mortality.

Bloodstream infections remain a heavy burden in developing countries where there is an estimated prevalence of 20–50% in hospitalized children as compared to much lower rates in developed countries. Contributing factors include limited healthcare resources, ineffective infection control guidelines, and limited access to advanced diagnostic capability and appropriate antimicrobial treatment. Additionally, the rise in antimicrobial resistance is stressful as it limits the effectiveness of empiric therapies and inflates poor treatment outcomes. For this reason, early identification of bloodstream infections, timely institution of appropriate antibiotics, and improved infection control measures are important strategies to reduce the morbidity and mortality associated with pediatric bloodstream infections [2].

The risk factors that elevate the susceptibility of neonates and children to septicemia fall under categories, some related to the perinatal conditions, and some related to healthcare insecurity. Some of the risk factors for neonates include premature rupture or prolonged rupture of membranes, prematurity, and low birth weight, any of which can impact natural defenses and increase the likelihood of microbial invasion. In addition to prenatal conditions, maternal conditions, such as poor nutrition and urinary tract infections, also represent an important role in vertical transmission of pathogens [3].

Congenital defects and birth asphyxia also deplete the physiological reserve of neonates and increase the risk for life-threatening bloodstream infections. The risk underscores the need for preventive maternal health programs and appropriate neonatal intervention to reduce the burden of septicemia in this population [4]. Older children face additional risk from a combination of environmental exposure, underlying medical conditions, and influence of therapy. Infants and children with severe injury, and those on prolonged antibacterial treatment are specifically at increased risk due to either impaired immune defense, or changes in microbiota.

Systemic immunity is weakened by malnutrition and the presence of chronic medical conditions such as diabetes or congenital heart disease, while even more weakened by immunosuppressive therapy. At-risk populations may see more frequent cases of polymicrobial sepsis and its combination with multi-organ failure associated with intravascular catheters, gastrointestinal disease, neutropenia, or malignancies [5]. Polymicrobial cases are associated with not only more severity of illness, but also a larger challenge for diagnosis and treatment due to multi-organ involvement and the pattern of resistance leading to the need for close monitoring and alterations to therapy.

Bloodstream infections involve a variety of bacteria, with the most common bacteria belonging to certain groups, such as the group's streptococci, enterococci, staphylococci, or Enterobacteriaceae. Bacteria can originate from a variety of sources (skin, gastrointestinal tract, devices such as catheters, and IV lines) and invade the bloodstream relatively quickly under specific circumstances. For example, streptococci and enterococci may be involved in infections of surgical sites or with individuals who have a weakened immune system, while staphylococci, particularly Staphylococcus aureus, are infamously implicated in severe forms of bacteremia and sepsis [6]. Mycobacterial family, for example: Escherichia coli and Klebsiella species, have been frequently implicated in nosocomial infections and present an increasing concern of antibiotic-resistant strains.

These bloodstream infections have considerable clinical consequences and, if not recognized and treated in a timely manner, can escalate quickly to severe sepsis or septic shock. The specific type of bacterial pathogen can often direct and influence the symptomatology (or clinical scenario) and the choice of an appropriate antibiotic therapy, reinforcing the importance of recognizing the appropriate bacterial pathogen early in their health care encounters through an accurate and prompt identification of an organism and susceptibility profiling using blood culture. In addition, the emergence of multidrug-resistant strains, especially among staphylococci and Enterobacteriaceae, have created even larger challenges to the management of bloodstream infection, highlighting the balance between empirical and directed therapy [7]. Thus, it is necessary to understand the distribution of types and characteristics, to provide a guide in directed therapy of patients of such infections and improve outcomes in hospitalized patients.

Children with septicemia may show a range of non-specific but troubling signs and symptoms that may be alarming to caregivers, such as fever, tachypnea, tachycardia, malaise, lethargy, and not eating. These signs and symptoms indicate a systemic inflammatory response from the presence of bacteria or other pathogens in the bloodstream. The clinical progression of septicemia may be very rapid, often leading to more severe illness, including shock, multiple organ dysfunction, and disseminated intravascular coagulation [8]. Early recognition of these clinical signs is therefore very important because delays in diagnosis and therapy can significantly increase pediatric morbidity and mortality. Prompt therapy can reduce disease severity and improve clinical outcomes.

While clinical assessment through presentation of symptoms and physical exam allows a likely diagnosis of septicemia, it will not confirm the diagnosis of septicemia. Definitively diagnosing septicemia is reliant on diagnosis, which is isolating and identifying the pathogen responsible for the disease. The gold standard for identifying blood stream infections, which will in turn lead a clinician to the proper antimicrobial drug use, is blood cultures [9]. Monitoring for a pathogen specifically creates action by physicians monitoring antibiotic susceptibility patterns, effective regimens, and downstream effect of treatment. Management of pediatric septicemia is grounded in balance of attentive clinical judgement as well as physician decision making.

The current research project aims to evaluate the bacterial etiology of bloodstream infection in newborn and pediatric groups, to identify the predominant pathogens responsible for septicemia and to analyze patterns of antimicrobial susceptibility. The study will offer an understanding of the clinical features at the time of blood culture, which may facilitate early diagnosis, offer better guidance for empirical and targeted treatment, provide insight into

changing patterns of resistance, and contribute to increased management and decreased morbidity and mortality of children with bloodstream infection.

Methodology

Study Design: This was a hospital-based observation study designed to see the blood culture isolates and their patterns of antibiotic susceptibility in infants with suspected septicemia, clinically.

Study Area: The study was carried out in the Department of Microbiology at Patna Medical College and Hospital, Patna, Bihar, India.

Study Participants

Inclusion Criteria:

- Neonates who were aged 1 day to 28 days.
- Admitted to the hospital with clinical signs suggestive of septicemia (e.g., fever, lethargy, refusal to feed, respiratory distress).
- Parents/guardians who provided informed consent for participation.

Exclusion Criteria:

- Out-patient neonates.
- Neonates who received antibiotic treatment prior to hospital admission.
- Neonates with congenital anomalies incompatible with life.
- Neonates with incomplete or missing clinical data.

Sample Size: A total of 90 newborns that met the inclusion criteria were included in the study.

Study Duration: The study was conducted over one year from January 2024 to December 2024.

Procedure: Blood samples were obtained according to aseptic techniques. The venous site was cleaned with 70% alcohol before using povidone iodine. Blood was inoculated in commercially available BACTEC culture bottles at recommended volumes. Positive cultures were Gram stained immediately, and results were sent to the treating physician as outlined in the panic reporting protocol. Sub-cultures were performed on chocolate, blood, and Bromcresol purple agar plates, and incubated under appropriate conditions. Bacterial isolates were identified based on colony morphology, Gram stain, and biochemical tests including API identification strips. Antibiotic susceptibility testing was performed according to CLSI guidelines, using the disk diffusion method, testing classes of antibiotics including aminoglycosides, beta-lactams, cephalosporins, penicillins, fluoroquinolones, glycopeptides, macrolides, phosphonic acid derivatives, streptogramins, and sulfonamides. ESBL producing Gram-negative bacteria and MRSA were identified using the combination disk method and cefoxitin disk diffusion respectively. All quality control assessments were performed using standard ATCC strains.

Statistical Analysis: Data was entered and analyzed using Microsoft Excel. Qualitative variables were expressed as frequencies and percentages. Data analysis included transcription, preliminary inspection, content analysis, and interpretation. The study was approved by the institutional ethics committee.

Result

Table 1 demonstrates Staphylococcus aureus was the most common pathogen, representing 51.1% of the isolates. Coagulase-negative staphylococci and Klebsiella pneumoniae were each equally represented at 12.2%. Salmonella typhi was identified in 8.9% of cases, while Pseudomonas aeruginosa accounted for 6.7%. Less frequently isolated organisms included Acinetobacter species (3.3%), Escherichia coli, Enterobacter species, Proteus mirabilis, Viridans streptococci, and a mixed infection of Klebsiella sp. and Enterococcus sp., each representing 1.1% of the isolates. Overall, Gram-positive bacteria, particularly Staphylococcus species, dominated the bloodstream infections, whereas Gram-negative organisms were less common but still significant contributors to pediatric septicemia.

Table 1: Distribution and frequency of bacteria recovered from blood cultures (n = 90)

Bacterial isolates	Number (%)
Staphylococcus aureus	46 (51.1)
Coagulase negative staphylococci	11 (12.2)
Klebsiella pneumoniae	11 (12.2)
Salmonella typhi	8 (8.9)
Pseudomonas aeruginosa	6 (6.7)
Acinetobacter sp.	3 (3.3)
Escherichia coli	1 (1.1)
Enterobacter species	1 (1.1)
Proteus mirabilis	1 (1.1)
Viridans streptococci	1 (1.1)
Mixed (Klebsiella sp. and Enterococcus sp.)	1 (1.1)
Total	**90**

Table 2 presents the antibacterial resistance patterns of 90 Gram-positive bloodstream isolates, including S. aureus, coagulase-negative staphylococci (CoNS), and E. faecalis. Among the 60 S. aureus isolates, the highest resistance was observed to penicillin/ampicillin (63.3%), followed by oxacillin (30%), while no resistance was detected to vancomycin and low resistance rates were seen for gentamicin (5%) and amikacin (3.3%). In CoNS (n=25), penicillin resistance was 40%, oxacillin 32%, and notable resistance was observed to gentamicin (48%), whereas vancomycin resistance remained relatively low at 20%. All E. faecalis isolates (n=5) were resistant to penicillin, with 20% showing resistance to ceftriaxone, vancomycin, and gentamicin, and 40% exhibiting high-level aminoglycoside resistance. Overall, the data indicates that penicillin resistance is prevalent among Gram-positive bloodstream isolates, while vancomycin remains largely effective, though aminoglycoside resistance is emerging, particularly in E. faecalis and CoNS.

Table 2: Gram-positive bloodstream isolates' pattern of antibacterial resistance (n = 90)

Organism (n)	P/A n (%)	Ox n (%)	Cf n (%)	Va n (%)	G n (%)	Ce n (%)	Ak n (%)	Co n (%)
S. aureus (n=60)	38 (63.3)	18 (30.0)	7 (11.7)	0 (0)	3 (5.0)	7 (11.7)	2 (3.3)	7 (11.7)
Coagulase-negative staphylococci (n=25)	10 (40.0)	8 (32.0)	3 (12.0)	5 (20.0)	12 (48.0)	5 (20.0)	0 (0)	NT
E. faecalis (n=5)	5 (100)	NT	1 (20.0)	1 (20.0)	1 (20.0)	High-level aminoglycoside (HLA) 2 (40.0)	0 (0)	NT

Table 3 reveals significant variability among the bacterial groups. In Enterobacteriaceae (excluding S. typhi, n=40), resistance to antibiotic A (Ampicillin) was the highest resistance (70%). Enterobacteriaceae reported moderate resistance to Ca (Ceftazidime) (55%) followed by Ac (Amoxicillin Clavulanic acid) and Ce (Ceftriaxone) (45% each). Furthermore, Enterobacteriaceae exhibited less resistance to Cn (Gentamicin), Cf (Ciprofloxacin), and Pt (Piperacillin-Tazobactam) (7.5%–37.5%) and were completely susceptible to G (Imipenem) and Ak (Amikacin). Among S. typhi isolates (n=20), antibiotic A (45%) provided the most resistance followed limited resistance to Ac (Amoxicillin Clavulanic acid); Cu (Cefuroxime); and Cf (Ciprofloxacin) (15% each). S. typhi was completely susceptible to Ce (Ceftriaxone), C (Chloramphenicol), G (Imipenem), Ak (Amikacin), Co (Colistin), Pt (Piperacillin-Tazobactam), and I. Results in non-fermenters (n=30) showed resistance of A (Ampicillin) (73.3%) and Ca (Ceftazidime) (63.3%) as the most resistance again, moderate resistance was present for Ce (Ceftriaxone) (36.7%) and Co (Colistin) (50%), and little resistance was observed for Pt (Piperacillin-Tazobactam) (13.3%), with full susceptibility to G (Imipenem); Ak (Amikacin); Ac (Amoxicillin Clavulanic acid); Cn (Gentamicin); Cu (Cefuroxime); Cf (Ciprofloxacin); and I (Meropenem). Overall antibiotic A (Ampicillin) had the most resistance across groups and aminoglycosides (Ak) and some of the other agents had good activity; indicated the need for specific therapy based on bacteria susceptibility.

Table 3: Gram-negative bloodstream isolates' trend of antibacterial resistance (n=90)

Antibiotic	Enterobacteriaceae except S. typhi (n=40)	S. typhi (n=20)	Non-fermenters (n=30)
A (Ampicillin)	28 (70%)	9 (45%)	22 (73.3%)
Ac (Amoxicillin Clavulanic acid)	18 (45%)	3 (15%)	NT
Cn (Gentamicin)	15 (37.5%)	NT	NT
Cu (Cefuroxime)	NT	3 (15%)	NT
Ce (Ceftriaxone)	18 (45%)	0	11 (36.7%)
Ca (Ceftazidime)	22 (55%)	NT	19 (63.3%)
G (Imipenem)	3 (7.5%)	0	0
Ak (Amikacin)	0	0	0
Cf (Ciprofloxacin)	3 (7.5%)	3 (15%)	0
C (Chloramphenicol)	NT	0	0
Co (Colistin)	NT	0	15 (50%)
Pt (Piperacillin-Tazobactam)	3 (7.5%)	NT	4 (13.3%)
I (Meropenem)	0	NT	0

Discussion

This study indicates that Staphylococcus aureus is the most prevalent pathogen for blood stream infections affecting pediatric populations with 51.1% of all isolates being S. aureus. The next most frequent isolates were coagulase-negative staphylococci (CoNS) and Klebsiella pneumoniae, with both found in 12.2% of cases, followed by Salmonella typhi and Pseudomonas aeruginosa, with 8.9% and 6.7%, respectively. Isolates that represented an even smaller proportion of overall counts included Acinetobacter species, Escherichia coli, Enterobacter species, Proteus mirabilis, Viridans streptococci and a mixed infection of Klebsiella sp. and Enterococcus sp. (1.1% each). These findings are consistent with earlier studies reporting the predominance of Gram-positive organisms, particularly S. aureus, in pediatric septicemia, and while Gram-negative organisms are of clinical importance they still overall account for fewer isolates than Gram-positive pathogens. coagulase-negative staphylococci 10 (12.3%), K. pneumoniae 10 (12.3%), and S. aureus 41 (50.61%), were the most frequently isolated blood cultures in our investigation. The results align with those of earlier research (Onipede et al., 2009) [10].

Analysis of Gram-positive individual strains showed high levels of resistance against penicillin/ampicillin, especially against S. aureus (63.3%) and CoNS (40%). There was also a considerable rate of oxacillin resistance, most notably with CoNS (32%) and S. aureus (30%), suggesting contamination with methicillin-resistant organisms. Overall, the level of aminoglycoside resistance among S. aureus isolates was comparatively low, but was higher in CoNS and E. faecalis, including very high levels of aminoglycoside resistance in 40% of E. faecalis isolates. Importantly, vancomycin remained active against most Gram-positive organisms with excellent activity, which highlights how it should continue to provide a critical role in treatment for Gram-positive organisms and infections. They analyzed 410 blood samples that were positive for septicaemia in clinically suspected instances. The frequency of bacterial isolation from children's blood cultures in this study (12.2%) is consistent with several previous investigations conducted in different countries (Karki et al., 2010) [11]. Coagulase-negative Staphylococcus, Klebsiella, Staphylococcus aureus, Enterobacter, E. coli, Pseudomonas, Streptococcus and Serratia were among the frequently recovered bacterial species from blood cultures in this study. Also, 14 blood samples were utilized for isolation of Candida. The results of the study suggest infections with these agents pose a significant threat to child survival in the context of this study and are consistent with some findings of previous studies.

Enterobacteriaceae (with the exception of S. typhi), the Gram-negative isolates exhibited high resistance to antimicrobial A (ampicillin) (70%) and moderate susceptibility to Ca (ceftazidime), Ac (amoxicillin clavulanic acid), and Ce (ceftriaxone). The resistance of S. typhi was comparatively lesser, and several antibiotics, including aminoglycosides, had excellent susceptibility. Non-fermenters, including Pseudomonas and Acinetobacter species, had high resistance to various commonly used antibiotics, but demonstrated increased susceptibility to aminoglycosides and some of the cephalosporins. These results demonstrated the need for species-specific susceptibility testing to aid in guiding empiric therapy in pediatric septicemia. Similar findings of resistance pattern among species of Pseudomonas and other gram-negative bacilli were found in Rahman et al., (2002) [12] study in Pakistan. Data from Joshi et al., (2000) [13] indicated that gram-negative bacteria were predominant in India and were resistant to varying degrees to aminoglycosides (23–69%) and to cephalosporins (25–75%).

The growing resistance to use during antibiotics, such as penicillins and antibiotic A (Amipillin) in bloodstream infections among children, clearly demonstrates the growing concern of antimicrobial resisted (AMR). Vancomycin and aminoglycosides are still effective antibiotics, however, resistance has emerged in Community robbing Staphylococci (CoNS) and Enterococcus faecalis, while very high resistance has been observed among non-fermenters, which raises the source of potential treatment failure where therapy is not initiated or continued culture and sensitivity. There is a need for routine surveillance, are antimicrobial stewardship programme in paediatrics units. We processed 185 blood samples from blood septicaemia cases that were clinically diagnosed. The 43.78% rate of bacterial isolation from blood cultures from this study is like an earlier study by Meremikwu et al., (2005) [14]. The increased rate of isolation for children and neonates could be because they are more immunocompromised.

he variety of pathogens noted in this study (e.g., Gram-positive cocci, Gram-negative rods, and non-fermenters) further supports the multifactorial cause of pediatric septicemia, which includes, but is not limited to, aspects of hospital exposure, invasive procedures, and comorbidities. Timely recognition of the causative pathogen and appropriate antibiotic therapy is still important in reducing morbidity, length of hospital stay and mortality from bloodstream infections in children. This study concluded that Pseudomonas species were more resistant to cephalosporins than to quinolones and aminoglycosides. The study by Movahedian et al., (2006) [15] found a similar pattern of antibiotic resistance in pseudomonas in sepsis.

The study determined that S. aureus is still responsible for most cases of pediatric septicemia, with Gram-negative bacteria being responsible for a sizeable burden as well. The resistance patterns

observed highlighted the need for ongoing monitoring of antimicrobial resistance and optimization of antibiotic prescribing. Strategies to improve the clinical outcomes of pediatric bloodstream infections include optimizing empiric therapy using local susceptibility data and initiating culture-directed therapy as early as possible.

Conclusion

The present study demonstrates that most bloodstream infections in neonates and children are associated with Gram-positive bacteria with Staphylococcus aureus being the most common isolate and coagulase-negative staphylococci and Klebsiella pneumoniae becoming increasingly uncommon. Gram-negative pathogens such as Salmonella typhi, Pseudomonas aeruginosa, and other Enterobacteriaceae continue to have a significant contribution to pediatric septicemia. In addition, we discuss the high prevalence of antimicrobial resistance to penicillin, in addition to the frequent prescribing of antibiotics, except for vancomycin and the aminoglycosides that continue to be effective against most Gram-positive isolates. Conversely, resistance patterns in the non-fermenters and alarmingly high levels of aminoglycoside resistance among E. faecalis and CoNS make therapy and management increasingly difficult. Overall, we emphasize the need for timeliness of blood culture diagnostics, species-specific susceptibility testing, and an appropriate approach to antibiotic optimization for the start of empirical therapy, minimalize failure to treat, and improve clinical outcomes and survival of pediatric patients with bloodstream infections.

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