Original Article

Evolving Antibiotic Resistance in Neonatal Sepsis in a Tertiary Neonatal Care Unit in Eastern India: A Prospective Study

Turiyo Adhikary[1], Tapabrata Chatterjee[2]

From, [1]Senior Resident, [2]Professor, Department of Paediatrics, Vivekananda Institute of Medical Science, Kolkata, India

ABSTRACT

Introduction: The Indian National Neonatology Forum (NNF) advises using ampicillin and gentamicin as the initial empirical antibiotics for treating neonatal sepsis. However, with the ongoing rise in antibiotic resistance among pathogens, there is a pressing need to update and review the current antibiotic protocols. **Aims and Objectives:** This study aims to describe the spectrum of antibiotic sensitivity pattern of common pathogens causing blood culture-positive neonatal sepsis and propose a uniform first-line empirical antibiotic against them. **Methodology**: The study was conducted in the Neonatal Intensive Care Unit (NICU) over a period of 2 years. A total of 100 inborn neonates, who had blood culture proven sepsis, were included in the study. The antibiotic susceptibility pattern against commonly used antibiotics like Ampicillin, Gentamicin, Piperacillin-tazobactam, Amikacin and Meropenem were analysed. **Results:** The most common organism to be isolated from blood culture was *Klebsiella pneumoniae* (32%). None of them were sensitive to Ampicillin, and only 43.75% were sensitive to Gentamicin. Instead, Piperacillin tazobactam had 50% sensitivity, and Amikacin had 81.25% sensitivity. **Conclusion:** The study highlights the changing trends of antibiotic sensitivity patterns and the high degree of resistance to the conventional Ampicillin–Gentamicin combination. Instead, Piperacillin tazobactam - Amikacin can be a better alternative choice of empirical antibiotic.

Key words: Neonatal sepsis, Antimicrobial Resistance, Empirical antibiotic, Antibiotic stewardship

Neonatal sepsis has been a leading cause of neonatal mortality in developing countries like India, with literature reporting an incidence of 30 per thousand live births, approximately contributing to 19% of all neonatal deaths [1, 2]. The causative organisms in neonatal sepsis vary from place to place. Although Gram-positive organisms like Group B Streptococcus contribute to the majority of sepsis in the Western world, Indian studies show a pre dominance of Gram-negative organisms, particularly Klebsiella pneumoniae [3]. Various studies have been done in the past to determine the efficacy of different antibiotics in neonatal sepsis, and different guidelines were proposed addressing the appropriate choice of first-line empirical antibiotic. Most International guidelines, along with the Indian National Neonatology Forum (NNF), recommend a combination of β-lactams (usually Ampicillin) and an aminoglycoside, particularly Gentamicin, as the initial choice of antibiotic in neonatal sepsis [4].

However, recent studies have shown a very high level of resistance to benzyl penicillin and ampicillin [5]. The widespread emergence and spread of multidrug-resistant organisms (MDRO) such as Extended spectrum beta lactamase producers (ESBL), Gentamicin-resistant Gram-negative bacteria, Carbapenem-resistant Enterobacteriaceae (CRE), Methicillin-resistant Staphylococcus aureus (MRSA), Vancomycin-resistant enterococcus (VRE) pose a significant threat to global health [6]. The alarming antimicrobial resistance threatens the return of the pre-antibiotic era in Indian NICUs. Therefore, continuous assessment of antimicrobial susceptibility patterns should be done to select the appropriate antibiotic therapy as well as to reduce antimicrobial resistance, thereby improving sepsis outcomes. The present study is intended to highlight the changing trends in sensitivity patterns of the etiologic agents against common antimicrobial drugs and the need to revise the existing antibiotic policy.

METHODS

This prospective observational study was conducted in the NICU of a tertiary neonatal care unit in Kolkata for a period of 24 months from July 2022 to June 2024 after obtaining necessary institutional ethical approval (ECR/62/Inst/WB/2013/RR-19). During the study period, 257 inborn neonates under 28 days of age, clinically diagnosed with sepsis and for whom the treating physician requested blood culture and sensitivity testing, were included in the study.

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Correspondence to: Turiyo Adhikary, Department of Paediatrics, Vivekananda Institute of Medical Science, Kolkata, India

Email: madanadhikari62@gmail.com

Taking all universal precautions, blood was collected from them into sterile liquid culture bottles and immediately transported to the laboratory for incubation. After detecting positive growth, the lab technician performed Gram staining and subcultured the culture broth by inoculating it onto blood agar, chocolate agar, and MacConkey agar plates for further identification and isolation of bacteria. On the next day, the culture plate would be read for their colony characteristics, and additional biochemical tests were conducted to identify the microorganisms, and their antibiotic susceptibility was determined using the Kirby-Bauer disc diffusion method.

Kirby-Bauer disc diffusion technique involves placing an antibiotic-impregnated paper disc on an agar plate, seeded with a bacterial lawn and observing the formation of a zone of inhibition around the discs after incubation [7]. The organisms were then classified as Sensitive, Intermediate or Resistant based on the standardised Minimum Inhibitory Concentration (MIC) values. "Sensitive" implies that the organism is inhibited by the serum concentration of the drug that is achieved using the usual dosage; "Intermediate" implies that the organisms are inhibited only when higher concentrations than the usually recommended dosages are achievable; and "Resistant" implies that the organisms are resistant to the usually achievable serum drug levels [8].

These interpretive standards have been established by the Clinical and Laboratory Standards Institute (CLSI). As per protocol, the blood culture bottles that did not reveal any growth even after remaining in the incubator for 5 days were taken out, and the report was declared negative. All the culture sensitivity reports were collected, and the sensitivity patterns of the common microorganisms causing neonatal sepsis were analysed to reach our conclusions.

All such cases of probable sepsis that were declared negative by the lab were excluded and cases that had a positive growth in blood culture out of the initial 257 cases were included in this study. The sample size was set at 100, assuming the confidence interval to be 95%, prevalence of neonatal sepsis to be 30% [1] and considering a relative margin of error of 10%. The collected data was then entered into Microsoft Excel and analysed using the OpenEpi, Version 3.

RESULTS

There was a predominance of Gram-negative organisms (70%) headed by *Klebsiella pneumoniae* (32%), followed by *Serratia marcescens* and *Acinetobacter baumanii*, accounting for 12% each. *Escherichia coli* only accounted for 6% of cases (Table 1). The gram-positive groups (30%) were largely due to *Staphylococcus hemolyticus* (12%) and *Methicillin-resistant Staphylococcus aureus (MRSA)* (8%). *Staphylococcus aureus* was detected in 5% of cases. We also found some novel agents like *Elizabethkingia meningoseptica* (2%), *Streptococcus mitis* (2%), *Achromobacter xylosoxidans* (1%), which are considered as rarer causes of neonatal sepsis (Table 1).

Table 1: Distribution of microorganisms isolated from blood culture in neonatal sepsis according to Gram stain

Bacterial strain (n)	*Name of the bacteria*	Frequency (%)
Gram-negative (70)	*Klebsiella pneumoniae*	32
	Serratia marcescens	12
	Acinetobacter baumanni	12
	Escherichia coli	6
	Pseudomonas aeruginosa	4
	Elizabethkingia meningoseptica	2
	Achromobacter xylosoxidans	1
	Acinetobacter hemolyticus	1
Gram positive (30)	*Staphylococcus aureus*	5
	Staphylococcus hemolyticus	12
	Methicillin-resistant Staphylococcus aureus	8
	Streptococcus mitis	2
	Enterococcus faecalis	3
Total (100)		100

The most common organism, *Klebsiella pneumonia*e was 100% resistant to conventionally used ampicillin and 56.25% resistant to gentamicin. On the contrary, Piperacillin tazobactam demonstrated a 50% sensitivity, and Amikacin had a higher 81.25% sensitivity to *Klebsiella*.

Table 2: Antibiotic Susceptibility pattern of *Klebsiella pneumoniae (n=32)*

Resistance Pattern		Sensitivity Pattern	
Antibiotic	**n (%)**	**Antibiotic**	**n (%)**
Ampicillin	32 (100%)	Ampicillin	0 (0%)
Gentamycin	18 (56.25%)	Gentamycin	14 (43.75%)
Piperacillin	16 (50%)	Piperacillin	16 (50%)
Amikacin	6 (18.75%)	Amikacin	26 (81.25%)
Meropenem	12 (37.5%)	Meropenem	20 (62.5%)

Similarly, *Serratia marcescens* and *Acinetobacter baumanii* were 100% resistant to Ampicillin, whereas Piperacillin-tazobactam had 33% sensitivity for *S. marcescens* and 83% sensitivity for *A. baumannii*. Besides, Amikacin demonstrated a higher sensitivity for *S. marcescens* (100%) and *A. baumannii* (50%) than gentamicin, which had 67% and 0% sensitivity for them, respectively (Tables 3 and 4).

Table 3: Antibiotic Susceptibility pattern of *Serratia marcescens (n=12)*

Resistance Pattern		Sensitivity Pattern	
Antibiotic	**n (%)**	**Antibiotic**	**n (%)**
Ampicillin	12 (100%)	Ampicillin	0 (0%)
Gentamycin	4 (33%)	Gentamycin	8 (67%)
Piperacillin	8 (67%)	Piperacillin	4 (33%)
Amikacin	0 (0%)	Amikacin	12 (100%)
Meropenem	0 (0%)	Meropenem	12 (100%)

Table 4: Antibiotic Susceptibility pattern of *Acinetobacter baumanni (n=12)*

Resistance Pattern		Sensitivity Pattern	
Antibiotic	**n (%)**	**Antibiotic**	**n (%)**
Ampicillin	12 (100%)	Ampicillin	0 (0%)
Gentamycin	12 (100%)	Gentamycin	0 (0%)
Piperacillin	2 (17%)	Piperacillin	10 (83%)
Amikacin	6 (50%)	Amikacin	6 (50%)
Meropenem	2 (17 %)	Meropenem	10 (83%)

As far as gram positive *Staphylococcus hemolyticus* is concerned, the isolate had 100% resistant to ampicillin but strikingly none of them were resistant to Piperacillin tazobactam (Table 5).

Table 5: Antibiotic Susceptibility pattern of *Staphylococcus hemolyticus (n=12)*

Resistance Pattern		Sensitivity Pattern	
Antibiotic	**n (%)**	**Antibiotic**	**n (%)**
Ampicillin	12 (100%)	Ampicillin	0 (0%)
Gentamycin	12 (100%)	Gentamycin	0 (0%)
Piperacillin	0 (0%)	Piperacillin	12 (100%)
Amikacin	12 (100%)	Amikacin	0 (0%)
Meropenem	2 (17%)	Meropenem	10 (83%)

DISCUSSION

In the western world, the commonest organism for neonatal sepsis has been *Group B Streptococcus* [9], which has been universally sensitive to benzyl penicillin. However, the majority of Indian data, along with this study, points out a higher prevalence of Gram-negative sepsis among neonatal nurseries, particularly *Klebsiella pneumoniae* [10, 11]. *Group B Streptococci* were isolated rarely in the Indian subcontinent. A large multicentric study in Delhi by investigators of the Delhi Neonatal Infection Study (DeNIS) showed *Acinetobacter*, *Klebsiella* and *Escherichia coli* as major pathogens [12]. In contrast, Sharma P et al. showed a preponderance of gram-positive organisms, of which *Staphylococcus aureus* was the most prevalent [13]. Another study by Shrestha et al. in Nepal showed Coagulase-negative Staphylococci (CONS) as a major pathogen [14]. The disparity in major isolates may be attributed to variations in study context, population characteristics and hand hygiene practices.

A rare opportunistic nosocomial pathogen, *Serratia marcescens*, has emerged as a common isolate in our cohort. Although a few recent studies from India and other low-income and middle-income countries did report it as one of the isolates [15, 16], none have so far reported such a high degree of dominance. The growing burden of *Serratia* sepsis might pose an impending threat in upcoming years due to its rapid clonal expansion and antibiotic evasion property, streamlined by suboptimal infection control practices, including inadequate hand hygiene compliance. Our results are mostly consistent with other studies in the Indian literature regarding the high degree of resistance to the frequently used first-line options, such as Ampicillin and Gentamicin.

In our study, Klebsiella isolates exhibited complete (100%) resistance to conventional ampicillin. In contrast, sensitivity to piperacillin and amikacin was observed in 50% and 81.25% of Klebsiella sepsis cases, respectively. A study conducted in Orissa by Panigrahi P et al. similarly reported a very high level of resistance to ampicillin, while resistance to amikacin and gentamicin was found to be extremely low [5]. In another study in a tertiary care hospital in South India, it was found that *Klebsiella*, the most common agent causing neonatal sepsis, was resistant to most common antibiotics tested except Amikacin and Meropenem. Hence recommends the use of Amikacin in gram-negative neonatal sepsis [17, 18].

Though in most of these studies, Amikacin sensitivity is reasonably high, ideally for neonatal sepsis, we prefer to use a combination therapy for initial coverage [19]. Furthermore, aminoglycosides may be associated with important adverse effects, and they require frequent monitoring of blood levels [20]. However, most government hospitals in Kolkata do not have the facility to perform drug level monitoring. There is often a question of blood-brain barrier penetration of Amikacin [21], temporarily masking the brain infection, hence few physicians do not prefer to use it empirically in neonatal sepsis. But the literatures suggest that early onset neonatal sepsis (EONS) is mostly a respiratory involvement [22] rather than a CNS involvement. In late onset neonatal sepsis (LONS), it is prudent to do a lumbar puncture and based on cytology, we may shift to higher antibiotics like Meropenem.

Our study has several limitations that warrant consideration. Firstly, this is a single-centre study with a relatively small sample size. Secondly, although rigorous skin preparation protocols were adhered to before blood culture collection, there is always a chance of contamination leading to false-positive growth. Lastly, sensitivity against other antibiotics like fluoroquinolones and cephalosporins, which are infrequently used in other settings, has not been studied here.

CONCLUSION

This study highlights the high-grade resistance to the conventional Ampicillin and Gentamicin, which are still being commonly used as a first-line empirical antibiotic in neonatal sepsis. Instead, Piperacillin tazobactam and Amikacin can be a better choice of first-line empirical antibiotic. This study also shows the growing concern of resistance to higher antibiotics like Meropenem, underscoring the need for judicious antibiotic use and enhanced surveillance.

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