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Emerging Antibiotic Resistance Pattern in a Neonatal Intensive Care Unit in Pokhara, Nepal

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ABSTRACT

Background: Treating neonatal sepsis in Nepal remains difficult given the high rates of antimicrobial resistance. The objective of this study is to determine the antibiotic resistance pattern of culture-proven infections in neonates admitted to a neonatal intensive care unit.

Methods: This cross-sectional prospective observational study was performed at the Neonatal Intensive Care Unit of Pokhara Academy of Health Sciences from 15th july 2022 to 15th july 2023. We included all neonates admitted with positive cultures grown from blood, cerebrospinal fluid, endotracheal tube, and pus. Demographic, clinical, and microbiologic data were collected from the medical record. We reviewed antimicrobial susceptibility testing of all isolates.

Results: There were 51 culture-positive infections among 1327 neonates admitted, among which 23 cases from blood culture, 2 cases from cerebrospinal fluid, 14 cases from endotracheal tube samples, and 12 cases from pus samples. Gram-negative infections were predominant amounting to 35 (68.6%) including *Pseudomonas* in 12 (23.5%), and *Acinetobacter species in* 9 (17.6%) cases. Gram-positive infections were seen in 14 (27.4%) in which Methicillin-Resistant *Staphylococcus aureus* accounted for 6 (11.8%) cases. Yeast cells other than *Candida albicans* accounted for two (5.4%). For all Gram-negative isolates, resistance to Third-generation cephalosporin and aminoglycosides was reported in 75.0% (12 of 16 isolates tested) and 87.0% (24/31), respectively. Fluoroquinolone resistance was seen in 61% (8/13), resistance to penicillin was 59.3% (10/19), and resistance to carbapenem was in 100.0% (7/7) cases.

Conclusions: There were high rates of antimicrobial resistance even with the reserved drugs among gram-negative pathogens. This alarms for the need for rationale prescribing of antimicrobials.

Keywords: Antimicrobial resistance; bloodstream infection; meningitis, neonatal sepsis.

INTRODUCTION

Neonatal sepsis causes a large proportion of all neonatal deaths worldwide.[1] Nepal reports higher rates of neonatal infections among lower and middle-income countries (LMICs),[2]and a recent review of neonatal infections in Nepal reported approximately 40 times higher incidence rates of neonatal infection and double the mortality rates.[2,3] Despite improvements in infant mortality and under-5 mortality, from previous surveys, the neonatal mortality (NMR) rate has remained unchanged since 2016, and currently, it is 21/1000 live births in Nepal.[4] Now it is very difficult to treat neonatal sepsis in NICU as antimicrobial resistance (AMR) cases are emerging more frequently and broader spectrum antibiotics are more common in their treatment.[5]

A recent systematic review from 2019 on AMR in neonatal sepsis in South Asia (including 69 studies from India) shows that challenges are ongoing and more widespread.[6] This review reported rates of resistance between 67% and 86% to first-line drugs such as ampicillin, gentamicin, and third-generation cephalosporins, as well as high (50 to 70%) degrees of MDR in isolates throughout India and South Asia.

Given the worsening trends in AMR in our country also, the objective of this study is to determine the antibiotic resistance pattern of culture-proven infections in neonates admitted to a neonatal intensive care unit (NICU) in western Nepal.

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METHODS

This is a cross-sectional prospective observational study conducted in the NICU, of Pokhara Academy of Health Sciences (PoAHS), a tertiary care referral center of Gandaki Province, Nepal, with a 30-bedded Level II & III NICU.

All neonates admitted to the NICU between July 15, 2022, and July 15, 2023, with a documented positive culture from blood, cerebrospinal fluid (CSF), endotracheal tube(ETT), and pus were included in this study and babies whose samples could not be taken, and whose culture were negative were excluded.

We have included all 51 culture-positive cases during the study period following the census sampling technique.

Culture specimens were collected and processed according to routine laboratory standards in the nationally accredited microbiology laboratory of PoAHS. Microbiological data including isolated organisms and results of antibiotic susceptibility test (AST) was manually extracted and collected from the hospital's electronic medical record.

The CSF samples were examined microscopically by Gram stain and was further inoculated on Chocolate, Blood and MacConkey agar. Blood samples were first collected in Brain Heart Infusion Broth, which was incubated overnight and were sub-cultured in Blood agar and MacConkey agar in the following day and the subculturing was repeated in the next day as well. The incubation of the BHI broth was extended for five to seven days. Furthermore, pus and endotracheal tube tip and aspirates were inoculated in Blood agar and MacConkey agar. All the inoculated samples were incubated at 35°-37°C. Bacterial isolates if grown were further identified on the basis of their colony characteristics, gram reaction, morphology, biochemical features. Antibiotic sensitivity testing was then performed with Kirby Bauer Disk Diffusion method following CLSI 2021 guidelines. Data was entered into MS Excel and analyzed with SPSS-23. This study was approved by the Institutional Review Committee (IRC) of PoAHS -ID No:190/080.

RESULTS

Among a total of 1327 samples sent for the culture test during the study period, 51 (3.84 %) had culture-positive microorganisms. We have presented the profile of the 51 cases.

Table 1. Distribution of sex among patients.

Distribution of Sex	Gram Positive n(%)	Gram Negative n(%)	Fungi n(%)	Total n(%)
Female	5(35.7)	14(40.0)	1(50.0)	20(39.2)
Male	9(64.3)	21(60.0)	1(50.0)	31(60.8)
Total	9(5.0)	16(95.0)	25(100.0)	51(100.0)

Table 1. shows the sex distribution of patients among the population. The majority of the population was male 31(60.8%) followed by females 20(39.2%).

Table 2. Type of Sample according to the organisms.

Organisms	Type of sample				Total N (%)
	Blood	CSF	ETT	Pus	
Pseudomonas aeruginosa	2	0	9	1	12 (23.5)
Acinetobacter species	6	0	0	3	9 (17.6)
Klebsiella pneumoniae	2	1	0	2	5 (9.8)
Citrobacter freundii	2	0	1	1	4 (7.8)
Escherichia coli	1	0	1	1	3 (5.8)
Enterobacter species	1	0	0	0	1 (1.9)
Serratia marcescens	1	0	0	0	1 (1.9)
Staphylococcus aureus	5	0	1	2	8 (15.7)
Methicillin Resistant *Staphylococcus aureus*	3	1	0	2	6 (11.7)
Yeast cells other than *Candida albicans*	0	0	2	0	2 (3.9)
Total	23	2	14	12	51 (100)

Among the culture-positive cases, 23 cases from blood culture, 14 cases from Endotracheal Tube Tip (ETT) samples, 12 cases from pus samples, and two cases from Cerebrospinal Fluid (CSF) samples. The growth of organisms grown with cross tabulations with different specimens is presented in table 2.

Table 3. Distribution of pathogens in early onset and late onset culture-positive infections.

	Early Onset n=37 n (%)	Late Onset n=14 n (%)	Total N=51 n (%)
Gram Negative organisms n (%)	29(78.4)	6(42.9)	35(68.6)
Pseudomonas species	10(27.0)	2(14.3)	12(23.5)
Acinetobacter species	7(18.9)	2(14.3)	9(17.6)
Klebsiella pneumoniae	4(10.8)	0(0.0)	5(9.8)
Citrobacter freundii	4(10.8)	0(0.0)	4(7.8)
Escherichia coli	2(5.4)	0(0.0)	3(5.9)
Enterobacter species	1(2.7)	0(0.0)	1(2.0)
Serratia marcescens	1(2.0)	0(0.0)	1(2.0)
Gram Positive organisms n (%)	6(16.2)	8(57.1)	14(27.5)
Staphylococcus aureus	3(8.1)	5(35.7)	8(15.7)
MRSA	3(8.1)	3(21.4)	6(11.8)
Fungi	2(5.4)	0(0.0)	2(5.4)
Yeast cells other than Candida Albicans	2(5.4)	0(0.0)	2(5.4)

Gram-negative infections were predominant and were demonstrated in 35 (68.6%) cases.(Table 3). The most commonly isolated Gram-negative pathogens were *Pseudomonas* species in 12((23.5%) cases, *Acinetobacter* Species in 9 (17.6%), *Klebsiella pneumoniae in 5* (9.8%) *Enterobacter* and *Serratia marcescens* accounted for 1 (2.0%). *Staphylococcus aureus* accounted for 8 (15.7%) of Gram-positive infections and *MRSA* accounted for 6 (11.8%). Yeast cells other than *Candida albicans* accounted for 2 (5.4%).

Table 4. Antimicrobial susceptibility for culture-positive Gram-negative bacteria.

	Aminoglycoside n=16(%)	3rd Generation Cephalosporin n=31(%)	Fluoroquinolone N=13(%)	Penicillin n=32(%)	Carbapenems n=7 (%)
Gram-negative organism, n resistant/ isolates tested (%)	12/16(75.0)	27/31(87.0)	8/13(61.5)	19/32(59.3)	7/7(100.0)
Pseudomonas aeruginosa	6/12(50.0)	8/27(29.6)	5/8(62.5)	10/19(52.6)	4/7(57.1)
Acinetobacter species	4/12(33.3)	9/27(33.3)	1/8(12.5)	2/19(10.5)	3/7(42.9)
Citrobacter freundii	1/12(8.3)	2/27(7.4)	2/8(25.0)	2/19(10.5)	0(0)
Klebsiella pneumoniae	1/12(8.3)	4/27(14.8)	0(0)	3/19(15.8)	0(0)
Escherichia coli	0(0)	3/27(11.1)	0(0)	1/19(5.3)	0(0)
Enterobacter species	0(0)	0(0)	0(0)	1/19(5.3)	0(0)
Serratia marcescens	0(0)	1/27(3.7)	0(0)	0(0)	0(0)
Gram Positive organism, n resistant/ isolates tested (%)	4/16(25.0)	4/31(13.0)	5/13(38.4)	13/32(40.6)	0/0(0.0)
MRSA	4/4(100.0)	0/4(0.0)	3/5(60.0)	5/32(15.6)	0/0(0.0)
Staphylococcus Aureus	0/4(0.0)	4/4(100.0)	2/5(40.0)	8/32(25.0)	0/0(0.0)

For all Gram-negative bacteria, resistance to commonly used antibiotics as of the penicillin group was 59.3% (10/19), aminoglycosides was 87.0% (24/31), and for third-generation cephalosporin 75.0% (12 of 16 isolates tested). Fluoroquinolone resistance was 61% (8/13). Even resistance to carbapenems was found 100.0% (7/7), especially for *Pseudomonas aeruginosa* and *Acinetobacter species*.

For all Gram-positive isolates resistance to penicillin was 40.6% (13/32), aminoglycosides 25.0% (4 of 16 isolates

tested), and to 3rd Generation Cephalosporin 13.0% (4/31). Fluoroquinolone resistance was 38.4% (5/13), and resistance to carbapenems was not found at all.

DISCUSSION

Neonatal sepsis is a driver of neonatal deaths worldwide especially in low- and middle-income countries such as Nepal. Changing antimicrobial resistance patterns compound the challenges of treating sepsis in neonates in resource-limited communities. Our study showed Gram-negative infections were the predominant pathogen for NNS and among them, *Pseudomonas*, *Acinetobacter, Klebsiella pneumonia, Citrobacter,* and *Enterobacter* are the most common pathogens whereas *Staphylococcus* and *MRSA* were the only gram-positive pathogens accounting for not only NNS but the terrible surge in neonatal mortality in our Hospital.

A study done in Gujrat, India showed 31.57% culture-positive NNS which was quite high in comparison to our 3.84% but most of the pathogens were gram-negative as of our study and 90% gram-negative isolates were resistant to ampicillin and gentamycin which emphasizes to update the current sepsis management of NICU.[7]

A study done in Ethiopia showed *Klebsiella pneumoniae*, the most prevalent gram-negative species followed by *Escherichia coli* but we found *Pseudomonas* spp. and *Acinetobacter* spp. more common than *Klebsiella* spp. in our study. Antibiotic resistance was more common in ampicillin (95%), gentamicin (85%), cefotaxime (87%), amikacin (8.4%), piperacillin-tazobactam (39%), and Imipenem 1 (0.8%).[8] The huge difference in the resistant pattern between gentamicin and amikacin in their study needs to be studied more.

A study done in the teaching hospital of Nepal in 2015 showed the emergence of multidrug-resistant (MDR) *Acinetobacter species* in NICU. Of the 246 *Acinetobacter* spp. isolates, 122 (49.6%) were MDR *A. baumannii,* with the majority being resistant to aminoglycosides, carbapenems, and fluoroquinolones but not to colistin and tigecycline.[9] But in our study even the carbapenems group antibiotics were resistant to isolated gram-negative bacteria which emphasizes the burden of antimicrobial resistance (AMR).

A similar, study was done in the Neonatal Care Unit (NCU) of Western Regional Hospital (which is now upgraded to the NICU of PoAHS), from 14th August 2012 to 14th July 2014 but the results showed Gram-positive *Staphylococcus aureus* was the most common organism (56%) followed by *Escherichia coli* (12%).[10] *Staphylococcus aureus* was resistant to penicillin group antibiotics in 3% of cases only but now our study showed 64.2% resistance. This addresses the gravity of AMR to our NICU and the whole country.

The review article published in 2017 also showed the prevalence of carbapenems-resistant Gram-negative bacteria like *Acinetobacter* and *Enterobacteriaceae* among countries of Southeast Asia including Nepal, Bhutan, India, and Bangladesh where the rates of resistance were found uppermost in the world.[11]

Inappropriate recommendations of antibiotics by clinicians, complex health systems, as well as extensive multinational travel probably cause the rapid spread of these microbes among hospitals. Huge political drive and effort from the concerned country, along with the coordination with WHO plays a greater role in reducing the prevalence of multidrug-resistant microbes in Southeast Asia to prevent their worldwide spread.

In addition, sufficient manpower allocation and recurrent training in the laboratory leading to appropriate sample collection and processing of the specimen can play a major role in reporting the true scenario of infection and resistance patterns in a tertiary center like PoAHS. Moreover, initiation of a functioning antimicrobial stewardship program and vigilant infection control committee advocating for strict infection control practices is indeed, the need of the hour. The limitations of this study include the absence of confirmatory cultures for potential contaminant pathogens.

CONCLUSIONS

The emergence of high rates of antimicrobial resistance (AMR), including resistance to last-resort antibiotics, among gram-negative pathogens responsible for neonatal infections is a matter of significant concern. This highlights the critical need for the rational and judicious use of antimicrobials in clinical practice. The limited availability of narrow-spectrum empiric regimens with sufficient coverage for neonatal sepsis often necessitates the use of broad-spectrum agents as initial therapy, thereby contributing to the escalation of antimicrobial resistance. As the utilization of reserved antibiotics becomes increasingly common, it is essential for clinicians to adopt more stringent antimicrobial stewardship practices. The outcomes of this study will contribute to strengthening infection prevention and control strategies within the Neonatal Intensive Care Unit (NICU) of the Pokhara Academy of Health

Sciences (PoAHS) and are expected to play a vital role in mitigating the burden of AMR in Nepal.

CONFLICTS OF INTEREST

All authors declare no conflict of interest.

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