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THE RESISTANCE PATTERN AND PHENOTYPIC DETECTION OF ESBL, MBL AND AMPC B-LACTAMASE AMONG GRAM-NEGATIVE BACTERIAL ISOLATES FROM BLOOD CULTURES OF NEONATES WITH NEONATAL SEPSIS ADMITTED IN A TERTIARY CARE HOSPITAL

Medical Microbiology

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ABSTRACT

Neonatal sepsis with Gram-negative bacteria remains to be among the important reasons of newborn mortality or morbidity increasingly exhibiting multidrug resistance through the production of β-lactamase enzymes. Early identification of resistant pathogens is important for effective antimicrobial therapy or improved clinical outcomes. To regulate the antimicrobial resistance pattern and phenotypically perceive AmpC β-lactamase, Extended-Spectrum β-Lactamase (ESBL), and Metallo-β-Lactamase (MBL) in blood cultures of newborns suspected of having neonatal sepsis yielded isolates of gram-negative bacteria. One hundred Isolates of gram-negative bacteria from blood cultures of infants suspected of having sepsis who were hospitalized to a tertiary care centre were the subject of a hospital-based cross-sectional study. Standard microbiological procedures were used to classify the isolates, and the Kirby-Bauer disc diffusion technique was used in accordance with CLSI guidelines to screen for antibiotic susceptibility. MBL, AmpC β-lactamase, and ESBL phenotypic detection production was done using standard confirmatory methods. Klebsiella pneumoniae was the predominant isolate (74%), Acinetobacter spp. (12%) and Escherichia coli (10%) came next. ESBL, MBL and AmpC production were detected in 92%, 89%, and 53% of isolates, correspondingly. Colistin demonstrated 100% susceptibility, whereas high resistance was observed against ampicillin, cephalosporins, carbapenems, fluoroquinolones, and a number of other commonly used antibiotics, suggesting widespread multidrug resistance. The research shows a high load of Gram-negative bacteria that are resistant to several antibiotics and cause neonatal sepsis, with widespread β-lactamase production limiting therapeutic options. Routine phenotypic detection, antimicrobial stewardship, continuous resistance surveillance, and evidence-based empirical antibiotic policies are essential to optimize neonatal sepsis management.

KEYWORDS

Neonatal sepsis; Gram-negative bacteria; Extended-spectrum β-lactamase; Metallo-β-lactamase; Antimicrobial resistance.

1. INTRODUCTION

Neonatal sepsis, a potentially fatal systemic infection that appears during the first 28 days of life, continues to be one of the main causes of morbidity and mortality among newborns worldwide. Sepsis arises as an outcome of the body's dysregulation in responding to the invasion of microbes and can lead to serious organ dysfunction if it is not detected and treated in a timely manner. Depending on the time of presentation after birth when neonatal sepsis develops, it is divided into two types: While late-onset sepsis (LOS) develops after 72 hours of birth and is typically acquired in a hospital or community setting, early-onset sepsis (EOS) happens within the first 72 hours of life and is associated with maternal or perinatal risk variables. Maternal infection or colonization of the pathogen represents one of the key aspects of EOS transmission, thus the prevention measures must be taken antenatally and perinatally to reduce the disease burden.[1] Despite some advances in neonatal care, the burden of neonatal sepsis remains very high, especially in low and middle-income countries.[2]

Neonatal sepsis is a problem that has been a serious issue in India due to its high frequency and importance as the major reasons of death among neonates. It is a common problem in tertiary care institutions and neonatal ICU where critically ill neonates need long-term hospital stay and intensive management. The clinical presentation can be in the form of septicaemia, pneumonia, meningitis, osteomyelitis, septic arthritis, and UTI, with a large number of infants showing non-specific symptoms. Early diagnosis and appropriate use of antibiotics hence required to save lives of neonates.[3] Moreover, hospital-based research from India has shown that culture-proven neonatal sepsis still has a high rate of mortality, indicating the importance of ongoing microbiological surveillance and appropriate antibiotic policies.[4] Gram-negative bacteria are a key group of pathogens causing bloodstream infections among neonates. The outer layer of their cell membranes contains lipopolysaccharides and endotoxins which help them become highly virulent and cause a strong systemic inflammatory reaction. Moreover, there are other factors that make Gram-negative bacteria virulent and allow them to adhere to host tissue, evade immune responses and survive in hospital settings. They can resist drugs by developing different mechanisms like alteration in cell membrane permeability, efflux systems and inactivation of antibiotics.[5] Early diagnosis based on laboratory tests is crucial for the management of neonatal sepsis cases.[6]

There have been notable changes in the epidemiology of neonatal sepsis over recent years, with multidrug-resistant Gram-negative pathogens being increasingly seen in neonatal ICU patients. Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, Citrobacter species, together with Pseudomonas aeruginosa are some of the isolated Gram-negative bacteria from the bloodstream infection in neonates. Some of the factors that make neonates prone to sepsis caused by Gram-negative bacteria include prematurity, low birth weight, prolonged admission to the hospital, invasive procedures, and maternal colonization.[7] One of the mechanisms for resistance to antibiotics that have been found to be important is β-lactamase production as it makes β-lactam antibiotics ineffective.[8] Systematic reviews have revealed that prematurity and invasive procedures in neonates increase the chances of having severe infections.[9] The occurance of antimicrobial resistance has increasingly developed a excessive task in the handling of neonatal sepsis as it narrows the therapeutic options and causes prolonged stays in hospitals, treatment failure, and death. There is recent research showing that Gram-negative microorganisms have emerged as the most common causative agents of neonatal sepsis and have been known to be resistant to various categories of antimicrobial drugs.[10] The problem becomes greater among preterm and low birth weight infants, which are predisposed to infections due to their weak immune response and susceptibility to invasive procedures.[11] Studies done in low and lower-middle income countries have shown the dominance of multidrug resistant Gram-negative bacteria among neonatal bloodstream infections, and the need for local surveillance in order to guide empirical antibiotic prescription.[12] Similarly, various systematic reviews have shown that there is high prevalence of antimicrobial-resistant Gram-negative microorganisms among cases of neonatal sepsis.[13]

Of all the resistance mechanisms that exist, β-lactamases are clinically important. The β-lactamases called the AmpC confer resistance to penicillins, cephalosporins, and cephamycins, limiting the effectiveness of widely-used antibiotics.[14] The metallo-β-lactamases (MBLs) act on the carbapenems, which are considered to be the last choice of drugs for treatment of the resistant Gram-negative infections.[15] ESBLs, AmpC β-lactamases, and MBLs are today known to be the principal enzyme mechanisms causing the resistance to β-lactams in Gram-negative organisms and becoming increasingly prevalent in the healthcare sector.[16] Accurate diagnosis through laboratory analysis is imperative for the effective action of neonatal sepsis. Identification of the etiological pathogen through blood culture remains the gold standard; meanwhile, the results of antimicrobial susceptibility tests

and phenotypic detection of ESBL, AmpC, and MBL will be useful for antimicrobial therapy and Antimicrobial Stewardship Programme (ASP).[17] The results of standardized antimicrobial susceptibility test along with CLSI interpretation of resistant pattern make accurate reporting possible and facilitate the decision-making process.[18] Hence, the research has been done to determine the antimicrobial resistance pattern and MBL, AmpC β-lactamase, and ESBL phenotypic detection in isolates of Gram-negative bacteria from the blood cultures of neonates with neonatal sepsis at a tertiary care facility.

AIM

To detect the antimicrobial resistance pattern and phenotypically detect MBL, AmpC β-lactamase, and ESBL among Gram-negative bacterial isolates from blood cultures of neonates admitted to a tertiary care facility who were suspected of having neonatal sepsis.

OBJECTIVES

1. To identify Gram-negative bacterial isolates from blood culture-positive samples of neonates with suspected neonatal sepsis.
2. To detect the production of Metallo β-lactamase (MBL), Extended Spectrum β-lactamase (ESBL) and AmpC β-lactamase among Gram negative isolates
3. To determine the antimicrobial susceptibility pattern of the Gram-negative bacterial isolates using the Kirby–Bauer disc diffusion method.

2. METHODOLOGY

2.1 Study Design

This cross-sectional study was done at a tertiary healthcare facility's Department of Microbiology over a period of eighteen months. The sample size estimated by calculations was 74 with a proportion of 25.84% and an absolute error of 5%, but the sample size was rouded up and taken as 100.

2.2 Study Population

The research population comprised suspected cases of neonatal sepsis that were brought to the tertiary care facility.

2.3 Inclusion and Exclusion Criteria

Neonates suspected with neonatal sepsis based on laboratory and clinical criteria were used in the study. Those neonates with a history of antibiotic intake within the last 48 hours were excluded from the investigation.

2.4 Sample Collection

The blood cultures were taken from the newborns that satisfied the criteria of inclusion in the study period. The samples were aseptically drawn by healthcare providers by following standard protocols. After sterilizing the venipuncture site for one minute with a 70% alcohol and 2% iodine solution, the skin was allowed to dry. A disposable needle and syringe were used to draw 0.5 to 2 mL of blood, which was then transferred to blood culture bottles that contains Brain Heart Infusion (BHI) broth.

2.5 Isolation and Identification of Gram-negative Bacteria

2.5.1 Gram Stain

In the Microbiology Laboratory, blood culture specimens were examined. After being cultivated in the BHI broth, blood culture specimens were incubated at 37°C. The blood cultures were then sub-cultured after 18 to 24 hours on Blood Agar and MacConkey Agar plates. Biochemical assays, Gram's staining, and colony morphology were used to classify the bacterium. Gram's staining test was performed using the conventional method and the Gram-stained smear was observed under the 100x oil immersion lens of a light microscope.

2.5.2 Biochemical Tests

The identification was carried out based on Gram staining and colony morphology; after that, the isolates underwent biochemical reactions as a confirmatory test. The biochemical tests conducted were Catalase test, Oxidase test, Indole test, Methyl red (MR) test, Voges-Proskauer (VP) test, Citrate utilization test, Urease test and Triple sugar iron (TSI) test.

2.6 Antimicrobial Susceptibility Testing

The CLSI guidelines were used to evaluate the antimicrobial sensitivity pattern of the isolated Gram-negative bacteria using the Kirby-Bauer disk diffusion test in Mueller-Hinton agar. The bacterial culture suspension whose density matched 0.5 McFarland standards was swabbed onto Mueller-Hinton agar. As quality control organisms, Pseudomonas aeruginosa ATCC 27853, Klebsiella pneumoniae ATCC 700603, and Escherichia coli ATCC 25922 were used. Different classes of antibiotics were used to study antimicrobial resistance patterns, and the interpretations were done using clinical breakpoints in CLSI 2022 guidelines.

2.7 ESBL Detection

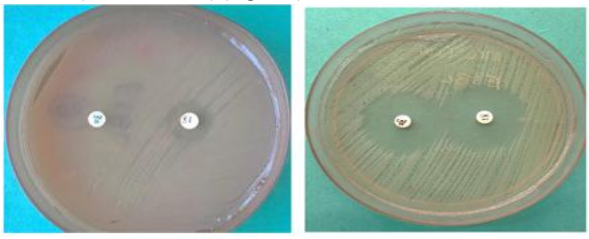
2.7.1 Screening

Cefotaxime (30 µg), Ceftazidime (30 µg), and Ceftriaxone (30 µg) were tested for ESBLs using disk diffusion method as per CLSI guidelines. The isolates that had smaller zones of inhibition were considered as ESBL producers and taken for confirmatory test.

2.7.2 Confirmatory

The Double Disk Synergy Test (DDST), sometimes referred to as the disk potentiation test, was used to validate the presence of ESBL-producing bacteria. Ceftazidime was tested both alone and in combination with clavulanic acid using Kirby-Bauer disk diffusion method. For sixteen to eighteen hours, the plates were incubated at 37°C. A ≥ 5 mm increase in a zone diameter for Ceftazidime tested in combination with Clavulanic acid versus its zone when tested alone indicates ESBL production

Phenotypic validation in accordance with CLSI standards, DDST indirectly detects the hydrolysis of the screening agent (ceftazidime or cefotaxime) by an ESBL producer in the presence of β-lactamase inhibitor (clavulanic acid) (Figure 1).



ESBL Positive

Figure 1. ESBL Production Phenotypic Identification Using the Double Disc Synergy Test (DDST)

2.8 AmpC Detection

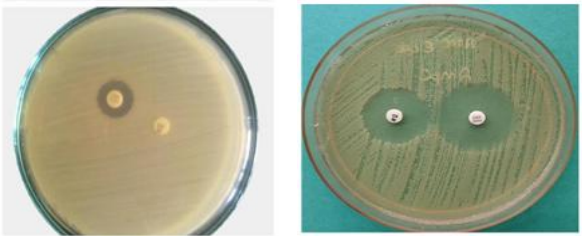
2.8.1 Screening

AmpC β-lactamases were detected using the 30 µg cefoxitin disc test on inoculated Mueller-Hinton agar. Organisms with inhibition zone sizes of ≤18 mm were regarded as AmpC producers and were subjected for further confirmatory tests.

2.8.2 Confirmatory

The production of AmpC β-Lactamase was determined using the Cefoxitin–Cloxacillin Double-Disc Synergy Test (CC-DDS). Mueller-Hinton Agar was streaked with the test strain's 0.5 McFarland suspension, and the agar plate's surface was covered with discs containing 30 µg of Cefoxitin along with 30 µg of Cefoxitin plus 200 µg of Cloxacillin. Then plates were incubated overnight at 35°C for 16–18 hours. The difference in the Cefoxitin-Cloxacillin inhibition zones and the Cefoxitin zone alone, if measured ≥4 mm, then considered as positive for AmpC production

Phenotypic detection of production of AmpC β-Lactamase was carried out with the CC-DDS. (Figure 2) which demonstrated that the cloxacillin inhibited the AmpC enzyme activity, confirming the AmpC producer.



AmpC Positive

Figure 2. Phenotypic Detection of AmpC β-Lactamase by CC-DDS

2.9 MBL Detection

2.9.1 Screening

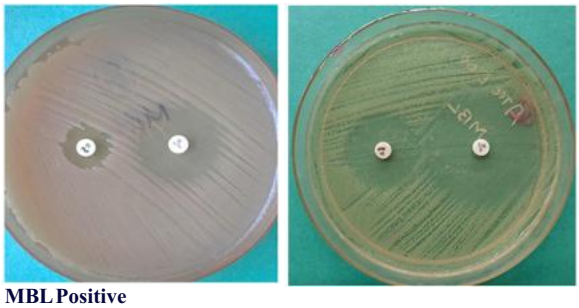
The detection of MBL was done using a 10 µg Imipenem disk onto inoculated Mueller-Hinton agar. Those isolates that showed inhibition

zones of ≤ 20mm were suspected as MBL producers and were subjected for further confirmation test.

2.9.2 Confirmatory

The production of MBL was detected by the Imipenem-EDTA Combined Disk Test. Imipenem and Imipenem-EDTA disks were placed on Mueller-Hinton Agar inoculated with the test isolate and kept for eighteen hours at 37°C. Increase zone of inhibition around combined Imipenem- EDTA disk by ≥ 7mm than that of Imipenem disk alone is taken as positive MBL producer.

The Imipenem-EDTA Combined Disk Test was used to identify phenotypic MBL production. (Figure 3). EDTA diffuses from the Imipenem disk and also act as a chelating agent that binds to the enzyme inhibiting its activity.



MBL Positive

Figure 3. Phenotypic Detection of MBL by Imipenem–EDTA Combined Disc Test

2.10 Quality Control

Rigorous quality control procedures were applied throughout the investigation, including standardized laboratory procedures, regular calibration of equipment, and periodic review of results by senior microbiologists.

2.11 Statistical Analysis

The collected data for the study was analyzed using the correct statistical approach. Descriptive statistics were applied in the presentation of the circulation pattern of Gram-negative bacteria, their antimicrobial susceptibility patterns and the frequency of ESBL, AmpC and MBL producers between the isolates.

3. RESULTS

3.1 Distribution of Study Participants by Age

Age distribution among study participants has been outlined in Table 1 below. Out of the 100 infants considered for the study, the largest percentage was represented by those aged 3 days (45.0%), then 5 days (31.0%), 4 days (17.0%), 6 days (5.0%), and 2 days (2.0%).

Table 1. Distribution of research participants by age (n = 100)

Age (Days)	Frequency	Percent
2	2	2.0
3	45	45.0
4	17	17.0
5	31	31.0
6	5	5.0
Total	100	100.0

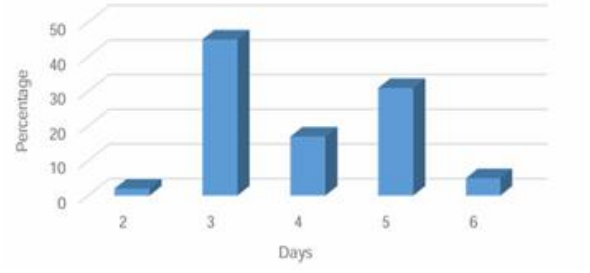


Figure 4. Age-wise distribution

The most frequent age group among neonates was 3 days (45%) followed by 5 days (31%) and 4 days (17%), showing distribution of neonatal sepsis cases in first week of life as demonstrated in Figure 4.

3.2 Distribution of Study Participants by Gender

The gender-wise distribution of the investigation subjects is shown in Table 2. Of the 100 neonates included in the investigation, 51 (51.0%) were females and 49 (49.0%) were males.

Table 2. Distribution of Study Participants by Gender (n = 100)

Gender	Frequency	Percent
Female	51	51.0
Male	49	49.0

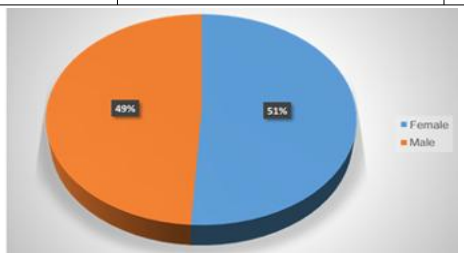


Figure 5. Gender-wise distribution of the study

The study population showed a nearly equal gender distribution, with a slight predominance of female neonates (51%) compared with male neonates (49%) as shown in Figure 5.

3.3 Distribution of Isolates of Gram-negative Bacteria

The proportion of Gram-negative.

bacteria isolates from blood culture samples is illustrated in Table 3. The most prevalent bacteria causing neonatal sepsis was K. pneumoniae (74.0%), followed by A. baumannii (12.0%), E. coli (10.0%), Citrobacter spp. (3.0%), and P. aeruginosa (1.0%).

Table 3. Distribution of isolates of Gram-negative bacteria (n = 100)

Bacterial isolate	Frequency	Percent
Klebsiella pneumoniae	74	74.0
Acinetobacter spp.	12	12.0
Escherichia coli	10	10.0
Citrobacter spp.	3	3.0
Pseudomonas aeruginosa	1	1.0

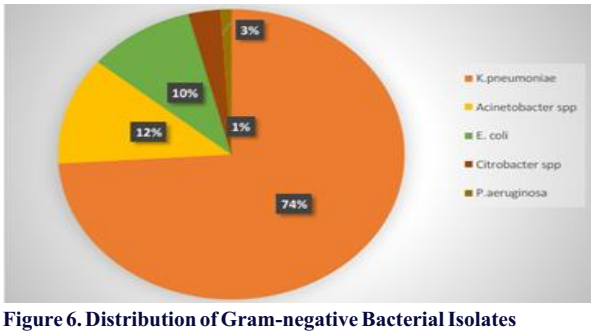


Figure 6. Distribution of Gram-negative Bacterial Isolates

Most common organism isolated was Klebsiella pneumoniae (74%), followed by Acinetobacter species (12%) and Escherichia coli (10%). Least common isolates were Citrobacter species (3%) and Pseudomonas aeruginosa (1%) as shown in Figure 6.

3.4 Distribution According to Resistance Pattern

The proportion of MBL, AmpC β-lactamase, and ESBL producers between the identified Gram-negative bacteria is presented in Table 4. For ESBL resistance, 8 subjects out of the 100 (8.0%) was found to be negative (N), while the vast majority, 92 subjects (92.0%), tested positive (P) for ESBL. Similarly, for MBL resistance, only 11 subjects (11.0%) tested negative (N), and 89 subjects (89.0%) tested positive (P) for MBL. Regarding AmpC Beta-Lactamase resistance, 47 subjects (47.0%) tested negative (N), while 53 subjects (53.0%) tested positive (P) for AmpC.

Table 4. Distribution according to resistance pattern (n = 100)

Organism	Total	ESBL (%)	MBL (%)	AmpC (%)
Klebsiella pneumoniae	74	71 (95)	70 (94)	31 (42)
Acinetobacter spp.	12	10 (91)	9 (83)	10 (91)
Escherichia coli	10	8 (80)	7 (70)	9 (90)
Citrobacter spp.	3	1 (66)	1 (66)	1 (66)
Pseudomonas aeruginosa	1	0	0	0
Total	100	92	89	53

Figure 7 A. Overall Phenotypic Identification of AmpC β-Lactamase, MBL, and ESBL

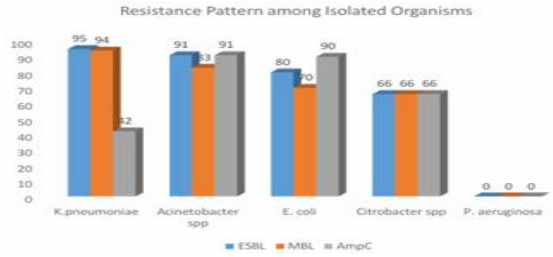


Figure 7 B. Phenotypic Resistance Pattern Between Gram-negative Bacterial Isolates

Among the 100 isolates, 92% were ESBL positive, 89% were MBL positive, and 53% were positive for AmpC. Out of 74 K. pneumoniae isolates, 95% produced ESBL, 94% produced MBL, and 42% produced AmpC. Out of 12 Acinetobacter spp. isolates, 91% produced ESBL, 83% produced MBL, and 91% produced AmpC. 80% of the 10 E. Coli isolates produced ESBL, 70% produced MBL, and 90% produced AmpC. Among the 3 isolates of Citrobacter spp., 66% were ESBL, MBL, and AmpC producers. P. aeruginosa, on the other hand, which had one isolate, was negative for ESBL, MBL, and AmpC production as shown in Figure 7 A and Figure 7 B.

3.5 Antibiotic Sensitivity Pattern of Total Isolated Gram-negative Bacteria

Table 5 displays the antibiotic susceptibility profile of every isolate of Gram-negative bacteria.

Table 5. Antibiotic Susceptibility Pattern of total isolated Gram-negative bacteria (n = 100)

Antibiotic	Response	Frequency	Percentage
AMPICILLIN	IS	1	1.0
	R	98	98.0
	S	1	1.0
AMOXYCILLIN-CLAVULANATE	R	92	92.0
	S	8	8.0
COTRIMAXAZOLE	R	21	21.0
	S	79	79.0
AMIKACIN	IS	66	66.0
	R	23	23.0
	S	11	11.0
CIPROFLOXACIN	IS	10	10.0
	R	88	88.0
	S	2	2.0
OFLOXACIN	IS	1	1.0
	R	27	27.0
	S	72	72.0
TETRACYCLINE	IS	1	1.0
	R	95	95.0
	S	4	4.0
GENTAMICIN	R	45	45.0
	S	55	55.0
DOXYCYCLINE	R	95	95.0
	S	5	5.0
CEFUROXIME	IS	1	1.0
	R	98	98.0
	S	1	1.0
CEFOTAXIME	R	92	92.0
	S	8	8.0
CEFTAZIDIME	R	92	92.0
	S	8	8.0
CEFEPIME	R	92	92.0
	S	8	8.0
CEFTRIAXONE	IS	1	1.0
	R	91	91.0
	S	8	8.0
CEFOXITIN	R	53	53.0
	S	47	47.0
PIPERACILLIN-TAZOBACTAM	IS	5	5.0
	R	86	86.0
	S	9	9.0
IMIPENEM	R	88	88.0
	S	12	12.0
AMPICILLIN-SULBACTAM	IS	2	2.0
	R	86	86.0
	S	12	12.0
CHLORAMPHENICOL	R	41	41.0
	S	59	59.0
COLISTIN	S	100	100.0

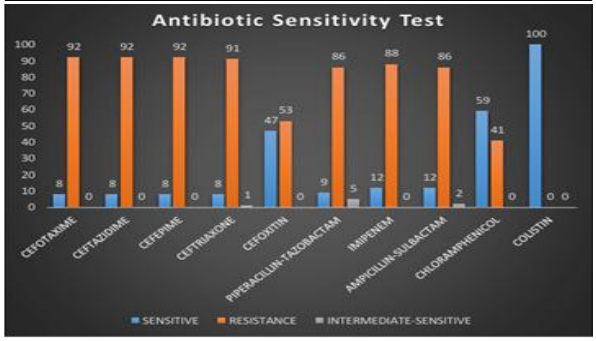


Figure 8 A. Antibiotic Sensitivity Pattern of isolated Gram-negative bacteria

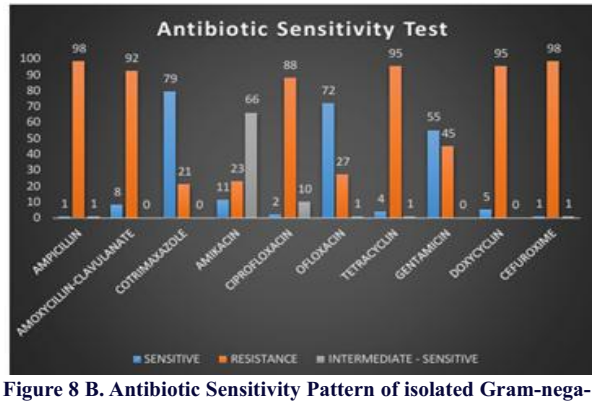


Figure 8 B. Antibiotic Sensitivity Pattern of isolated Gram-negative bacteria

All 100 Gram-negative bacterial isolates were susceptible to Colistin (100%). The highest susceptibility among the remaining antibiotics was observed with Cotrimoxazole (79%), followed by Ofloxacin (72%), Chloramphenicol (59%), and Gentamicin (55%). Conversely, the highest resistance was observed against Ampicillin (98%) and Cefuroxime (98%), followed by Tetracycline (95%), Doxycycline (95%), Amoxycillin–clavulanate (92%), Cefotaxime (92%), Ceftazidime (92%), Cefepime (92%), Ceftriaxone (91%), Imipenem (88%), Ciprofloxacin (88%), Piperacillin–tazobactam (86%), and Ampicillin–sulbactam (86%), demonstrating a high level of multidrug resistance among the isolates as shown in Figure 8 A and Figure 8 B.

4. DISCUSSION

The study was undertaken to detect distribution of Gram negative bacteria, their resistance pattern and ESBL, MBL and AmpC produ-ction in 100 Gram-negative isolates from blood cultures of suspected neonatal sepsis patients. The results showed the occurrence of multidrug resistant Gram-negative bacteria, mostly Klebsiella pneumoniae, along with a very high rate of β-lactamase production. It is worth noting that this finding supports the rising threat of antibiotic resistance in neonatal intensive care units, as β-lactamase-producing Gram-negative bacteria limit the efficacy of widely used β-lactam drugs.[19] Most neonates who were included in the current study were three days old, thus it can be

concluded that most cases of bloodstream infection develop during the early days of neonatal period. This result is associated with the increased vulnerability of newborns in the first week of life due to an under-developed immune system, perinatal contact with pathogens, and necessity of intensive treatment measures. Early diagnosis and timely administration of adequate therapy are important for improving survival rates of critically ill neonates.[20]

Of the isolated pathogens, the most predominant, Klebsiella pneumoniae, Acinetobacter species, and Escherichia coli were among the gram-negative bacteria. The dominance of Klebsiella pneumoniae highlights its continuous significance as the main reason of neonatal sepsis in tertiary care centre, mostly because of its ability to grow easily in the hospital setting, colonize invasive devices, and develop resistance to antimicrobial agents. According to Muley et al.,[21] the bacteriology profile is consistent with theirs in which Klebsiella pneumoniae is found to be the major bacterial agent causing neonatal septicemia in tertiary care hospitals. In addition, this current study revealed extremely high rates of ESBL (92%), MBL (89%), and AmpC (53%) producers among Gram-negative bacteria. Our study showed that, Klebsiella pneumoniae was 95% were ESBL positive, 94% were MBL positive and 42% were AmpC positive. Similarly, Acinetobacter spp. 91% were ESBL positive, 83% were MBL positive and 91% were AmpC positive and E. coli 80% were ESBL positive, 70% were MBL positive and 90% were AmpC positive. The results obtained show widespread distribution of β-lactamase producing bacteria within the study population, indicating limitation of antibiotic choices for effective treatment of neonatal sepsis. Similar results were also reported by Weldu et al.[22] who found extremely high rate of multidrug resistance among Gram-negative neonatal pathogens and high resistance against β-lactam antibiotics.

Multidrug-resistant Klebsiella pneumoniae seen in the existing investigation is also found with previous research done in other developing countries, where Klebsiella pneumoniae remains the maximum frequent cause of neonatal septicemia. Likewise, Pokhrel et al.[23] have found that Klebsiella pneumoniae was the greatest prevalent microorganism isolated in cases of sepsis in neonates and showed wide resistance to various antimicrobial drugs. Comparison of the findings indicates that multidrug-resistant Klebsiella remains one of the vital pathogens related with hospital-acquired infections. Antimicrobial susceptibility profiling in the current study showed, all the 100 isolates were susceptible to Colistin, while higher susceptibility levels were recorded for Cotrimoxazole, Ofloxacin, Chloramphenicol, and Gentamicin. On the other hand, very high levels of resistance were shown against Ampicillin, Cefuroxime, Tetracycline, Doxycycline, Amoxicillin-clavulanate, third and fourth generation Cephalosporins, Ciprofloxacin, Imipenem, Piperacillin-tazobactam, and Ampicillin-sulbactam. The outcomes of the present investigation clearly show that the majority of Gram-negative strains were multidrug resistant, thus making treatment options very difficult for neonatal sepsis. This is similar to a prior investigation by Chelliah A et al.,[24] in which there is higher number of β-lactamase producing Gram-negative bacteria that were extremely resistant to widely used antibiotics and susceptible to a small number of reserve antibiotics.

This study found that the existence of wide spread β-lactamase production along with high levels of antimicrobial resistance indicates the absolute necessity of ongoing monitoring of resistance mechanisms within neonatal intensive care units. This is consistent with the findings of Zakariya et al.[25] who found that Gram negative pathogens showed high resistance and the significance of ongoing antimicrobial susceptibility testing was highlighted.

The results obtained during the current study have significant clinical implications. Predominance of Gram-negative bacteria that are resistant to many drugs and the remarkably high frequency of ESBL, MBL, and AmpC suggests that empirical antimicrobial treatment is supposed to be based on antimicrobial susceptibility results. Standardized phenotypic detection of β-lactamase producing pathogens, antimicrobial stewardship programs, regular update of hospital antibiotic policies, and adherence to the principles of infection prevention and control are desirable in order to combat multidrug-resistant bacteria and to improve the treatment of neonatal sepsis.

5. CONCLUSION

According to the current study, the age-wise distribution of neonates diagnosed with neonatal sepsis provides valuable insights into the timing of diagnosis and emphasizes the necessity of early detection and intervention strategies, particularly within the first week of life. To improve early detection and intervention, future research should focus on developing specific biomarkers, rapid diagnostic tests, and preventive measures particularly for neonatal populations.

Regarding bacterial isolates from blood cultures, our study identified Klebsiella pneumoniae as the predominant pathogen causing neonatal sepsis, followed by Acinetobacter spp. and Escherichia coli. These findings show the diversity of pathogens contributing to neonatal sepsis in hospital settings and emphasize the importance of understanding bacterial species for making appropriate treatment strategies and recommended measures to prevent nosocomial infections particularly in NICUs. Finally, our analysis of antibiotic resistance patterns revealed a concerning prevalence of resistance among Gram-negative bacterial isolates, posing challenges in effectively treating neonatal sepsis. Routine screening for ESBL, MBL and AmpC β-lactamases together with antimicrobial sensitivity analysis based on cultures should become an integral part of the routine laboratory work to enable prompt identification of resistant strains and development of evidence-based antibiotic therapy. Continuous surveillance, effective hospital infection control measures and appropriate antibiotic stewardship are essential to tackle antimicrobial resistance and ensure effective treatment outcomes for neonates. Further research should focus on exploring alternative treatment options and implementing targeted infection control measures to address this pressing issue in neonatal care.

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