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RESEARCH ARTICLE
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SPECTRUM AND ANTIBIOTIC SUSCEPTIBILITY OF GRAM NEGATIVE ORGANISMS ASSOCIATED WITH NEONATAL SEPSIS

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Abstract:
This study involved the analysis of 492 blood samples to assess the prevalence of sepsis, specifically focusing on Gram-negative bacterial (GNB) neonatal sepsis. Out of the total cases diagnosed with culture-proven sepsis (96 patients), 54 (56.25%) were identified as GNB neonatal sepsis. Among the GNB neonatal sepsis cases, Pseudomonas aeruginosa was the predominant pathogen, isolated in 32 patients (59.26%), and followed by Klebsiella species in 12 patients (22.22%), E. coli in 6 patients (11.1%), and Acinetobacter species in 4 patients (7.41%).

Detailed antibiotic susceptibility profiles were determined for each identified pathogen. Notably, Carbapenem such as Imipenem (IPM) and Meropenem (MEM) exhibited robust efficacy, with over 80% susceptibility observed. The highest susceptibility was observed for Meropenem against Pseudomonas aeruginosa (87%), and the lowest susceptibility was noted for Ciprofloxacin against Klebsiella species (10%). However, Tazobactam-piperacillin (TZP) demonstrated varied effectiveness across pathogens, and only 31.25% of Pseudomonas aeruginosa isolates showed sensitivity to TZP. Commonly used antibacterial agents, including Ampicillin, Co-amoxiclav, and third-generation cephalosporin, displayed limited efficacy against isolates. In conclusion, Pseudomonas aeruginosa emerged as the most common Gram-negative organism causing neonatal sepsis. High resistance was observed in current empiric treatment choices like Amikacin (AK) and Tazobactam-piperacillin (TZP). Routinely used antibacterial agents, including Ampicillin, Co-

amoxiclav, ceftriaxone, cefotaxime, and ciprofloxacin, were found to be ineffective against Gram-negative isolates, emphasizing the need for careful consideration in neonatal sepsis treatment.

Keywords: Neonatal sepsis, Gram negative bacteria, Neonatal intensive care unit, Blood culture, Anti-bacterial agents

Introduction

Despite significant decrease in infant mortality rates in past 2 decades, the mortality burden is still high. According to 2019 reports the child mortality rate below the age of 5 years was 38/1000 live births and 98% of them were recorded in low and middle income countries (LMICs).[1]Neonatal infections account for >30% of the total neonatal deaths, among these infections, sepsis is the commonest one. Neonatal sepsis accounts for 1 to 4 per 1000 live births in developed nations.[2, 3]Majority of the affected neonates are pre-term accounting for 30 to 40% of total cases and mortality rate of 30% to 40% depending upon the etiologic agent.[4, 5] The outcome in terms of mortality associated with Early onset neonatal sepsis is much poorer than that of Late onset neonatal sepsis.[6] Mortality rate is higher among neonates affected with gram negative bacilli (GNB) in comparison to those affected with gram positive bacilli (GPB).[7] Neonatal sepsis has now been declared as global epidemic by World health organization (WHO).[8]

Despite higher incidence figures, the reliable information about the incidence and etiology of sepsis is scare in LMICs. The spectrum of causative organisms varies over time and from region to region. This owes to excessive antibiotic use and resultant antimicrobial resistance.[9]Certain gram negative bacteria such as *Klebsiella pneumoniae, Enterobacter sp, Acinetobacter sp and Pseudomonas aeruginosa* have been recently found as highly drug resistant pathogens implicated in neonatal sepsis.[10] Continuous surveillance regarding pathogens of neonatal sepsis and their susceptibility pattern would enable the policy makers and pediatricians for appropriate antibiotic selection for management of neonatal sepsis.[11]Blood culture is the most dependable and reliable test for detection of microbial pathogens in hospitals worldwide and still remains the gold standard for detecting bloodstream infections.[12]Since septicemia by drug resistant gram negative bacteria have become a challenge for management of neonatal sepsis. The present study was designed to analyze blood cultures of neonates with clinical suspicion of sepsis, to recognize the gram negative organisms causing bloodstream infections and assess their antimicrobial sensitivity pattern. This would help the pediatricians to commence empirical antibiotic therapy when there is clinical suspicion of neonatal sepsis by having knowledge of common neonatal pathogens and their drug sensitivity pattern.

Materials and methods

Study design and Area

In this cross-sectional prospective study, conducted in a tertiary care hospital Lahore from July 2020 to June 2021.

Specimen Collection

We processed the blood cultures from neonates sent to the pathology laboratory of hospital for determination of neonatal sepsis causative organisms and their susceptibility pattern.

Specimen Processing, Morphology & Microscopy

All specimens were received in blood culture bottles. The specimens were placed in incubator for 5 days at 35 °C temperature. The specimens were first sub-cultured on blood agar and Mac Conkey agar after 48 hours of incubation. If negative, the blood sample was sub-cultured for second time on same media on day 5. In case of no growth after 2 subcultures, negative blood culture was reported after 5 days. In positive cases, the identification of micro-organisms was done using the colony morphology by Gram staining and then Analytical profile index (API) was used for identification. Gram staining,

directly from culture bottles and re-culture was also conducted for confirmation of the causative organisms.

Antibiotic Susceptibility Testing (AST)

The antibiotic susceptibility testing of the causative bacteria was carried out by Kirby Bauer disc diffusion assay. Bacterial suspension was prepared by suspending colonies of the organism in the normal saline and getting turbidity equivalent to 0.5 McFarland turbidity standards. The suspension was lawned over the Mueller-Hinton agar plates using the sterile swab and antibiotic discs were placed on the plates with aseptic technique. The plates were incubated for 18-24 hours at 35°C and the zone diameters of antibiotics were interpreted according to the Clinical & Laboratory Standards Institute (CLSI) 2020.

CLSI Panel-2020

The antibiotics were reported as susceptible, resistant, or intermediate. The antibiotic panel applied for gram negative bacteria included ampicillin, amoxicillin, co-amoxiclav, ceftazidime, cefotaxime, ceftriaxone, cefepime, imipenem, meropenem, tazobactam-piperacillin, aztreonam, amikacin and ciprofloxacin, ampicillin-sulbactam, co-trimoxazole, and doxycycline.

Data Interpretation:

All the gathered information was entered in SPSS version 25. Frequency and percentages were calculated to present the pattern of causative organisms and their susceptibility pattern.

Results

Distribution of Blood Specimen Results

Out of 492 blood samples received in microbiology laboratory, culture proven sepsis was diagnosed in 96 patients. Fifty four (56.25%) patients were diagnosed with GNB neonatal sepsis.

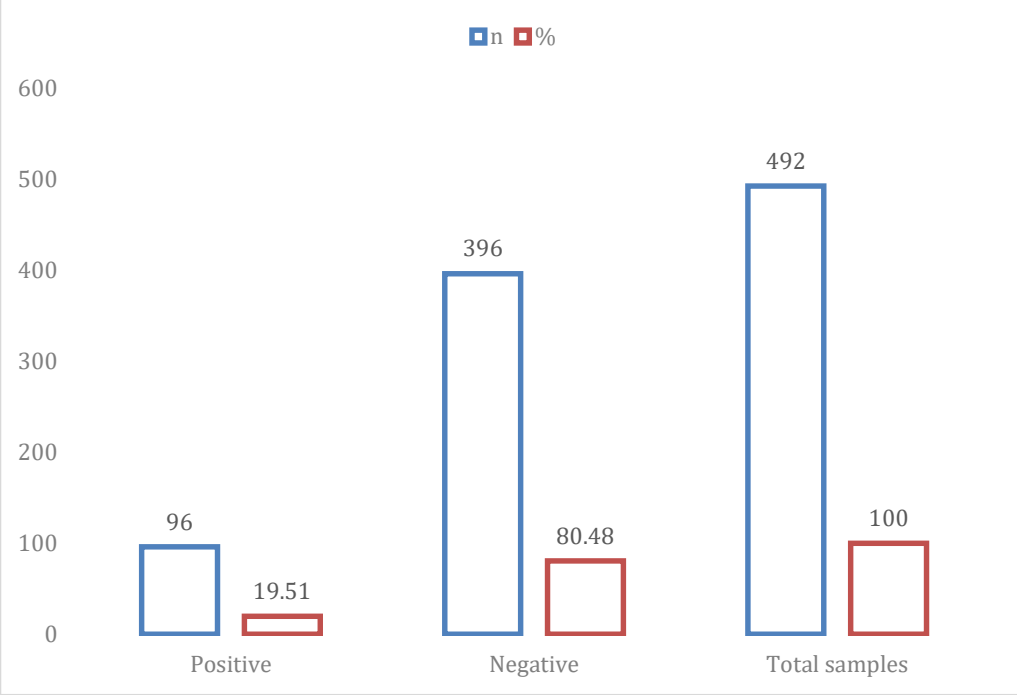

Figure 1: Distribution of Blood specimen

Distribution of Clinical Isolates

Out of 54, *Pseudomonas aeruginosa* was diagnosed in 32 (59.26%) patients, *Klebsiella species* in 12 (22.22%) patients, *E. coli* in 6 (11.1%) patients and *Acinetobacter species* in 4 (7.41%) patients.

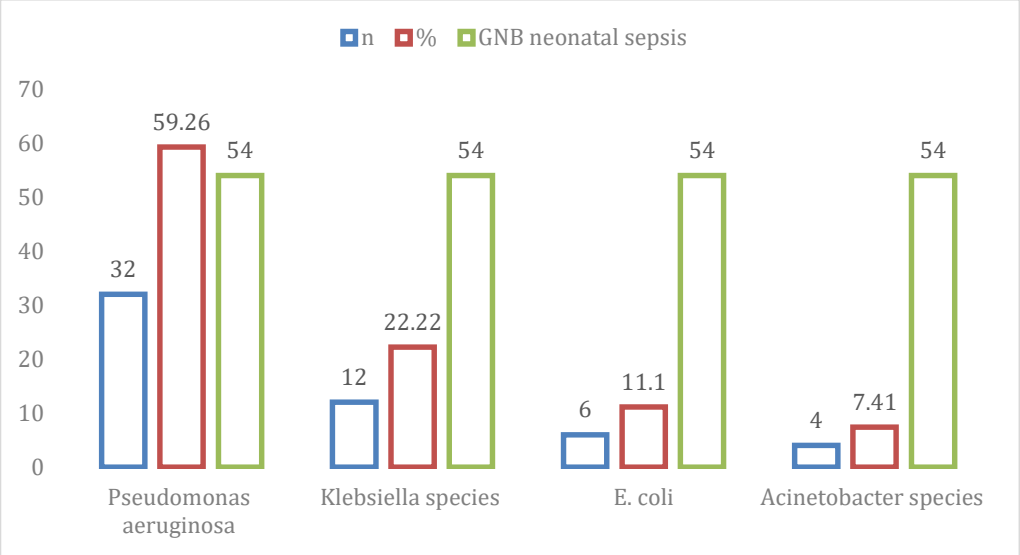

Figure 2: Distribution of clinical isolates among GNB neonatal sepsis

Antibiotic Susceptibility CLSI Panel-2020

Table 1: Antibiotic Susceptibility Distribution in *Pseudomonas aeruginosa*

Antibiotics	***Pseudomonas aeruginosa* (N=32)**				
	S (%)	R (%)	**M ± SD (%)**		**P-values**
Ceftazidime	52	48	50 ± 2.89		0.065
Cefepime	55	45	50 ± 4.18		0.032
Imipenem	63	37	50 ± 9.90		0.001
Meropenem	87	13	50 ± 26.27		<0.001*
Piperacillin/Tazobactam	32	68	50 ± 15.81		0.003
Amikacin	25	75	50 ± 22.36		0.012
Ciprofloxacin	10	90	50 ± 31.62		<0.001*
Levofloxacin	11	89	50 ± 32.47		<0.001*
Piperacillin	34	66	50 ± 15.81		0.004
Gentamicin	20	80	50 ± 28.87		<0.001*
Aztreonam	83	17	50 ± 26.27		<0.001*

P-value <0.001 (highly statistically significant), <0.05 (statistically significant).

Table 2: Antibiotics Susceptibility Distribution in *Klebsiella species*

Antibiotics	***Klebsiella species (N=12)***			
	S (%)	**R (%)**	**M ± SD (%)**	**P-values**
Amoxicillin/Clavulanate	15	86	50 ± 34.6	<0.001*
Ceftazidime	10	90	50 ± 15.8	<0.001*
Cefotaxime	20	80	50 ± 15.8	0.054
Ceftriaxone	20	80	50 ± 24.5	0.054
Cefepime	15	85	50 ± 7.1	<0.001*
Imipenem	55	45	50 ± 7.1	<0.001*
Meropenem	55	45	50 ± 1.4	<0.001*
Piperacillin/Tazobactam	51	49	50 ± 7.1	0.752
Amikacin	43	57	50 ± 34.6	0.008
Ciprofloxacin	10	90	50 ± 34.6	<0.001*
Levofloxacin	10	90	50 ± 14.1	<0.001*
Trimethoprim/Sulfamethoxazole	40	60	10 ± 0	0.001
Ampicillin/Sulbactam	10	10	50 ± 5.0	0.156
Gentamicin	45	55	50 ± 11.2	0.002
Aztreonam	38	62	50 ± 11.2	0.048

P-value <0.001 (highly statistically significant), <0.05 (statistically significant).

Table 3: Antibiotics Susceptibility Distribution in *Acinetobacter species*

Antibiotics	***Acinetobacter species (N=4)***			
	S (%)	**R (%)**	**M ± SD (%)**	**P-value**
Ceftazidime	25	75	50 ± 5.0	<0.001*
Cefepime	25	75	50 ± 5.0	<0.001*
Imipenem	58	42	50 ± 7.1	0.003
Meropenem	58	42	50 ± 7.1	0.003
Piperacillin/Tazobactam	56	46	50 ± 5.7	0.014
Amikacin	42	58	50 ± 7.1	0.003
Ciprofloxacin	10	90	50 ± 34.6	<0.001*
Levofloxacin	10	90	50 ± 34.6	<0.001*
Trimethoprim/Sulfamethoxazole	20	80	50 ± 15.8	0.002
Gentamicin	43	57	50 ± 7.1	0.005
Tobramycin	80	20	50 ± 28.9	<0.001*

P-value <0.001 (highly statistically significant), <0.05 (statistically significant).

Table 4: Antibiotics Susceptibility Distribution in *E. coli*

	E. coli (N=6)			
Antibiotics	**S (%)**	**R (%)**	**M ± SD (%)**	**P-value**
Amoxicillin/Clavulanate	30	70	50 ± 14.1	<0.001*
Ceftazidime	15	85	50 ± 24.5	<0.001*
Cefotaxime	20	80	50 ± 15.8	0.002
Ceftriaxone	20	80	50 ± 15.8	0.002
Cefepime	15	85	50 ± 24.5	<0.001*
Imipenem	70	30	50 ± 14.1	<0.001*
Meropenem	70	30	50 ± 14.1	<0.001*
Piperacillin/Tazobactam	63	37	50 ± 9.9	0.004
Amikacin	55	45	50 ± 7.1	0.010
Ciprofloxacin	20	80	50 ± 15.8	0.002
Levofloxacin	20	80	50 ± 15.8	0.002
Trimethoprim/Sulfamethoxazole	35	65	50 ± 12.0	0.006
Ampicillin/Sulbactam	20	80	50 ± 15.8	0.002
Gentamicin	55	45	50 ± 7.1	0.010
Doxycycline	75	25	50 ± 14.1	0.001

P-value <0.001 (highly statistically significant), <0.05 (statistically significant).

Discussion

Regarding neonatal sepsis, in the current study 96 neonates were suffering from culture proven sepsis. A very high count of 1.3 million annual cases of neonatal sepsis have been documented by Global Burden of Disease (GBD) Study 2016/2017. Sepsis is the commonest infection among neonates which can occur independently or may present with some diseases of neonates.[13] Neonatal sepsis has a vast risk factors profile. Pre-term and infants with low birth weight have increased chances of infections because of immature immune profile, prolonged hospitalizations, and not getting mother feed during intensive care units stay.[14, 15]

Despite availability of newer and more effective antibiotics neonatal sepsis still results in high morbidity and mortality. Neonatal infections especially during hospitalization have been previously observed mostly to be caused by Gram positive cocci (GPC).[16] However, in the recent past an increasing trend in neonatal sepsis by Gram negative bacteria is observed.[1] A high mortality rate is reported in sepsis caused by GNBs in comparison to GPC.[7] In this study, among total 96 patients of culture proven sepsis, GNB were isolated in 54 (56.2%) patients. Among these 54, the commonest organism was *Pseudomonas aeruginosa* isolated in 32 (59.26%) cases, *Klebsiella species* in 12 (22.2%) cases, *Acinetobacter species* in 4 (7.41%) and *E. coli* in 6 (11.1%) cases. Similar results were shown by a study conducted in Karachi, 2020 reporting *Pseudomonas aeruginosa* as the commonest

Gram negative bacterium causing neonatal sepsis. [7] A latest study conducted in Sep 22, exhibited that Gram-negative rods were responsible for 60.3% of all pathogens isolated from a total of 73 positive blood cultures.[17]
A recent study by BARNARDS network evaluators on characterization of GNB positive neonatal sepsis in seven African and Asian countries reported including 2483 cases of culture proven sepsis, reported *Klebsiella pneumoniae* as the commonest organism responsible for neonatal sepsis isolated in 258 (10.39%) cases, followed by *Serratia marcescens* in 151 (6%) cases, *Escherichia coli* in 75 (3.0%) and *Enterobacter cloacae* complex in 57 (2.29%) cases.[1]

Another study by Nordberg et al. including the data of 107 neonates with diagnosis of GNB neonatal sepsis reported *E. Coli* as the commonest organism responsible for neonatal sepsis isolated in 47 (43.9%) cases, *Klebsiella pneumoniae* in 20 (18.7%) cases, and *E. cloacae* in 14 (13%) cases.[18] However, a study in Nepal concluded *Klebsiella sp* to be the most prevalent pathogen among Gram negative neonatal sepsis.[17]

Regarding antimicrobial susceptibility pattern, for *E. coli*, IPM, MEM and AK were sensitive in 100% cases, while TZP and LEV were sensitive in 66.7% cases. For *Acinetobacter species*, IPM, MEM were sensitive in 100% cases, while SAM and DO in 75% cases. None of the *Acinetoacter* isolates exhibited susceptibility to fluoroquinolones, while 50% isolates were susceptible to CAZ, FEP, AK, SXT. For *Klebsiella species* IPM, MEM, TZP, AK were sensitive in >80% cases. As for as *Pseudomonas aeruginosa* is concerned CAZ, FEP, MEM, and IPM were sensitive in >75% cases. Even higher resistance has been reported in another study with Gram negative bacteria accounting for 75% of neonatal sepsis, exhibiting 74% of aminoglycoside resistance, and 95% resistance to third and fourth generation cephalosporins. [19] Nordberg et al. reported very low prevalence of antibiotic resistance, in their study *Pseudomonas aeruginosa* was sensitive to all antibiotics and *E. Coli* resistance was reported to gentamicin in only 2 cases.[18]

Conclusion:
Pseudomonas aeruginosa is the commonest isolated organism causing GNB neonatal sepsis and has high resistance among the current choice for empiric treatment like AK, TZP. The routinely used drugs like Ampicillin, co-amoxiclav, ceftriaxone, cefotaxime and ciprofloxacin are not effective against gram negative isolates and shouldn't be used for neonatal sepsis. In our study there was a high prevalence of antibiotic resistant organisms in comparison to existing literature, emphasizing the revision of empirical therapy and judicious use of antibiotics

Future prospect:
This study will help not only in timely selection of the empirical antibiotic treatment in our local population but also would slow the rise in anti-microbial resistance.

Limitations and recommendations:

- It is a single center study and has taken into account only the causative agent along with their susceptibility pattern**.**
- Large multi-center studies should be conducted in Pakistan as the spectrum of causative GNB of neonatal sepsis and their antimicrobial susceptibility varies from region to region. This would enable us to know spectrum of GNB neonatal sepsis and their anti-microbial spectrum for our local population.
- This study also highlights the role of infection control practices in preventing the spread of such drug resistant pathogens in the neonates. So it is highly recommended for the health care workers to meticulously follow infection control measures while delivering the baby and handling the neonate.

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Authors' contribution:
Dr. Saima Inam: Conception, planning of research, Data Collection, and writing of manuscript, Discussion., **Dr Asma Inam, Dr. Sadia Ikram**, **& Dr Muhammad Tahir Saeed**: Discussion and Proof Reading. **Dr Sadaf Munir, Dr Almas Raza & Dr Aroosh Shabbir**: Help in references, and Data analysis. **Dr. Syed Zeeshan Haider**: Critically revised the paper in keeping with important intellectual content.

Conflict of interest:
Authors state no conflict of interest.

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