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ORIGINAL ARTICLE

Spectrum and Antibiotic Sensitivity Pattern of Bloodstream Bacterial Isolates from Septicemic Neonates

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ABSTRACT

Aim: To evaluate the etiology of neonatal sepsis. We aimed to explore the antimicrobial sensitivity patterns among neonates.
Methodology: This observational-based study was conducted in POF hospital Wah Cantt from September 2020 to March 2021. We selected 300 sepsis patients based on the WHO neonatal sepsis screening tool. Blood samples were collected by trained laboratory technicians using the aseptic technique. Laboratory technicians prepared a 5cm (in diameter) patch of skin over the proposed site of venipuncture. We drew one ml sample of blood from a fresh venipuncture site. This blood was added to a bottle containing 5–10 mL of blood Agar culture media.
Results: We observed that babies with less than 5 Apgar scores reported a high risk of neonatal sepsis. We observed gram-positive bacteria in 49 (55.7%) cases while 39 (44.3%) were reported gram-negative bacteria. At the time of diagnosis, we observed that 67.3% of cases of gram-positive bacteria were highly reported in the LONS group. Around 23 (63.3%) gram-negative bacterial isolates were from EONS. Regarding bacterial pathogen, we observed 22 (25%) cases of Coagulase-negative staphylococcus aureus and 18 (20%) were of E. coli which results in neonatal sepsis.
Conclusion: Our results concluded that third-line antimicrobials can be a beneficial treatment for managing neonatal sepsis. However, failure of irst two antimicrobials was observed due to high utilization before an accurate diagnosis of neonatal sepsis.
Keywords: Antimicrobial, Sensitivity Patterns,. Neonatal sepsis

INTRODUCTION

In developing countries, the neonatal mortality rate due to various conditions gives rise to the infant mortality ratio[1]. However, neonatal sepsis is one of the contributing factors to neonatal deaths[2]. Approximately, 26 to 50% of deaths are reported in developing countries[3,4]. Neonatal sepsis is defined as any kind of sepsis that is diagnosed within the first 28 days of life. Neonatal sepsis is further classified into two major categories including early-onset sepsis which arises within 6 days after birth and late-onset sepsis which is diagnosed after 7 to 28 days of life[5]. Gram-positive bacteria and fungi are the major reasons for neonatal sepsis. These bacterial infections causing sepsis evolve due to significant geographical diversity[5,6,7].

Antibiotic resistance is one of the major challenges in neonatal sepsis. Increased ratio of neonatal sepsis in developing countries occurs due to multidrug-resistant bacteria. Clinical signs of neonatal sepsis are nonspecific which creates massive obstacles in early diagnosis. This leads to the consumption of many antibiotics and results in the spreading antimicrobial resistance strain of bacteria. Information related to causative agents and antimicrobial sensitivity could help select neonatal therapy[5,6]. Targeted antibiotics therapy plays a great role in the reduction of antimicrobial resistance.

Our study was designed to evaluate the etiology of neonatal sepsis. We aimed to explore the antimicrobial sensitivity patterns among neonates.

METHODOLOGY

This observational-based study was conducted in POF hospital Wah after permission from IRB, during 2020-2021. Study participants were recruited from the neonatal emergency department. The neonatal intensive care unit (NICU) is one of the biggest wards of the hospital with the capacity of dealing 500_700 neonates per year. This NICU ward has a microbiology hematology and biochemistry diagnostic laboratory. The sample size was estimated from four previous studies which study neonatal sepsis in the 20% to 40% population[1,8,7,10]. By applying a single population proportion and estimated 5% margin of error at a 95% confidence interval, a total of 322 patients are selected. We selected 322 sepsis patients based on the WHO neonatal sepsis screening tool[5,7]. This WHO tool was then augmented with the manual of the neonatal intensive care unit. We excluded all the critically ill patients with sepsis from the study. Neonates which need further laboratory examinations or medical procedures were also excluded. Finally, 303 subjects were selected for analysis who fulfilled the inclusion criteria. We defined early-onset sepsis (EONS) as diagnosed within the first six days of life. Late-onset sepsis was defined as sepsis which was diagnosed between the 7th to 28 days of life. Other variables like premature rupture of membrane (PROM) were defined as membrane rupture before labor onset however we included analysis related to prolonged premature rapture which lasted more than 18 hours. We performed antenatal screening of all mothers to evaluate the cause of neonatal sepsis. Neonates were treated with three-line antibiotics. In first line antibiotics, we used Ampicillin and Gentamycin second line included third-generation Cephalosporins and the third line antibiotics involved Vancomycin, Amikacin, and Ciprofloxacin[11]. We prepared a questionnaire regarding demographic information, risk factors, and clinical features of sepsis after reviewing books, and relevant literature related to neonates. After the admission of neonates, we interviewed mothers in comfortable and convenient areas. For diagnosis of bloodstream infection, blood cultures are the gold standard test and should be measured in all sepsis cases before administration of antibiotics. We collected blood cultures from all neonates after clinical diagnosis. Blood samples were collected by trained laboratory technicians using the aseptic technique. Laboratory technicians prepared a 5cm (in diameter) patch of skin over the proposed site of venipuncture. We used 70% isopropyl alcohol along with povidone-iodine for cleaning this area. Before venipuncture we allowed the skin to dry for at least 1 min. We drew one ml sample of blood from a fresh venipuncture site. This blood was added to a bottle containing 5–10 mL of blood Agar culture media. At 37°C blood samples were incubated aerobically and observed regularly for three days for checking the findings on culture media. These findings included hemolysis, air bubbles (gas production), and coagulation of broth[12]. We made subcultures during three consecutive days on enriched and selective media

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including blood agar, chocolate agar, MacConkey agar, and mannitol salt agar plates, and analyzed the fot growth after 24–48 h of incubation. In case of no growth after 7 days these blood cultures were reported as sterile. We used standard techniques (Gram stain reaction, biochemical reaction properties, and morphological characteristics) for the identification of isolated bacteria[13]. By using the Kirby Bauer diffusion method we performed antimicrobial sensitivity testing with incubation of 24h at 37°C. This test was performed according to Clinical Laboratory Standard Institute standards (CLSI)[8]. Testing was done for three-line treatments. We used EPI-INFO version 3.5.1 for cleanup and anthropometric interpretation. After interpretation data was shifted to SPSS software for statistical analysis. We used frequencies and summary statistics for relevant variables. P values of 0.05 were considered as statistical significance.

RESULTS

A total of 300 subjects were recruited for analyzing neonatal sepsis. Out of these 300 subjects, 122(40.6%) were culture-proven. Out of these culture-confirmed cases, 86 were male while 36 were females. During antenatal scanning we did not find a single abnormality which cause sepsis. A total of 47(38.5%) isolate cultural bacteria were found in the EONS group while 75(61.4%) were from LONS. We found statistically significant differences between both groups (p = 0.001). Antibiotics during labor reduced two-fold risk of acquiring EONS than LONS. Adjustable odd ratios between both groups were noted as 2.02 at 95% C. I range 1.17–3.50. A total of 11(9%) culture-proven neonates were born at home whereas 111(91%) were born at our labor ward. A total of 25 neonates developed RDS right after birth and need mechanical ventilation. However, initially, chest radiographs reflect mild RDS but after two weeks of life, we observed disseminated streaky patches infiltrates. A total of 10 cases of LONS revealed parenchymal changes on chest radiographs. We observed that babies with less than 5 Apgar scores reported a high risk of neonatal sepsis (Tabel 1). We observed gram-positive bacteria in 68 (55.7%) cases while 54 (44.3%) were reported gram-negative bacteria. At the time of diagnosis, we observed that 67.3% of cases of gram-positive bacteria were highly reported in the LONS group. Around 23 (63.3%) gram-negative bacterial isolates were from EONS. Regarding bacterial pathogen, we observed 36 (29.5%) cases of Coagulase-negative staphylococcus aureus and 30 (24.5%) were of E. coli which results in neonatal sepsis. The majority of the gram-positive bacterial isolates of the LONS group acquired infection from the hospital. We observed high resistance of these bacteria against first and second-line antibiotic therapy. We observed that 27 (90%) cases of CoNS, 16 (66.6%) cases of aureus, and 2 (33.3%) cases of Enterococcus show resistance against Ampicillin. On the other hand, these organisms show 23 (63.8%), 14 (58.3%), and 4(66.7%) respectively against Gentamycin. In gram-positive bacterial isolates, we observed high resistance to third-generation cephalosporins while Ceftriaxone drug show resistance of 28 (60%), Ceftazidime show 23(47%) resistance, and Cefotaxime reported 31(64%). Gram-positive bacterial isolates show high sensitivity patterns for Vancomycin, Clindamycin, Ciprofloxacin, and Chloramphenicol. We detected a significant methicillin resistance rate in Staphylococcus aureus (16, 66.6%%) and CoNS (36, 100%) (Table 2). Evaluating gram-negative bacterial isolates, we observed high resistance against empiric antibiotics. We observed that E. coli and Klebsiella species show high resistance against Ampicillin (20(66.7%) and 10(91%) and Gentamycin (17 (55.6%) and 9(82%) respectively] (Table 3).

Table 1: Frequency distribution of isolated bacteria at time of diagnosis[13]

Variables	Late onset of sepsis (LONS) (n= 75)	Early onset of sepsis (EONS) (n= 47)
Others including Streptococcus pneumoniae, Listeria monocytogenes and candida	2 (40%)	3 (60%)
Coagulase negative staphylococcus	28 (77.7%)	8 (22.7%)
Citrobacter spp	2 (66.7%)	1 (33.3%)
Staphylococcus aureus	20 (83.3%)	4 (16.6%)
Enterococcus spp.	3 (50%)	3 (50%)
E. coli	8 (26.6%)	22 (73.3%)
Enterobacter spp.	5 (71.4%)	2 (28.6%)
Klebsiella spp	7 (63.6%)	4 (36.4%)

P value 0.001

Table 2: Antimicrobial sensitivity patterns for gram-positive bacteria[13]

Drugs	Enterococcus spp.(n=6)	Coagulase negative staphylococcus (n=36)	Staphylococcus aureus ((n=24)
Cloxacillin	Not tested	36 (100%)	16 (66.6%)
Ampicillin	2 (33.3%)	33 (91.6%)	16 (66.6%)
Erythromycin	5 (66.7%)	24 (66.6%)	13 (54.1%)
Gentamycin	4 (66.7%)	23 (63.8%)	14 (58.3%)
Clindamycin	2 (33.3%)	7 (19.4%)	3 (12%)
Ceftriaxone	3 (50%)	26 (72.2%)	13 (54.1%)
Chloramphenicol	2 (33.3%)	16 (44.4%)	9 (37%)
Ciprofloxacin	1 (16.7%)	13 (36.2%)	6 (25%)
Vancomycin	1 (16.7%)	10 (27.7%)	5 (20.8%)
Cotrimoxazole	4 (66.7%)	26 (72.2%)	16 (66.6%)

Table 3: Antimicrobial sensitivity patterns for gram-negative bacteria[13]

Variables	Enterobacter spp (n= 7)	E.coli (n=30)	Citrobacter (n= 3)	Klebsiella spp (n=11)
Erythromycin	Not tested	20 (66.7%)	Not tested	9 (72%)
Ampicillin	6 (85.5%)	20 (66.7%)	Not tested	10 (91%)
Amikacin	3 (43%)	7 (23.3%)	Not tested	4 (36%)
Gentamicin	6 (85.7%)	17 (56.6%)	2 (66.7%)	9 (82%)
Chloramphenicol	Not tested	15 (50%)	Not tested	6 (56%)
Cefotaxime	3 (43%)	11 (61.1%)	1 (33.3%)	9 (82%)
Cotrimoxazole	5 (71.4%)	19 (63.3%)	Not tested	8 (73%)
Ciprofloxacin	2 (28.6%)	7 (23.3%)	1 (33.3%)	3 (27%)

DISCUSSION

Our observations reported that one-third neonatal population had clinical sepsis with or without bacterial growth in blood cultures. These growths were the major reason for neonatal morbidity. Our findings are consistent with the previous studies conducted on developing countries[1,2,3].

We observed a lower rate of sepsis in patients of the early onset sepsis group as compared to the late sepsis group. Routine utilization of antibiotics during obstetric care could be the major reason for mortality differences. Routine consumption of antibiotics might affect the blood culture yields of neonates due to significant transplacental transfer of these antibiotics into a fetus. We observed that Gram-positive bacteria was one of the most reported isolated oraganism which enhanced neonatal sepsis. These results

are parallel to the results of studies conducted on Egypt, Uganda, and other developing countries[3,4,9,14,15].

As we set a score of less than 7 to describe the low Apgar score, we observed that babies with a low Apgar score of 5 had more chance of developing Gram-positive neonatal sepsis. The positive culture of gram-positive demonstrates that these babies might underwent resuscitation and enhanced the risk of gram-positive bacteria. Our findings are correlated with the previous study of Ethiopia and Tanzania[1,2]. We found that majority of LONS reported more gram-positive isolates as compared to EONS. The study of Ramesh[16] and Tumaini[1] reported similar results in which the LONS group had more isolated S. aureus which results in sepsis. Previous studies of Egypt, Tanzania, Uganda, and other developing countries reported a high rate of isolated gram-positive bacteria causing neonatal sepsis[6,7,11,17,18,19] similar to our results. The management guideline of neonatal sepsis recommended Ampicillin and Gentamycin as first-line treatment[20]. However, we found high resistance of bacteria against first-line therapy. The study of Egypt and India reported high resistance of isolated bacteria against Ampicillin and Gentamycin[6,7]. Their study reported 85%-95% resistance against Ampicillin however the resistance against Gentamicin was comparatively low (57.3–72%). High antimicrobial resistance (AMR) was reported due to increased consumption of these drugs for many other neonatal issues which were not even infectious. The majority of the neonates with positive isolated bacteria acquired infection from the hospital. We also observed a high resistance ratio against third-generation Cephalosporines. Similar results were reported from Iran, Tanzania, Egypt, Georgia, and many other developing countries[11,12,16,18,21,22,24]. In our study, we observed high methicillin-resistant S. aureus (MRSA) and MDR against both, Gram-positive and Gram-negative bacteria isolates. These results are in correspondence with many previous studies[1,2,18,19,25].

In our study, we reported better results of third-line therapy against isolated gram-positive bacteria. These results are comparable with the results of previous Indian and many other studies.[1-4,9,14,16,18,25]. These antibiotics were less consumed previously due to their less availability at our center. However, in one-fifth of affected neonates, we observed resistance of S. aureus against vancomycin. These results are in contradiction to the previous study of Vietnam and Egypt in which they reported nill resistance strain against vancomycin[7,22]. Resistance could occur due to failure of first and second-line therapy which enhances the consumption of vancomycin drugs. The failure of first and second-line antibiotic therapy is also reported in many other studies[22-28].

CONCLUSION

We observed that CoNS, S. aureus, E. coli, and Klebsiella spp were the most contributing factors of neonatal sepsis due to high resistance against first and second-line antimicrobials. Our results concluded that third-line antimicrobials can be a beneficial treatment for managing neonatal sepsis. However, the failure of the first two antimicrobials was observed due to high utilization before an accurate diagnosis of neonatal sepsis.

Conflict of interest: Nil

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