Original Article

Aerobic Bacteria and Their Antibiotic Resistance Profile in Neonatal Septicaemia: A cross Sectional Study in a Tertiary Care Hospital of Rajshahi, Bangladesh

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Abstract

Objective: Neonatal sepsis is an infection of the bloodstream in infants younger than 28 days old. It continues to be the major cause of morbidity and mortality among newborns, particularly in middle- and low-income nations. The objective of this study was to isolate and identify aerobic bacteria of neonatal sepsis by blood culture using FAN method followed by subculture and relevant biochemical tests.

Methods: It was a cross sectional type of descriptive study where the sample size was 95 and data were collected purposively from the clinically suspected neonatal septicaemia cases from inpatient Pediatric department in Rajshahi Medical College Hospital, Rajshahi.

Results: Most 34 (35.79%) of the cases were culture positive, whereas 61 (64%) were culture negative. 7 (21%) of the 34 positive cases were Gram-positive, while 27 (79%) were Gram-negative. S. aureus was found in 6 (17.65%) of the culture-positive isolates, followed by CoNS with 1 (02.94%), E. coli with 14 (41.17%), Klebsiella spp. with 6 (17.65%), P. aeruginosa with 6 (17.65%), and Acinetobacter with 1 (02.94%). Gram-positive bacteria were extremely susceptible to Vancomycin (90%), Gentamicin (90%), and Ciprofloxacin (80%) based on antibacterial susceptibility testing. Colistin (100%) and ampicillin (100%) exhibited the greatest resistance. Meropenem (90%), Amikacin (90%), and Colistin (89%) were the most effective antibiotics against Gram-negative bacteria, whereas Ceftazidime (60%) was the least effective. Vancomycin (100%) and ampicillin (100%) exhibited the highest degree of resistance.

Conclusion: Both Gram-positive and Gram-negative aerobic bacteria were related with newborn septicaemia, and a significant proportion of them were resistant to multiple medicines.

Key words: Neonatal septicaemia, Aerobic bacterial profile, FAN method, Bangladesh

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Introduction

Neonatal phase, or the first 28 days of a newborn's existence, is a key period in which the infant must adapt to a new environment. The undeveloped immune system and inadequate skin barrier of neonates render them extremely susceptible to infection.[1] Neonatal sepsis is an invasive, mostly bacterial infection that occurs during the neonatal era.[2] According to the WHO (World Health Organization), more than 40 percent of all deaths among children under the age of five occur during the newborn period.[3] Recently, the Global Burden of Disease (GBD) Study (2016/2017) predicted 1,3 million yearly incidence cases of newborn sepsis, resulting in 203 thousand fatalities owing to sepsis.[4] As a rising south Asian nation, Bangladesh is not an exception to this trend. In Bangladesh, Ethiopia, and Iran, the prevalence of newborn sepsis was 69.35%, 79%, and 51.8%, respectively.[5]

Neonatal surveillance in affluent nations identifies Group B Streptococcus (GBS) and Escherichia coli as the predominant EONS and LONS pathogens, respectively, followed by GBS and Staphylococcus.[6] In a study of the bacteriological profile of neonatal septicaemia in

Bangladesh, Klebsiella pneumoniae (31.03%), Escherichia coli (27.59%), Serratia (24.14%), Acinetobacter (10.34%), and Pseudomonas (6.90%) were identified.[7] For neonatal sepsis patients to have a favorable prognosis, antimicrobial treatment must be initiated promptly. Several Tertiary Care Hospitals in Bangladesh prescribe Amikacin and Ceftazidime as second-line antibiotics and Meropenem, Vancomycin, Ciprofloxacin, piperacillin/Tazobactam, and Imipenem/Cilastatin as third-line antibiotics. The advent and spread of pathogenic microorganisms resistant to many or all of the available antibiotics is a major public health problem.[8] Methicillin-resistant Staphylococcus aureus (MRSA), extended-spectrum -lactamase (ESBL)-producing, and multidrug-resistant Gram-negative organisms are the primary worry for clinicians who combat infections.[2] According to reports, Gram-negative bacteria, specifically Klebsiella, E. coli, and Acinetobacter, are the primary causes of neonatal sepsis in Bangladesh, and nearly all of them are resistant to Ampicillin, Gentamicin, and third-generation Cephalosporine.[9] Resistance to new medications such as Carbapenems is one of the greatest problems related with antibiotic therapy. About 89% of MBL-producing Gram-negative bacteria isolates in Bangladesh are resistant to imipenem, posing a challenge to infection control in the country.[10] Before initiating antibiotic therapy, it is crucial to undertake blood culture and antimicrobial susceptibility testing in neonatal septicemia.[11] To avoid newborn sepsis-related morbidity and mortality, it is required to strengthen the existing capacity for antenatal screening for early detection and treatment of maternal infection during pregnancy, as well as identification of high-risk pregnancies for effective perinatal therapy. Use of antibiotics in accordance with local epidemiology and culture and sensitivity data can reduce the dangers of antibiotic resistance.

Methods

This descriptive cross-sectional study was conducted between July 2021 and June 2022. During the study period, the study population comprised all clinically suspected cases of newborn septicemia from the Pediatric department of the Rajshahi Medical College Hospital, Rajshahi. After determining the sample size, 95 was determined to be the sample size. In this study, a purposeful sampling strategy was employed.

2.1 Inclusion criteria: The study included clinically suspected neonatal septicaemia cases having two or more of the following criteria:[12]

1. Unable to feed, 2. Lethargy, 3. Hypothermia/ hyperthermia, 4. Abdominal distension, 5. Respiratory distress with fast breathing, 6. Convulsions, 7. Slow/ fast heart rate

2.2 Exclusion criteria: Extreme low birth weight baby (<1 kg), extreme prematurity (<28 weeks of gestational age), resuscitated baby and parents or guardian refused to include in this study. Statistical analysis was carried out using SPSS software (IBM, version 21.0).

Collection of Blood:

After discussing the process and obtaining written authorization from the patient's legal guardian, the venipuncture site was cleaned with 10% povidone iodine and 70% alcohol using aseptic precautions. In addition, a single 2 ml blood sample was collected and put into a BacT/ALERT PF Plus bottle (BioMerieux, Inc., Durham, North Carolina) for isolation and identification of the organism by automated blood culture. Initially, the needle used for venipuncture was discarded and a new, sterile needle was inserted. The top of the BacT/ALERT PF Plus bottle was cleansed with 70% alcohol and allowed to air dry after the cap was removed. The bottle was then inoculated with 2 ml of blood by puncturing the rubber cap. The bottle of BacT/ALERT PF Plus was then gently shaken to combine the blood with the medium. Each bottle was labeled with pertinent patient information, including sample number, patient name, collection date and time, and registration number. The bottle was immediately transported to the RMCH Microbiology laboratory, where it was processed utilizing the BacT/ALERT 3D 60 Microbial Detection System (bioMerieux, Inc, Durham, North Carolina). The bottles of BacT/ALERT PF Plus contain 30 ml of complicated medium and 1.6 g of polymeric absorbent beads. After seven days, non-positive bottles were eliminated from the system.[13]

Gram staining and microscopy:

From a culture-positive sample, a thin, uniform smear was created. It was then air-dried and fastened with flames. The fixed smear was stained with gram stain to observe the gram reaction, morphology, and organization of bacteria under an oil immersion objective microscope. The observations were recorded on the predesigned data sheet.[14]

Subculture on:

After sanitizing the top of the positive vial with 70% alcohol, a small volume of blood was aspirated using a sterile disposable syringe, and then 2-3 drops of blood were dispensed onto blood agar, MacConkey agar, and nutritional agar media. Before injection, all culture plates were dried for 30 minutes in an incubator. The inoculating wire loop was sterilized using the red heat procedure and cooled in unused media before being placed to the dispensing drop to create a seed (A). The inoculating wire loop was warmed and then drawn in two or three parallel lines from the seed to the new medium surface (B,B,B). This procedure was repeated as B,B,B to C,C,C, C,C,C to D,D,D, and D,D,D to E,E,E, with the inoculating wire loop being reheated between each iteration.

At each phase, the inoculum was obtained from the most distal portion of the stroke that immediately before it. The blood agar, nutritional agar, and MacConkey agar plates were incubated aerobically at 37oC for 24 hours after inoculation (24 hours). After 24 hours, the culture plates were checked for microbial growth; if no growth was observed, the plates were incubated again and examined after another 24 hours. If a microbial colony grew, each organism was identified based on colony form, hemolytic criteria, and pigment production. For each positive culture, the vial and time of the initial discovery were recorded. After confirming the presence of microorganisms, the bottles and plates were discarded per the safety protocol. If no development was observed after three days, the plates were thrown in the same manner.[14]

Identification of Bacteria:

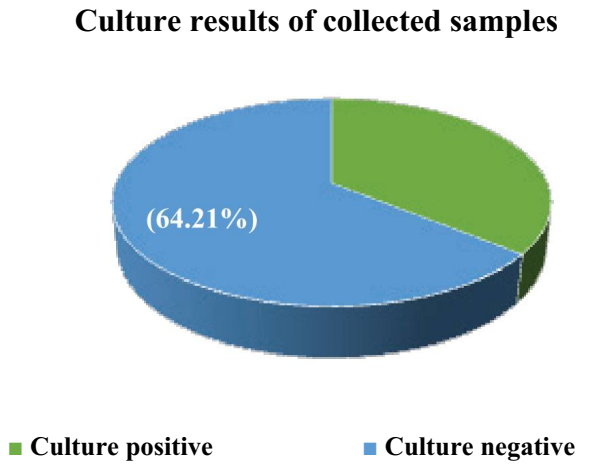
On the following day, bacterial isolates which yield significant growth were identified by Gram stain and microscopy, colonial morphology, lactose fermentation, pigment productions and hemolysis on blood agar plate. Motility test and relevant biochemical tests like catalase, coagulase, oxidase, citrate utilization test, Motility Indole Urease test and Triple sugar iron agar were also done. The identified bacteria were sub- cultured and processed for drug susceptibility test and preserved in Trypticase Soya Broth with 20% glycerol for further use.[14]

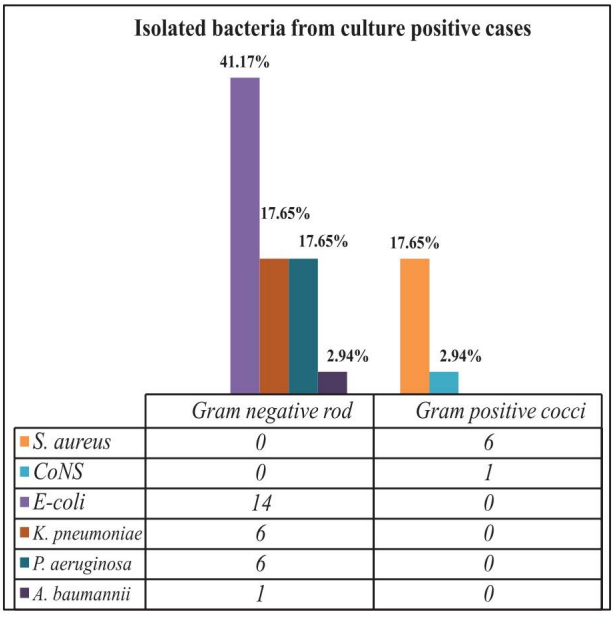
Results

Table 01 showed the different neonatal characteristics of the neonates. It revealed that maximum 55 (57.89%) neonates were male. About three-fifth (63.15%) neonates were born in hospital and by caesarian section. More than half (52.63%) of the neonates developed early-onset sepsis. Out of 95 samples, 61 (64.21%) were culture negative and 34 (35.79%) were culture positive (Figure I). Among 34 isolates, E. coli was the predominant bacteria about 14(41.17%). Other Gram negative isolates were Klebsiella spp. 6(17.65%), P. aeruginosa 6(17.65%) and Acinetobacter spp. 1(02.94%).In case of Gram positive isolates S. aureus was 6(17.65%) and CoNS was 6 (2.94%) (Fig-1). Among 34 isolates, E. coli was the predominant bacteria about 14(41.17%). Other Gram negative isolates were Klebsiella spp. 6 (17.65%), P. aeruginosa 6(17.65%) and Acinetobacter spp. 1(02.94%). In case of Gram positive isolates S. aureus was 6(17.65%) and CoNS was 6(2.94%) (Fig-2). Meropenem and Amikacin showed highest susceptibility (90%) followed by Colistin (87%) Ciprofloxacin (60%), Ceftazidime (50%), Gentamicin (17%), Piperacillin / Tazobactam (10%) towards most prevalent bacteria E. coli. Highest intermediate susceptibility was shown against Ciprofloxacin (30%), Ceftazidime (30%) followed by Piperacillin / Tazobactam (20%). Ampicillin showed highest resistance (100%) followed by Gentamicin (87%), Piperacillin / Tazobactam (70%), Colistin (13%) and Ceftazidime (20%) (Figure III). Among the above mentioned 9 drugs, Gentamicin Showed 90% susceptibility followed by Colistin (89%), Ciprofloxacin (70%) and Amikacin (60%) and towards Pseudomonas. Highest intermediate susceptibility was shown against Amikacin (30%), followed by Piperacillin / Tazobactam (10%) and Ciprofloxacin (10%). Ceftazidime, Vancomycin, Meropenem and Ampicillin showed highest resistance (100%) followed by Piperacillin/ Tazobactam (70%), Colistin (11%), Amikacin (10%) and Gentamicin (10%) (Figure V). Colistin showed highest susceptibility (100%) towards Acinetobacter. There were no intermediate susceptible and resistant drug towards Acinetobacter. 100 % resistant drugs were Ceftazidime, Amikacin, Vancomycin, Meropenem, Piperacillin / Tazobactam, Ciprofloxacin, Gentamicin and Ampicillin (Figure VI). Gentamicin and Vancomycin showed highest susceptibility (90%) followed by Ciprofloxacin (80%) Amikacin (80%) and Piperacillin/Tazobactam (40%) towards most prevalent bacteria S. aureus. Intermediate susceptibility was shown against Ciprofloxacin and Vancomycin (10%) in case of both drugs. 100% resistant drug were, Colistin and Ampicillin followed by Ceftazidime (80%), Amikacin (20%) and Ciprofloxacin (10%) (Figure VII). Vancomycin showed highest susceptibility (100%) towards CoNS. There were no intermediate susceptible and resistant drug towards CoNS. 100% resistant drug was Ceftazidime, Amikacin, Meropenem, Piperacillin/ Tazobactam, Ciprofloxacin, Gentamicin, Colistin and Ampicillin (Figure VIII). Highest susceptible drug for Gram positive bacteria were Vancomycin and Gentamycin. Highest susceptible drug for Gram negative bacteria were Meropenem, Amikacin and Colistin (Table 02). Out of 95 samples, 34 (35.79%) were culture positive and 15 (15.79%) showed intermediate susceptibility towards selected antibiotics such as Ceftazidime, Amikacin, Vancomycin, Meropenem, Piperacillin/ Tazobactam, Ciprofloxacin(Table 03).

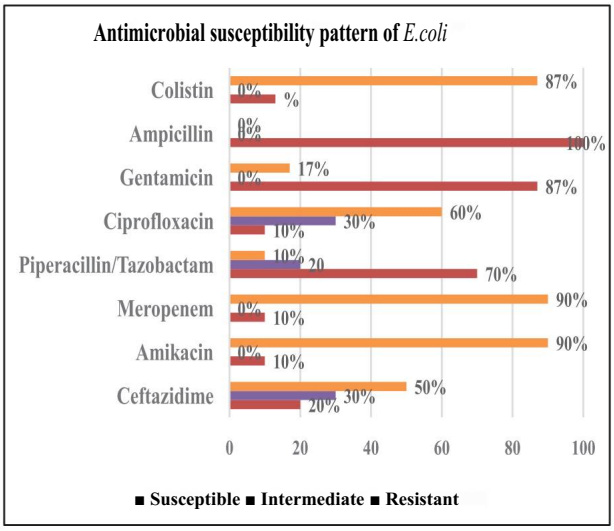
Table 01: Distribution of neonate according to neonatal characteristics (n=95)

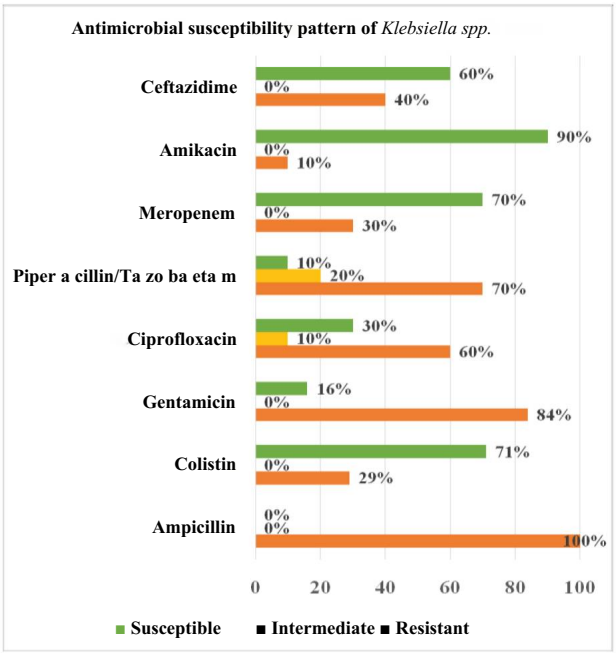
Neonatal characteristics		Frequency (%)
Sex	Male	55 (57.89)
	Female	40 (42.10)
Place of delivery	Hospital	60 (63.15)
	Home	35 (36.84)
Mode of delivery	C/S	60 (63.15)
	NVD	35 (36.84)
Type of sepsis	Early onset	50 (52.63)
	Late onset	45 (47.37)

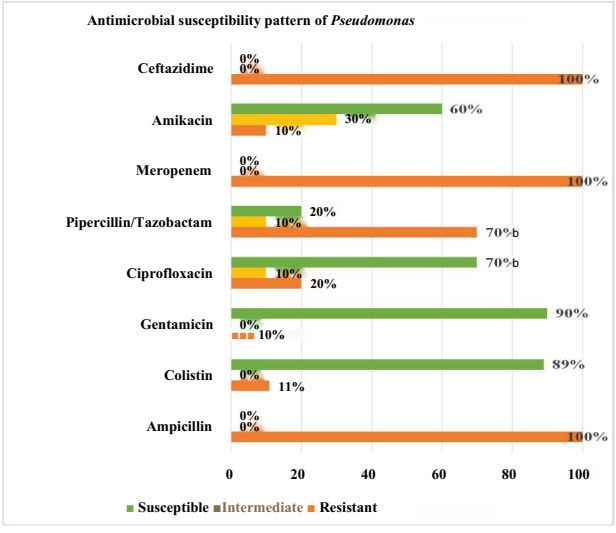


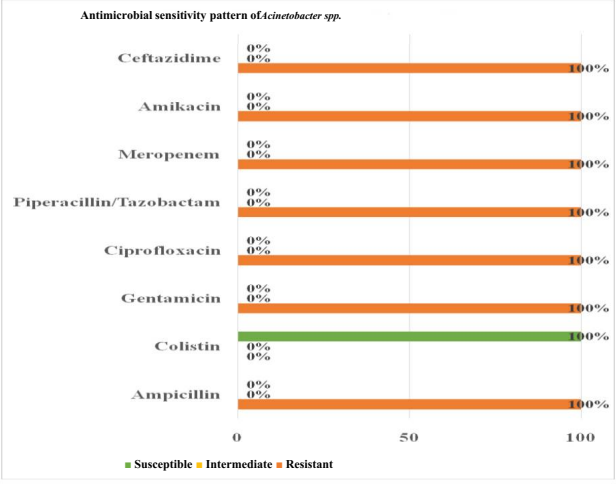


	Gram negative rod	Gram positive cocci
S. aureus	0	6
CoNS	0	1
E-coli	14	0
K. pneumoniae	6	0
P. aeruginosa	6	0
A. baumannii	1	0









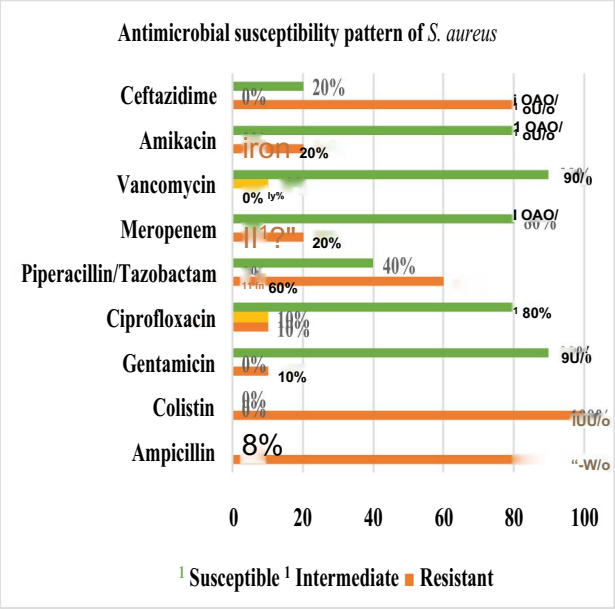


Table 02: Highest susceptibility drugs for each type of bacteria causing neonatal septicaemia

SI No	Name of bacteria	Highest susceptible drugs
01	*E. coli*	Meropenem, and Amikacin
02	*Klebsiella spp.*	Amikacin and Meropenem
03	*P. aeruginosa*	Gentamicin and Colistin
04	*Acinetobacter spp.*	Colistin
05	*S. aureus*	Gentamicin and Vancomycin
06	*CoNS*	Vancomycin

Table 03: Number of intermediate isolates (n=95)

No of total samples	No of culture positive case	No of intermediate isolates
95	34 (35.79%)	15(15.79%)

Discussion

This cross-sectional type of descriptive study was aimed to isolate and identify aerobic bacteria of neonatal sepsis by blood culture using FAN method followed by subculture and relevant biochemical tests. The present study was done with 95 cases of suspected neonatal septicaemia, attending in Department of Pediatrics, Rajshahi Medical College Hospital, Rajshahi.

In this study, different neonatal characteristics of the neonates revealed that 55 (57.89%) neonates were male and 40 (42.10%) were female. Nearly similar findings found in a study done in Bangladesh where male 55.26% and female 44.74%[15] and in Egypt where male 56.7% and female 43.3%.[12] Increased male septicemic neonates in this study may be due to gender biasness for hospital care in Bangladesh like other developing countries. Moreover, males are more prone to infection as genetic loci on the X chromosome. Presence of one X chromosome in the male baby confers less immunological protection compared to the female counterpart.[15] In this study, 60 (63.15%) neonates were bom in hospital by caesarian section and 35 (36.84) delivered in home by NVD. Nearly similar findings were found in a study in Nepal, where caesarean section delivery was 63.3%.[16] In the current study, more than half 50 (52.63%) of the neonates developed early onset sepsis and 45 (47.37%) developed late onset neonatal sepsis. In the current study, out of 95 samples 34 (35.79%) were culture positive and 61 (64.21%) were culture negative. A study was done in Bangladesh[17] and in India[18] reported that culture positive septicaemia was (31%) and (26.6%) respectively which were nearly similar with our findings. In previous studies in Bangladesh were conducted by Begum et al., (2016),[9] Hafsa et al., (2011),[19] were 7.45%,15.8 % respectively which were much lower. But other studies done by Rahman et al., (2020) in Bangladesh,[20] Misra et al., (2013)[21] and Awoniyi et al., (2009)[22] reported that culture positive isolates were 71.69%, 65.21% and 78% respectively which were far different from our study findings. The comparatively higher isolation rates in this study than previous studies in Bangladesh might be due to the fact that automated blood culture system was used in this study (FAN method) but other researchers used conventional blood culture method. In this study, according to Gram staining characteristics, 7 (20.59%) isolated bacteria from neonatal septicaemia were Gram positive cocci and 27 (79.41%) bacteria were Gram negative rods. Nearly similar findings were found in a study in Bangladesh[17] and in India[18] where Gram-negative isolates were 22 (70.97%), (68.7%) and Gram-positive 9 (29.03%) and (31.3%) respectively. This extreme rate of isolation may be due to bacterial pathogens of neonatal septicaemia are variable and differs

from place to place, various factors related to it like gestational age, birth weight, child health care facilities, maternal nutrition and maternal vaginal flora, perinatal care and hygienic conditions of mother etc. In the current study, among Gram negative rods, E. coli, Klebsiella spp., P. aeruginosa and Acinetobacter spp. were responsible for neonatal septicaemia in 41.18%, 17.65%, 17.65% and 2.94% cases respectively. Similarities was also found in a study, in Nepal where E. coli 35%. [23] In Nepal where Klebsiella pneumoniae (15.3%) was second leading cause of neonatal septicaemia.[16] These finding were not correspondent to the study in Bangladesh [19] where Klebsiella pneumoniae (50.0%) was the commonest bacterial pathogen and P. aeruginosa (1.9%) was the least isolated, while in this study. E. Coli (41.18%) was the most common organism. These findings were not resembling with a study in Bangladesh[9] where K. pneumoniae was the most common etiologic agent. In another study in Iran on 242 neonates found that P. aeruginosa (43%) was the leading cause of neonatal sepsis that was also dissimilar with the study findings.[24] The high prevalence of E. coli in this study may be due to the fact that it is commonly found as part of the intestinal and vaginal flora that were contaminated during deliveries at home presumably under conditions of poor hygiene.[23] These differences could be attributed to geographic location and with the time of onset of illness.

In this study, among Gram positive cocci Staphylococcus aureus and CoNS were responsible for 17.65% and 2.94% cases respectively. These findings were similar with a study in Bangladesh, in India which showed that Staphylococcus aureus were 15.4% and 22.9% respectively. This could be because Staphylococcus aureus is commonly associated with nosocomial sepsis as seen in LOS as well as in immunocompromised patients like the preterm babies.[18, 19] The pattern of bacterial resistance is important for epidemiological and clinical purposes. The obvious implication is that clinicians have to treat most of the cases empirically and this causes high degree of resistance to the commonly used antibiotics. This increasing antimicrobial resistance is a matter of concern with limited treatment options available for multidrug resistant strains. In the present study, highest susceptible drug for Gram positive bacteria were Vancomycin and Gentamycin. A study in Nepal reported that most effective antibiotic against Gram positive bacteria was found to be Gentamicin (93%) which was similar with the study findings.[16] In Bangladesh reported that Gram positive isolates were highly sensitive to vancomycin (83.3%) which was also similar with the findings.[17] In the current study highest susceptible drug for Gram negative bacteria were Meropenem, Colistin and Amikacin and moderate susceptible was Ceftazidime.

Similar studies done in Bangladesh[17] and in Nepal[16] showed highest (100%) effectiveness to Amikacin and Meropenem. In this study Ampicillin showed 100% resistance against Gram positive and Gram-negative organisms. The findings were in accordance with the study done in Bangladesh where all these three common isolates showed 100% resistance to Ampicillin.[9,17] In the present study, regarding antimicrobial susceptibility pattern of Gram negative bacteria E. coli showed that Colistin, Meropenem and Amikacin were the most effective drugs exhibiting 90% susceptibility followed by Ciprofloxacin (60%), Ceftazidime (50%) and Piperacillin/Tazobactam (10%). Klebsiella spp. was highly susceptible to Amikacin (90%) followed by Colistin (71%), Meropenem (70%) Ceftazidime (60%), Ciprofloxacin (30%), Gentamicin (16%) and Piperacillin/ Tazobactam (10%). This study was nearly similar to the study in Pakistan,[25] who reported that 94.12% to Meropenem, 64.7% to Ceftazidime. In Bangladesh showed very poor sensitivity to Gentamicin to all the common isolates which was also accordance with the findings.[17] Antimicrobial susceptibility pattern of P. aeruginosa showed highest susceptibility to Gentamicin (90%) followed by Ciprofloxacin (70%), Amikacin (60%), Colistin (89%). Ampicillin, Ceftazidime showed 100% resistance. However, the isolation rate was very low, still sensitivity of some drugs of this study was similar to the study in Nepal, who observed that 100% sensitive to Amikacin, Ciprofloxacin, Gentamicin. [23] In this study only one Acinetobacter spp. was isolated and was only susceptible to Colistin (100%). There was no single intermediate susceptible drug observed. In Bangladesh about 10.8% neonatal sepsis was responsible for Acinetobacter spp.[9] In current study the sensitivity pattern of Colistin towards Gram negative isolates was Acinetobacter spp. (100%), P. aeruginosa (89%), E. coli (87%) and Klebsiella spp. (71%).

Vancomycin and Gentamicin showed highest susceptibility (90%) followed by Ciprofloxacin (80%), Amikacin (80%) and Meropenem (70%) towards S. aureus. Highest intermediate susceptibility was shown against ciprofloxacin and vancomycin (10%) in case of both drugs. 100% resistant drug were Colistin, Piperacillin/ Tazobactam, Ampicillin followed by Ceftazidime was (80%). Antimicrobial susceptibility pattern of CoNS towards Vancomycin showed highest sensitivity (100%). In this study E-coli and Staphylococcus aureus were the most common Gram negative and Gram positive organisms causing neonatal septicaemia.

Conclusions

Newborn sepsis greatly contributes to neonatal morbidity

and mortality and is a serious global public health concern. The variables that contribute to its etiology remain poorly known. Patients in this study are newborns. Gram-negative bacteria predominate among culture-positive cases, with Escherichia coli being the most prevalent in this study. Gram-positive bacteria are more vulnerable to Vancomycin (100%) and Gentamicin (90%) while Gram-negative bacteria are most susceptible to Meropenem (90%), Amikacin (90%), and Colistin (88%). This effort will facilitate the development of more effective therapy guidelines, thereby preventing consequences of neonatal septicemia.

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Consent for publication: Not applicable for this study.

Ethics approval and consent to participate: The study got ethical clearance from the Ethical Review Board from Rajshahi Medical College, Rajshahi, Bangladesh.

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