Frequencies, sensitivity pattern and molecular characterization of bacterial isolates in blood in neonatal sepsis.

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ABSTRACT… Objective: To determine frequency, molecular characterization and sensitivity of bacterial isolates against commonly used antibiotics in neonatal sepsis. **Study Design:** Cross Sectional study. **Setting:** Department of Pathology Sahara Medical College Narowal. **Period:** October 2019 to March 2020. **Material & Methods:** Neonates admitted in neonatal intensive care unit (NICU) of study institution having signs and symptoms of neonatal symptoms such as fever, irritability, seizures, anorexia and lethargy, were included in the study using consecutive sampling technique. Blood sample from all study patients taken and sent for culture to determine bacterial isolates and antibiotic sensitivity against commonly used antibiotics for neonatal sepsis. Bacterial isolates identification was done using standard bacteriological technique performed by modified Kirby & Bauer disc diffuse method as per Clinical and Laboratory standards institute (CLSI) guidelines. **Results:** Total 200 cases were studied having neonatal sepsis and admitted in NICU including 58% female and 42% male children. Bacterial growth occurred in 10.5% samples and in 89.5% samples no bacterial growth seen. There were 1% samples with gram positive and 9.5% samples with gram negative bacterial isolates. Klebsiella was the commonest organism isolated in 38.1% cases out of total positive isolates. There were 72.5% neonates having age 1-14 days and 27.5% neonates having age 15-28 days. **Conclusion:** Gram negative bacteria are common cause of neonatal sepsis, out of which Klebsiella is the commonest organism. Antimicrobial drug resistance in different infections is a serious emerging issue.

Key words: Antibiotic Resistance, Antibiotic Sensitivity, Gram Positive Bacteria, Gram Negative Bacteria, Neonatal Sepsis.

INTRODUCTION

Septicemia in first month of child is called neonatal sepsis. Sepsis is a systemic condition characterized by bacteremia causing dysfunction of body organs. It is a lethal condition which should be immediately treated by sensitive and effective antibiotics.[1] Signs and symptoms of sepsis are fever, seizures, anorexia, lethargy, tachycardia, tachypnea etc. There are two types of neonatal sepsis first is early onset sepsis occurring from birth to 7th day of age, late onset sepsis occurs from 7th till completion of first month of life. Incidence of neonatal sepsis is high in developing and underdeveloped countries and low incidence in developed countries. According to a report its incidence is 1-4/1000 live births due to septic deliveries and good neonatal care.[2] Mortality rate of neonates in Pakistan is 49/1000 live births.[1,3] According to a study in neonatal sepsis gram positive bacteria are commonly found. In developing countries 1.6 million neonatal deaths occur each year due to neonatal sepsis.[2] Out of total neonatal deaths worldwide 7% occur in Pakistan and its main cause is neonatal infection occurring in 33% neonates.[3] Sepsis is very common in underweight children. In Pakistan 25% of cases with neonatal sepsis have either low weight or very low weight.[4] Early use of suitable empirical antibiotic regimen in neonatal sepsis reduces drug resistance and morbidity and mortality as well. A study conducted in Australia concluded gram positive bacteria as most common cause of neonatal sepsis and among them staphylococcus

were most common microbe.[5] A similar study conducted in India reported Klebsiella and E-coli as the commonest organisms in neonatal sepsis. Antibiotic susceptibility changes in developed and developing countries.[6]

Neonatal sepsis is a common cause of death in preterm neonates admitted in ICU. In 2010 7.6 million neonates died worldwide due to infections and neonatal sepsis.[7] In 1990 death rate of neonates was 37% out of total deaths in children under five years of age, which increased to 44% in 2013.[8,10] In developed countries despite much developed healthcare services, four out of ten neonates die due to sepsis or suffer from permanent disability.[8] In USA 36% of neonates born before 28 weeks of gestation suffer from one episode of infection at least during their birth hospitalization with 50% mortality rate. Compared to term babies, in preterm babies, sepsis is 1000 times more common.[9] According to a report published in 2016, mortality rate of under 5 years was 64.6 per 1000 live births in poorest households, and 31.3 per 1000 live births in richest households and those having in between socioeconomic status were having mortality rate between 64.6-31.3/1000 live births. Before prescribing antibiotic therapy it is very important to have knowledge of most common bacteria causing neonatal sepsis so that we may give proper treatment. There is very little data available from our country regarding common bacteria causing neonatal sepsis and most effective antibiotics against them, so this study was conducted so that we may treat children properly having neonatal sepsis and we may reduce mortality and morbidity rate as well.

MATERIAL & METHODS
This is a cross sectional study conducted in department of pathology Sahara Medical College Narowal. Study was started in October 2019 and completed after six months in March 2020. Inclusion and exclusion criteria were applied for selection of cases for study. Neonates admitted in neonatal intensive care unit (NICU) of study institution with age of 1-28 days having signs and symptoms of neonatal symptoms such as fever, irritability, seizures, anorexia and lethargy,

were included in the study using consecutive sampling technique. Neonates having congenital anomalies of heart, gastrointestinal tract and kidneys etc were excluded from the study. Base line and specialized investigations done. Blood sample from all study patients taken and sent for culture to pathology laboratory of study institution, to determine bacterial isolates and antibiotic sensitivity against commonly used antibiotics for neonatal sepsis. Bacterial isolates identification was done using standard bacteriological technique performed by modified Kirby & Bauer disc diffuse method as per Clinical and Laboratory standards institute (CLSI) guidelines. P-value < 0.05 was considered significant. Sample size was calculated using WHO sample size calculator with confidence level was 95% and margin of error was 5%, population anticipated proportion taken 8% and absolute precision was 4%. Sampling was done by non-probability consecutive sampling technique. Data calculated was documented on a performa and data analysis done on SPSS software. Percentage, frequency, means and standard deviation determined. Chi square test was applied on data. Consent was taken from all the patients for including their data into the study. Ethical approval was also taken from the institutional ethical review committee.

RESULTS
Total 200 cases were studied having neonatal sepsis and admitted in NICU including 116(58%) female and 42(42%) male children. Bacterial growth occurred in 21(10.5%) samples and in 79(89.5%) samples no bacterial growth seen. There were 2(1%) samples with gram positive (enterococcus) and 19(9.5%) samples with gram negative bacterial isolates. Gram negative bacteria included Acinetobacter in 3(1.5%) samples, E.coli in 4(2%) and Pseudomonas in 4(2%) samples. Klebsiella was the commonest organism isolated in 38.1% cases out of total gram-negative isolates. There were 72.5% neonates having age 1-14 days and 27.5% neonates having age 15-28 days.

25% Klebsiella were sensitive to Meropenem and Tazobactum, 25% Pseudomonas were sensitive to Amikacin and Ceftazidime, 50% were sensitive to Gentamycin and Meropenem and 100% were

sensitive to Tazobactum. 50-100% Enterococcus were sensitive to Ampicillin, Amikacin and Vancomycin. 33-66% Acinetobacter were sensitive to Ceftazidime and Meropenem. It was seen that Amikacin was effective against all these bacteria.

Micro-organism Isolated	Age (1-14 days)	Age (15-28 days)	Total	P-Value
E-coli (Negative)	3(14.3%)	1(4.8%)	4((19.1%)	
Acinetobacter (Negative)	3(14.3%)	0	3(14.3%)	
Pseudomonas (Negative)	4(19.1%)	0	4(19.1%)	<0.01
Klebsiella (Negative)	5(23.8%)	3(14.3)	8(38.1%)	
Enterococcus (Positive)	1(4.8%)	1(4.8%)	2(9.5%)	
Total	16(76.2%)	5(23.8%)	21(100%)	

Table-I. Age wise frequency of microbes isolated from in blood samples of patients showing bacterial growth (n=21).

Bacterial Sensitivity Pattern	E.coli n=4	Acinetobacter n=3	Pseudomonas n=4	Klebsiella n=8	Enterococcus n=2	P-Value
Amikacin	4 (100%)	1 (33.3%)	1 (33.3%)	1 (0%)	1 (50%)	
Ampicillin	1 (25%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)	
Ceftazidime	0 (0%)	1 (33.3%)	1 (33.3%)	0 (0%)	0 (0%)	
Cefotaxime	1 (25%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Meropenem	2 (50%)	2 (66.6%)	4 (100%)	1 (12.5)	1 (50%)	<0.05
Cloxacillin	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Vancomycin	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (100%)	
Tazobactum	0 (0%)	0 (0%)	4 (100%)	2 (25%)	0 (0%)	
Gentamycin	3 (75%)	1 (33.3%)	2 (50%)	0 (0%)	0 (0%)	

Table-II. Sensitivity of bacterial isolates against commonly used antibiotics for neonatal sepsis.
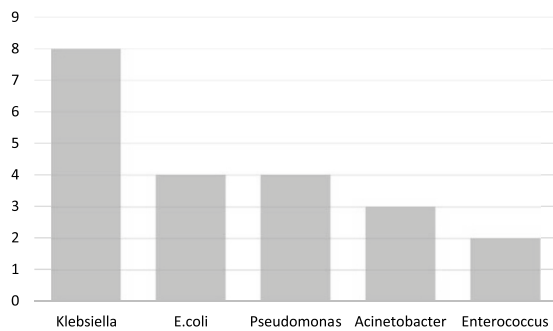

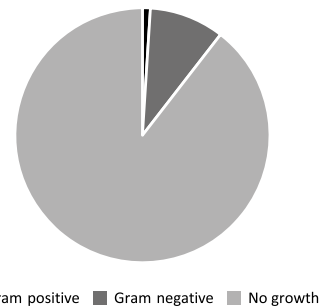**Figure-1. Frequency of various microbes isolated in blood. (n=21)**

Figure-2. Frequency of blood samples showing bacterial growth in Study group (n=200).
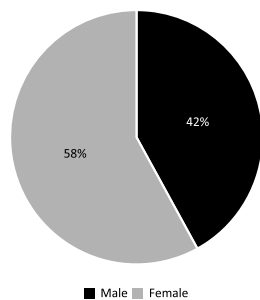

Figure-3. Sex distribution of neonates in study group (n=200).

DISCUSSION

Neonatal sepsis is a much common problem especially in preterm births.[10] Its indicators are fever, seizure, tachycardia, tachypnea and reluctant to feed. Elevated leucocyte count and acute phase protein and tachycardia are not specific to this condition as preterm babies mostly have such parameters as they are fighting with external environment before time.[11] If we wait to settle these signs and symptoms and delay antibiotic treatment then there is great chance

of developing antibiotic resistance and failure of treatment. So diagnosis should be clinical and immediately empirical antibiotic therapy should be given in neonatal sepsis.[12] Gold standard investigation for diagnosing neonatal sepsis is blood culture examination.[13] In a study done in Ethiopia by Moges et al 46.6% cases shows bacterial growth in blood samples suspected for neonatal sepsis. Most commonly gram-positive bacteria were found in 67.5% cases including staphylococcus in 40.8% cases and Klebsiella found in 15.8% cases. In their study 70% isolates were multidrug resistant.[14]

In our study most commonly gram negative bacteria were isolated from blood samples in 90.5% cases and gram negative bacteria were isolated in 9.5% samples out of total isolates showing bacterial growth. This difference may be due to geographical factor. In our study most common isolated organism was Klebsiella found in 38.1% of samples showing bacterial growth. A similar study conducted in Nigeria reported predominant gram-positive organisms in 59% and gram-negative in 41% cases of neonatal sepsis. In their study staphylococcus was most common and Klebsiella was second most common organism.[15] Similar study has been conducted in Islamabad, in which 8% samples showed bacterial growth and 92% samples did not show any growth. They concluded gram-negative bacteria as predominant organisms found in 92.3% samples and gram positive in 7.7% samples. Out of total 13 positive isolates, most common isolated organism in their study was Klebsiella found in 38.5% samples followed by pseudomonas found in 23.1% samples, E.coli and acinetobacter in 15.4% and enterococcus isolated in 7.7% isolates.[16] These results are similar to our study where gram negative organisms were predominant including Klebsiella as most common isolated organism. Other organisms in our study were E.coli found in 19.1%, Pseudomonas in 19.1%, Acinetobacter in 14.3% and enterococcus isolated in 9.5% samples out of total 21 positive isolates.

In their study 20% Klebsiella were sensitive to Meropenem and Tazobactum, 50-100%

E.coli were sensitive to Ampicillin, Amikacin, Gentamycin and Meropenem. 50% Acinetobacter were sensitive to Amikacin, Gentamycin and Meropenem.[16,17] These results are comparable to our study in which 25% Klebsiella were sensitive to Meropenem and Tazobactum, 25% Pseudomonas were sensitive to Amikacin and Ceftazidime, 50% were sensitive to Gentamycin and Meropenem and 100% were sensitive to Tazobactum. 50-100% Enterococcus were sensitive to Ampicillin, Amikacin and Vancomycin. 33-66% Acinetobacter were sensitive to Ceftazidime and Meropenem. It was seen that Amikacin was effective against all these bacteria. Abdul Rehsamn et al reported coagulase negative Staph. Aureus most common isolating organism in 58.5% cases, followed by Coagulase positive Staph. Aureus in 16.6% and E.coli in 7.6% cases.[18] In their study E.coli was common among Gram-negative organisms, but in our study it was second common organism after Klebsiella. In another study done by Shane et al, Klebsiella was isolated in 42% bacterial cultures followed by coagulase negative staph. aureus isolated in 19% cultures.[19] A study done in Nigeria by Moges et al found predominance of gram-positive bacteria staphylococcus aureus in 59% specimens as compared to 11% cultures having Klebsiella P. (Gram-negative).[23] A study done in Northwest Ethiopia reported Klebsiella the most common Gram negative organism found in 15.8% cultures. These results are similar to our findings.[20] Selium et al and other authors also found increasing resistance among gram negative bacteria, as reported 96.9% resistance reported against ampicillin, 90.6% resistance against amoxicillin-clavulanic acid and 84.4% resistance against cefotaxime and ceftazidime.[21,22] New developments are being done to reduce prevalence of neonatal sepsis such as sepsis prediction scores to diagnose it without culture examination, proper antimicrobial use and introducing preventive measures like maternal vaccines etc.[23]

CONCLUSION
Gram-negative bacteria are common cause of neonatal sepsis and use of proper antibiotics according to sensitivity pattern can reduce morbidity, mortality and resistance against

unnecessary use of antibiotics, so multidrug resistance in bacteria can be avoided. Antibiotic resistance is a main cause of treatment failure in neonatal sepsis, so isolation of bacteria from blood culture and starting antibiotic treatment according to sensitivity pattern can effectively treat the disease.

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**Weak people revenge.
Strong people forgive.
Intelligent people ignore.**

Albert Einstein

AUTHORSHIP AND CONTRIBUTION DECLARATION

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2	Ghulam Asghar Bhutta	Topic selection and data collection, Abstract and recording.	*Asghar*
3	Khushbu Farva	Data collection, Found additional sources of information.	*Khushbu*