Bacterial Isolate of Neonatal Sepsis and their Susceptibility Pattern in POF Hospital Wah Cantt.

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Abstract

Background
Neonatal sepsis is the occurrence of microorganisms in a normally sterile site (blood) of the neonates which accompanied by signs and symptoms of infection and systemic inflammatory response in the first month of life. Over world while 1.6 million deaths were recorded due to neonatal sepsis, caused by bacterial infection. The objective of the study is to find the frequency of baccteremia causing neonatal sepsis and their susceptibility pattern at Neonatal Intensive Care Unit (NICU) of Pakistan Ordinance Factory (POF) Hospital Wah Cantt.

Material & Methods
Study design: Descriptive Cross sectional study.
Study setting: Microbiology section of POF Hospital laboratory Wah Cantt.
Study sample: A blood sample of (n=168) patients (one each), clinically diagnosed as neonatal sepsis (both early and late onset), admitted to NICU were taken. Inclusion Criteria: Before administration of antibiotics, in six months' period from December 2014 to June 2015, exclusion Criteria: Patients who were already on antibiotics.
Data analysis: All blood samples were analyzed for bacterial pathogens and their antibiotic susceptibility was assessed by standard microbiological methods. By using SPSS, frequency chart and tables were developed to display the results.

Results
Out of N=168 blood samples, fifty one (30.4%) isolates were gram- positive and one hundred and seventeen (69.6%) were gram-negative bacteria. Among gram- positive isolates, *Staph. aureus (31.4%) and* Methicillin resistant coagulase negative staphylococcus (MRCoNS) (31.4%) were most frequently found while *Klebsiella* species (65.8%) was most frequent isolate in gram-negative bacteria. Gram-negative pathogens exhibited sensitivity mostly to amikacin (76.9%)and gram-positive isolates were sensitive to vancomycin (95.2%).

Conclusion
The present study concludes that gram negative bacteria predominantly as the causative agent of neonatal sepsis in our setting. Isolated bacteria showed high resistance to commonly prescribed antibiotics. Establishment and implantation of infection control practices are required to overcome this grave situation.

Keywords
Neonatal sepsis, Microorganisms, Antibiotic sensitivity.

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Background
Neonatal sepsis is the occurrence of microorganisms in a normally sterile site (blood) of the neonates and accompanied by signs and symptoms of fever, lethargy, poor cry, difficulty to arouse, refusal to suckle, abdominal distension and unstable body temperature associated with bacteremia or meningitis.[1] Neonatal septicemia is of two types, early onset sepsis (EOS) and late onset sepsis (LOS). During the first 5-7 days of life, the fulminant multisystem illness encountered is EOS whereas LOS is most commonly recognized after the first week of life.[2] Onset of infection within the first six days of life reflects vertical transmission from mother to infant, while at seventh day of life or later is likely to be acquired through horizontal transmission.[3] Over world while 1.6 million deaths were recorded due to neonatal sepsis, caused by bacterial infection.[4] This emergent medical condition required prompt diagnosis and relevant treatment, to prevent complications and death due to septicemia[5,6] The frequency of bacteria causing neonatal sepsis and their susceptibility pattern vary at different countries, even at different hospitals in same country.[7] The most common pathogens for neonatal sepsis in Europe and America were gram-negative organisms, in 1960. Which were replaced by group B *Streptococcus* in 1970`s, and coagulase negative staphylococci during late 1980`s and 1990`s. Gram negative organisms still remain the main cause of neonatal sepsis in most of the developing countries.[8]

In Asia, according to studies common organisms isolated from blood culture in neonatal sepsis were gram positive cocci including coagulase negative staphylococci (CoNS), *Staphylococcus aureus and enterococcus* while gram negative rods revealed, *E.coli*, *Pseudomonas* spp., *Enterobacteriaeceae* and *klebsiella* spp.[2,7]

Another study from Southern India, revealed that coagulase negative staphylococcus (CoNS) found to be the largest group 262 (37.6%) of isolates, followed by *Klebsiella* species 129(18.5%), *Pseudomonas* spp. *98*(14.1%), *Acinetobacter* spp.

49 (7%) and *Enterobacter* spp. 34(4.9%). Gram-negative organisms like *Enterobacter* spp., *Pseudomonas* spp. and *Klebsiella* spp., were found to be sensitive to amikacin and ciprofloxacin. In gram positive organism, coagulase negative staphylococcus (CoNS) was sensitive to ciprofloxacin.[9] In Pakistan isolated organisms in neonatal unit were, *E.coli* 22 (33.37%), *Klebsiella* spp. 22 (34.37%) and *Pseudomonas* spp. 8(12.5%), which were sensitive to ceftazidime, amikacin and imipenem.[10,11]

Neonates with sepsis are at high risk of losing life if there is delay in initiating treatment. The empirical antibiotic therapy should be started immediately to achieve the aim. So it is mandatory to identify the etiological agents and their antibiotic susceptibility pattern. Diversity of bacteria causing neonatal sepsis and their susceptibility pattern varies in time and region due to irrational use of antibiotics and change in life style.[5] The indecision regarding the choice of antibiotics can be reduced by regular survey of etiological agents and their antibiotic susceptibility patterns.

Awareness of predictors of positive blood culture and antimicrobial susceptibility pattern of common pathogens in a given area is crucial in guiding local empirical choice of antibiotics.[6]

The study was planned to identify the bacteria causing neonatal sepsis and their susceptibility pattern in POF Hospital Wah Cantt, so as to start specific, cost effective treatment at the earliest.

Materials & Methods

The descriptive Cross sectional study was designed at Microbiology section of POF Hospital laboratory Wah Cantt. A blood sample of (n=168) patients (one each), clinically diagnosed as neonatal sepsis (from birth till 28 days both early and late onset neonatal sepsis) admitted to NICU were taken.

Inclusion Criteria: Before administration of antibiotics, in six months' period from December 2014 to June 2015, without discrimination of gender & prematurity or birth weight.

Exclusion Criteria: Patients who were already on antibiotics. All blood samples were analyzed for bacterial pathogens and their antibiotic susceptibility was assessed by standard microbiological methods. By using SPSS, frequency chart and tables were developed to display the results.

Blood samples were collected in Brian heart infusion (BHI) broth for culture during period of study. The blood samples were incubated for 24 hrs at 37ºC under aerobic conditions. Subcultures were done by collecting the inoculums from BHI broth and inoculating on blood agar and MacConkey agar on three alternative days. The subcultures were incubated for 18-24 hrs at 37ºC under aerobic conditions. The agar plates were examined for growth of bacteria and their colonial morphology. The bacterial growth was subjected to gram stain and biochemical tests. Gram-negative bacilli were identified by using Oxoid Microbact 24E test strips. Gram positive cocci were identified by catalse and coagulase test. Antimicrobial susceptibility testing for gram-positive organism, was carried out on Muller Hinton agar using discs of penicillin (10 units), cefoxitin (30µg) (Oxoid, Basingstoke,UK), amoxicillin/clavulanic acid (20/10 µg), erythromycin (15 µg), linezolid (30 µg), ciprofloxacin (5 µg), clindamycin (2µg) and doxycycline (30 µg) and E strip of vancomycin (MIC). Gram-negative isolates were subjected to antimicrobial susceptibility test using discs of ampicillin (10µg), amoxicillin/clavulanic acid (20/10g), cefuroxime (30µg), ciprofloxacin (5µg), meropenem (10µg), cefepime (30µg), amikacin (30µg), gentamicin (10µg) and piperacillin/tazobactam (100/10µg), by Modified Kirby-Bauer disc diffusion method, according to CLSI recommendations.[12] ATCC 25923 *Staphylococcus aureus*, and ATCC 25922 *E.coli* were used as control strains.

Results

In a total of N= 168 positive blood culture isolates of neonatal sepsis, fifty one (30.4%) isolates were gram- positive and one hundred and seventeen (69.6%) were gram-negative bacteria. In the patients revealing isolation of gram-negative bacteria, the mean age was 4.8 + 5.4 days, and in gram-positive bacteria the mean age was 6.24 + 8.07 days. Out of 117 Gram-negative isolates, 63 (53.8%) were from male patients and 54 (46.2%) were from female patients. Gender distribution for 51 Gram-positive isolates were, as 36 (70.65%) and 15 (29.35%) for male and female patients respectively, (Table1).

Among gram- positive isolates, *Staph. aureus (31.4%)*, Methicillin resistant coagulase negative staphylococcus (MRCoNS) (31.4%) and methicillin resistant *Staphylococcus aureus* (MRSA) (23.5%), were frequently found (Figure1), while in gram-negative bacteria, *Klebsiella* species (65.8%) and *E.coli (24.8%)* were frequently isolated bacteria.

The major contribution of *Klebsiella* species found *Klebsiella oxytoca (34.2%) and Klebsiella pneumoniae (31.6%) (*Figure2*).*

Gram-negative organisms were found to be sensitive to amikacin (76.9%), piperacillin/tazobactam (75.2%), ciprofloxacin (63.2%) and cefepime (61.5%), and resistant to ampicillin (93.2%), gentamicin (60.7%), amoxicillin/clavulanate (75.2%), cefuroxime (74.4%) and meropenem (62.4%). (Table 2). Gram-positive isolates were sensitive to vancomycin (95.2%), linezolid (82.4%), amikacin (74.5%), and amoxicillin/clavulanate (60.8%), and resistant to penicillin (88.2%), doxycyclin (75.2%) and erythromycin (60.8 %). (Table 3). Twelve MRSA were isolated, among them 91% to vancomycin and linezolid, 50% to ciprofloxacin and amikacin, 41% were sensitive to clindamycin and erythromycin and 25% to doxycycline. (Table4)

Table 1: Demographic characteristics of study groups

Characteristics	Gram-negative isolates	Gram-positive isolates
Number of patients	117	51
Age of the patients(in days)	4.8 + 5.4	6.24+ 8.07
Gender (M/F)	53.8% /46.2%	70.65%/29.35%

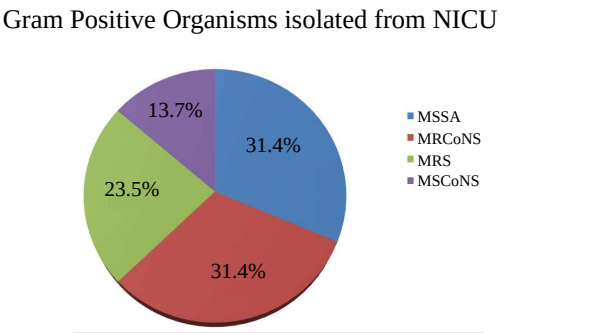

Fig 1. Percentage of Gram-Positive isolates from NICU

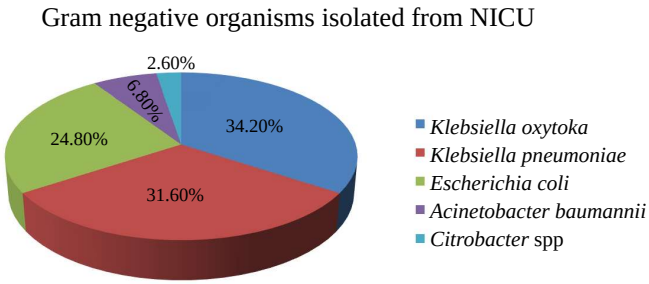

Fig 2. Percentage of Gram-negative isolates from NICU

Table 2: Antimicrobial sensitivity and resistance pattern of Gram-negative isolates

Antimicrobials	Sensitive	Resistance
Amikacin	76.9%	23.1%
Piperacillin/tazobactam	75.2%	24.8%
Ciprofloxacin	63.2%	36.8%
Cefepime	61.5%	38.5%
Gentamicin	39.3%	60.7%
Meropenem	37.6%	62.4%
Cefuroxime	25.6%	74.4%
Amoxicillin/clavulanate	24.8%	75.2%
Ampicillin	6.8%	93.2%

Table 3: Antimicrobial sensitivity and resistance pattern of Gram-positive isolates

Antimicrobials	Sensitive	Resistance
vancomycin	95.2%	4.8%
Linezolid	85.4%	14.6%
Amikacin	74.5%	25.5%
Amoxicillin/clavulanate	60.8%	39.2%
Erythromycin	39.2%	60.8%
Doxycycline	24.8%	75.2%
Penicillin	11.8%	88.2%

Discussion

Timely diagnosis and therapy are important for the avoidance of morbidity and mortality due to neonatal sepsis in NICU.[13] The precise prediction of likely pathogens and antimicrobial resistance patterns is a fundamental requirement for successful therapy. The distribution of the causative pathogens for sepsis in our study showed that these infections were caused mainly by gram-negative bacteria, mostly *Klebsiella oxytoca, Klebsiella pneumoniae*, and *E.coli*. The gram-positive bacteria included methicillin sensitive *Staph. auerus* (MSSA), methicillin resistant *Staphylococcus auerus* (MRSA), methicillin resistant coagulase negative staphylococci (MRCoNS), and less commonly with methicillin sensitive coagulase negative staphylococci (MSCoNS) while a study in Africa and Asia have shown *Klebsiella, pseudomonas, Enterobacteriaceae and E.coli as predominant gram negative organism and Staph. aureus as* common gram positive organism. [4, 14]

Coagulase negative staphylococci (CoNS) are the common colonizer of skin and mucous membranes of neonates, they are the common cause of blood culture contamination.[15] It is still hard to decide which blood isolates of CoNS represent true infection and which are contaminants.[16] Standard precautions were observed.[17] Historically the predominant organisms associated with neonatal sepsis have changed in years. In the past gram-positive bacteria dominated over gram-negative bacteria, but now the frequency of gram-negative organisms has increased in the recent years.[18]

Our study revealed the resistance pattern of gram negative isolates as ampicillin (93.2%), amoxicillin/clavulanic acid (75.2%) and cefuroxime (74.4%) and that of gram positive isolates as penicillin (88.2%), doxycyclin (75.2%) and erythromycin (60.8%). Effective antibiotics were amikacin (76.9%), Piperacillin/tazobactam 75.2%, ciprofloxacin (63.2%), and cefepime (61.5%) for gram negative bacteria, while vancomycin (95.2%), linezolid (82.4%), and amikacin (74.5%) for gram positive bacteria. MRSA showed highly sensitive pattern for vancomycin and linezolid i.e. eleven out of twelve were sensitive to vancomycin and linezolid. According to the

Table 4: Sensitivity/resistance pattern of antimicrobial against MRSA (n=12)

	Clindamycin	Erythromycin	Doxycycline	Ciprofloxacin	Amikacin	vancomycin	Linezolid
Sensitive	5 (41%)	5 (41%)	3 (25%)	6 (50%)	6 (50%)	11(91%)	11(91%)
Resistant	7 (59%)	7 (59%)	9 (75%)	6 (50%)	6 (50%)	1 (9%)	1 (9%)

study conducted in Tanzania, about 82% *Klebsiella pneumoniae* and 76% *E.coli were* resistant to gentamicin and ampicillin while methicillin resistant *Staphylococcus aureus* among gram-positive organisms was the second common isolate.[6] Another study from Uganda reported that most of the gram negative bacteria isolated in their study were resistant to ampicillin.[19] Where as a study in Pakistan conducted at NICU of Multan Hospital, gram negative isolates were sensitive to piperacillin/ tazobactam, cefoperazone/sulbactam and imepenem,[20] moreover imipenem was the most effective drug against gram-negative bacteria.[21]

Swift treatment with antibiotics is necessary for encouraging outcome of neonatal sepsis. In our study, *Staphylococcus* spp. and gram negative bacilli were frequently found to be resistant to ampicillin, thus indicating that the use of ampicillin to manage neonatal sepsis in our set up may be ineffective. Since *Staphylococci* are mostly resistant to penicillin therefore vancomycin will be a better choice for the coverage of the gram-positive pathogens. Gram-negative pathogens, *Klebsiella oxytoca*, *Klebsiella pneumoniae* and *E.coli* are resistant to commonly used antibiotics in our setup.

Conclusions

The present study concludes that gram negative bacteria predominantly as the causative agent of neonatal sepsis in our setting. Isolated bacteria showed high resistance to commonly prescribed antibiotics. Study suggested that amikacin and piperacillin/tazobactam should be given as empiric regimen.

Establishment and implantation of infection control practices are required to overcome this grave situation.

Recommendations

The knowledge of prevailing bacterial pathogens and their antibiotic sensitivity patterns in the region are essential to overcome the problem of neonatal sepsis. Continued surveillance is mandatory to assess the resistance pattern at a certain center and empirical antimicrobial therapy must be tailored according to the local as well as regional data.

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