https://doi.org/10.36570/jduhs.2021.3.1240

JDUHS

ORIGINAL ARTICLE

Bacteriological Profile and Antibiogram of Neonatal Sepsis

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ABSTRACT

Objective: To determine the frequency of common bacterial isolates and antimicrobial sensitivity pattern in patients with neonatal sepsis. Moreover, to assess the clinical profile of neonates with culture positivity.

Methods: This prospective cross-sectional study was conducted at emergency department of National Institute of Child Health, Karachi from September 2017 to March 2018. All clinical suspected neonates with sepsis of either gender were consecutively enrolled. All those neonates already taking antibiotic were excluded. Positive specimens were sub-cultured on blood agar and MacConkey agar and incubated at 37°c for 24hr. Isolated colonies was gram stained and biochemically identified. Common bacterial Isolates was also noted. This information along with certain important neonatal and maternal characteristics were noted.

Results: Of 151 neonates, the mean neonatal age was 11.41 ±6.76 days. There were 87 (57.6%) females and 64 (42.4%) males. Culture positivity was observed in 41 (27.15%) patients. Among 41 culture positive cases, Klebsiella Specie was found positive in majority 14 (34.1%) patients followed by staphylococcus aureus 13 (31.7%), Escherichia coli 8 (19.5%), Coagulative negative staphylococci 5 (12.2%) and group B streptococci 1 (2.4%). A significant association of culture positivity was observed with gestational age (p-value 0.002), low birth weight (p-value 0.039), low Appearance, Pulse, Grimace, Activity, and Respiration (APGAR) score (p-value 0.018), urinary tract infection in mother (p-value 0.026), and chorioamnionitis (p-value 0.005).

Conclusion: Culture positivity was observed in considerable number of suspected neonates. Current magnitude of common bacterial Isolates are Klebsiella Species and Staphylococcus Aureus while Meropenum and Amikacin are common antibiotics sensitivity pattern in patients with neonatal sepsis.

Keywords: Antimicrobial Sensitivity, Common Bacterial Isolates, Neonates, Sepsis.

INTRODUCTION

Neonatal sepsis is one of the significant life-threatening condition encountered by almost all neonates affected due to any communicable or non-communicable diseases particularly during hospitalization.[1] Various studies have reported that neonatal sepsis is a significant cause of morbidity and mortality worldwide.[1-4] Though, it is reported that sepsis is highly preventable, still, an estimate given by World Health Organization (WHO) revealed that on average, 1 million mortality among neonates was observed due to sepsis every year. In Pakistan, situation is also unsatisfactory.[5] According to a study, 25-50% of all neonatal mortality are attributable to infections.[6]

While, appropriate therapeutic options are now available, still, the neonatal sepsis problem is on the rise not only in early onset but in late onset neonatal sepsis as well. Literature has identified many causes for the adverse outcome amid early clinical intervention that involves drug resistance as one of the most common issues faced in most cases.[7,8] In developing country like Pakistan, drug resistant not only adds to difficulty in management and increased financial cause but a leading cause of neonatal death due to sepsis as well.[9]

Thus, the sensitivity pattern of the causative organisms must be studied frequently to determine ongoing resistance to antimicrobial therapy. As etiological agents and their pattern of antimicrobial susceptibility vary from region to region, knowledge of the prevalence of local isolates and their pattern of antimicrobial sensitivity is of utmost importance for prompt antimicrobial therapy. This study was conducted to determine the current magnitude of common bacterial isolates in patients with neonatal sepsis and common antibiotics sensitivity pattern. By virtue of this instant and suitable antibiotics could be started rather than long wait for culture reports.

METHODS

This prospective cross-sectional study was conducted at emergency department Of National Institute of Child Health Karachi, Pakistan from September 2017 to March 2018. All clinically suspected neonates for sepsis of either gender were consecutively enrolled. Clinical sepsis was labeled as positive based on the presence of neonates who have signs and symptoms of neonatal sepsis with or without risk factors.

Ethical clearance was taken from institution ethical review board. All information was kept confidential and was used only for research purpose.

Sample size was calculated at 95% confidence level with 4% precision, using WHO calculator. Sample size came out to be 151 calculated on the basis of previous study coagulase negative staphylococci (CoNS) 6.7%.[10]

A blood sample of 2.5ml was collected in pediatric blood culture bottles (bactec peads plus) by using aseptic technique (after collecting samples antibiotic were given accordingly and none of the patients had a delay in antibiotic use). Samples obtained in the microbiology laboratory of the National Institute of Child Health were incubated in the bactec 9120 instrument (Becton Dickinson, USA) for at least five days before being reported as negative. Positive specimens were sub-cultured on blood agar and macconkey agar and incubated at 37°c for 24 hours. Isolated colonies were gram stained and biochemically identified.

Antimicrobial Sensitivity Pattern was assessed by the measure of growth of an isolated microbe zone diameter ≥15 was taken as sensitive and ≤12 as resistance. Commonly used antibiotics in neonatal sepsis are Ampicillin, Amoxicillin-clavulanic acid, Amikacin, Ciprofloxacin Ceftriaxone Ceftazidime, Cefotaxime, cotrimoxazole, chloramphenicol, Gentamicin, Linezolid, Meropenem, Tanzocin (*Piperacillin* and Tazobactam), and Vancomycin. Sensitivity pattern to be assessed for these commonly used antibiotics. Commonly isolated microorganisms are mentioned in table 1. Statistical Package for Social Sciences (SPSS) version 24 was used for the purpose of statistical analysis. Quantitative variable like age was expressed as mean and standard deviation. While frequencies and percentages were computed for qualitative variables like gender, mothers' educational status, mode of delivery, hypertension, premature rupture of membranes (PROM), endometritis, and chorioamnionitis. Inferential statistics were explored using Chi-square test for comparison of culture positivity with neonatal maternal characteristics. The p-value of ≤0.05 considered as significant.

RESULTS

Of 151 neonates, the mean age of the patients was 11.41 ±6.76 days. Majority of the neonates 101 (66.9%) were presented with ≤15 days of age. There were 87 (57.6%) females and 64 (42.4%) males. Maternal characteristics showed that 49 (32.5%) of the mothers were illiterate, caesarean mode of delivery was observed in 79 (52.3%) patients, hypertension in 50 (33.1%), PROM in 32 (21.2%), endometritis in 22 (14.6%), and chorioamnionitis in 21 (13.9%) patients.

There were total 41 (27.15%) patients with positive culture sensitivity. A significant association of culture positivity was observed with gestational age (p-value 0.002), low birth weight (p-value 0.039), low Appearance, Pulse, Grimace, Activity, and Respiration APGAR score (p-value 0.018), urinary tract infection (UTI) in mother (p-value 0.026), and chorioamnionitis (p-value 0.005). (Tables 2 & 3)

Among 41 positive cases, Klebsiella spp was found

Table 1: Commonly isolated micro-organisms

- **Klebsiella spp, presence of all of the following:**
  - o Gram-negative
  - o Non-motile
  - o Encapsulated
  - o Lactose-fermenting
  - o Facultative anaerobic
  - o Rod-shaped bacterium
- **Group B streptococci: presence of all of the following:**
  - o Gram-positive
  - o Non spore forming bacteria
  - o pairs or chains of varying length
  - o Round or ovoid in shape
- **Escherichia coli: presence of all of the following:**
  - o Rod-shaped bacterium
  - o Approximately 0.5 µm in width by 2 µm in length
  - o Gram-negative bacterium
- **Staphylococcus aureus: presence of all of the following:**
  - o Gram positive
  - o Non-moving small round shaped or non-motile cocci
  - o Grape-like (staphylo-) clusters
- **Coagulase negative staphylococci (CoNS): presence of all of the following:**
  - o Gram positive
  - o Catalase Positive
  - o Immobile
  - o Non-capsulated spherical cocci

Table 2: Comparison of culture positivity with neonatal characteristics (n=151)

Neonatal Characteristics	Culture		p-value
	Positive (n=41) n (%)	Negative (n=110) n (%)	
Neonatal age, days			
≤15	27 (26.7)	74 (73.3)	0.869
>15	14 (28.0)	36 (72.0)	
Gender			
Male	17 (26.6)	47 (73.4)	0.889
Female	24 (27.6)	63 (72.4)	
Gestational age, weeks			
Preterm	31 (38.3)	50 (61.7)	0.002*
Term	10 (14.3)	60 (85.7)	
Mode of delivery			
Vaginal	24 (33.3)	48 (66.7)	0.103
Cesarean	17 (21.5)	62 (78.5)	
Low birth weight			
Yes	26 (34.7)	49 (65.3)	0.039*
No	15 (19.7)	61 (80.3)	
Low APGAR Score			
Yes	33 (33.3)	66 (66.7)	0.018*
No	8 (15.4)	44 (84.6)	
Resuscitation at birth			
Yes	30 (30)	70 (70)	0.271
No	11 (21.6)	40 (78.4)	

All data presented as number (%)

APGAR: Appearance, Pulse, Grimace, Activity, and Respiration

Chi-square test applied, *p-value <0.05 considered as significant

positive in majority 14 (34.1%) patients followed by Staphylococcus aureus 13 (31.7%), Escherichia coli 8 (19.5%), CoNS 5 (12.2%) and group B streptococci 1 (2.4%). Sensitivity was found higher Meropenum, i.e., 33 (89.2%) followed by Amikacin 29 (72.5%), Tazocin 19 (61.3) and Linzolid 13 (54.2%) whereas specificity was found to be higher in amoxicillin clavulanic acid 22 (75.9%). (Table 4)

DISCUSSION

Neonatal sepsis is a major public health concern, particularly in low- and middle-income countries, negligence and late reporting resulted in serious consequences. This study was conducted in National Institute of Child Health, Karahi, which is one of the largest tertiary care children hospital of Pakistan. This hospital caters children from almost all over country particularly Sindh and Baluchistan.

In the current study, antimicrobial sensitivity pattern of all neonates with sepsis were assessed. The findings showed that among 41 positive cases, Klebsiella spp. was found positive in majority thirty four percent patients followed by Staphylococcus aureus thirty two percent, Escherichia coli nineteen percent, Coagulative negative staphylococci twelve percent and group B streptococci two percent patients. However, in another study commonly isolated micro-organisms include Klebsiella spp., Streptococcus pneumonia, Escherichia coli, Staphylococcus aureus, Pseudomonas spp., Salmonella spp., CoNS and S. Pyogenic.[10,11] Group B streptococci.[12] Similar to the current study findings, a study conducted by Shaw *et al* in Nepal has shown Staphylococcus aureus and Klebsiella spp. as the common bacteriological profile.[13] Findings from recent studies conducted in Pakistan also revealed somewhat similar estimates like ours. In a study conducted in Karachi by Shaikh *et al.*, it was reported that Staphylococcus aureus was the commonest isolates.[14] While another recent study conducted in Bahawalpur by Atif *et al* has reported Klebsiella spp. as most common finding.[15] Moreover, Mudassar *et al.* from

Table 3: Comparison of culture positivity with maternal characteristics (n=151)

Characteristics	Culture		p-value
	Positive n (%)	Negative n (%)	
Maternal age, years			
≤25	29 (27.4)	77 (72.6)	0.930
>25	12 (26.7)	33 (73.3)	
Maternal Education			
Illiterate	10 (20.4)	39 (79.6)	0.198
Less than equal to matric	22 (27.5)	58 (72.5)	
More than equal to intermediate	9 (40.9)	13 (59.1)	
Antenatal Visits			
Yes	30 (32.6)	62 (67.4)	0.060
No	11 (18.6)	48 (81.4)	
HTN			
Yes	17 (34.0)	33 (66.0)	0.183
No	24 (23.8)	77 (76.2)	
UTI			
Yes	13 (43.3)	17 (56.7)	0.026*
No	28 (23.1)	93 (76.9)	
PROM			
Yes	11 (34.4)	21 (65.6)	0.301
No	30 (25.2)	89 (74.8)	
Endometritis			
Yes	7 (31.8)	15 (68.2)	0.594
No	34 (26.4)	95 (73.6)	
Chorioamnionitis			
Yes	11 (52.4)	10 (47.6)	0.005*
No	30 (23.1)	100 (76.9)	

All data presented as number (%)

HTN: Hypertension, PROM: Premature rupture of membranes, UTI: Urinary Tract Infection

Chi-square test applied, *p-value <0.05 considered as significant

Lahore has also reported Klebsiella spp. as most common specie.[16]

The findings of this study showed sensitivity was found higher in Meropenum, followed by Amikacin, Tazocin and Linzolid whereas specificity was found to be higher in amoxicillin clavulanic acid. In various studies antimicrobial susceptibility testing showed sensitivity for ampicillin as twenty five percent, amikacin thirty five percent, ciprofloxacin ninety percent, ceftrixone forty five percent, ceftazidime fourty percent, cefotaxime ninety four percent, gentamicin thirty percent, Llinozolid hundred percent, Meropenum hundred percent and Vancomycin hundred percent. Resistance for ampicillin was reported as seventy five percent, Amoxicillin-clavulanic acid thirty three percent, amikacin two percent, ciprofloxacin seven percent, ceftriaxone thirteen percent, ceftazidime fourteen percent, cefotaxime eleven percent, chloramohenicol two percent, gentamicin seventy percent, linezolid zero percent, Meropenum two percent, Tazocin (Piperacilin and Tazobactam) zero percent and Vancomycin zero percent.[10-12,17,18]

The sensitivity pattern of the causative organisms must be studied frequently to determine ongoing resistance to antimicrobial therapy. Since the etiological agents and their antimicrobial susceptibility pattern varies from region to region, so knowledge of prevalence of local isolates and their antimicrobial sensitivity pattern is of utmost importance for prompt application of effective antimicrobial therapy.

This study has limitation that certain important etiological agent that can largely influence the sensitivity pattern were not studied. These etiological factors may include sociodemographic characteristics like residence (rural/urban), prolong use of invasive ventilation, prolong use of catheter, failure of early

Table 4: Antibiotic susceptibility pattern and resistance profile of isolated organism

Antibiotics		Klebsiella Spp.	GBS	E. Coli	Staph. Aureus	CoNS
AMP	S	-	-	2 (100)	-	-
	R	-	-	-	9 (100)	-
AMC	S	7 (100)	-	-	-	-
	R	7 (31.8)	1 (4.5)	8 (36.4)	6 (27.3)	-
AMK	S	11 (37.9)	-	3 (10.3)	11 (37.9)	4 (13.8)
	R	3 (27.3)	1 (9.1)	5 (45.5)	1 (9.1)	1 (9.1)
CIP	S	11 (44.0)	-	4 (16.0)	7 (28.0)	3 (12.0)
	R	3 (20.0)	1 (6.7)	4 (26.7)	6 (40.0)	1 (6.7)
CRO	S	9 (52.9)	-	3 (17.6)	2 (11.8)	3 (17.6)
	R	5 (25.0)	1 (5.0)	5 (25.0)	7 (35.0)	2 (10.0)
CAZ	S	9 (69.2)	-	1 (7.7)	-	3 (23.1)
	R	5 (27.8)	1 (5.6)	5 (27.8)	5 (27.8)	2 (11.1)
CHL	S	6 (46.2)	-	1 (7.7)	4 (30.8)	2 (15.4)
	R	8 (36.4)	1 (4.5)	4 (18.2)	6 (27.3)	3 (13.6)
CMX	S	10 (52.6)	1 (5.3)	1 (5.3)	4 (21.1)	3 (15.8)
	R	4 (40.0)	-	4 (40.0)	1 (10.0)	1 (10.0)
GEN	S	5 (27.8)	-	3 (16.7)	6 (33.3)	4 (22.2)
	R	9 (90.0)	-	-	-	1 (10.0)
LZD	S	5 (38.5)	-	2 (15.4)	4 (30.8)	2 (15.4)
	R	9 (81.8)	1 (9.1)	-	-	1 (9.1)
MER	S	12 (36.4)	-	6 (18.2)	10 (30.3)	5 (15.2)
	R	2 (50.0)	1 (25.0)	1 (25.0)	-	-
TZP	S	5 (26.3)	1 (5.3)	4 (21.1)	6 (31.6)	3 (15.8)
	R	9 (75.0)	-	1 (8.3)	-	2 (16.7)
VAN	S	10 (47.6)	1 (4.8)	6 (28.6)	2 (9.5)	2 (9.5)
	R	4 (44.4)	-	1 (11.1)	1 (11.1)	3 (33.3)

AMC: Amoxicillin-clavulanic acid, AMP: Ampicillin, AMK: Amikacin, CAZ: Ceftazidime, CHL: Chloramphenicol, CMX: Cotrimoxazole, CIP: Ciprofloxacin, CoNS: Coagulase Negative Staphylococcus Species, CRO: Ceftriaxone, GEN: Gentamicin, LZD: Linzolid, MER: Meropenum, R: Resistant, S: Sensitive, TZP: Tazocin, VAN: Vancomycin

breast feeding, longer use of parenteral nutrition, hospitalization, surgery, cardiovascular diseases, and respiratory infections.[19-21] Despite of this limitation, this study is of significance as current magnitude of the sensitivity pattern is reported from the current study from a large public sector tertiary care hospital of metropolitan city Karachi. Further large-scale cross-sectional studies are recommended to preclude the findings of this study.

CONCLUSION

Current magnitude of common bacterial Isolates are Klebsiella Spp. and Staphylococcus aureus while Meropenum and Amikacin are common antibiotics sensitivity pattern in patients with neonatal sepsis. Thus, these antibiotics could be started as first line treatment in neonatal sepsis patients rather than long wait for culture reports.

ETHICAL APPROVAL: This study was approved by Institutional Review Board & Ethics Committee, National Institute of Child Health Karachi, Pakistan.

AUTHORS' CONTRIBUTION: HL, BN, & MK: Conception and design of work, interpretation of data for the work. BN, MA, & MK: Critical revision for important intellectual content. NT: Design of work, drafting the content. All authors approved final version of the manuscript to be published.

CONFLICT OF INTEREST: The authors declare no conflict of interest.

FUNDING: None declared by authors.

Received: September 06, 2021

Accepted: November 13, 2021

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