Original Article

Antibiotic Sensitivity pattern of Bacterial Isolates of Neonatal Septicemia in Peshawar, Pakistan

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Abstract

Background: Septicemia plays an important role in neonatal morbidity and mortality, especially in developing countries.

Objective: To investigate the bacterial pathogens causing neonatal sepsis and their antibiotic susceptibility profile.

Methodology: A total of 2,685 neonates aged 0–28 days were included in the study. Blood from each neonate was cultured and isolates were identified using standard biochemical tests. Antibiotic sensitivity pattern was analyzed using modified Kirby-Bauer disc diffusion method.

Results: Blood culture positivity was observed in 1,534 (57.1%) samples. Most of the cases (1089 counts - 71%) were of early onset sepsis while 445 (29%) were of late onset sepsis. The incidence of sepsis was higher in males 856 (55.8%) than females 678 (44.2%) with a 1:2 ratio. Similarly, 58.3% of septicemic patients were neonates with low birth weights. Twelve hundred and six (78.6%) isolates were gram negative while 328 (23.4%) were gram positive bacteria. *E. coli* was the dominant pathogen seen in 811 (52.8%) followed by *Staphylococcus aureus* 300 (19.5%), Pseudomonas 199 (13%), Klebsiella 102 (6.7%), Proteus 87 (5.7%), *Staphylococcus epidermidis* 28(1.8%) and Salmonella in 7 (0.5%) samples. All bacterial isolates showed high sensitivity to Imipenem, Enoxacin, Ofloxacin and Ciprofloxacin while low sensitivity was observed for other antibiotics (n = 16). The Proteus species showed high level of multiple resistances to all antibiotics (5.9%).

Conclusion: Imipenem, Enoxacin, Ofloxacin and Ciprofloxacin can be used as an effective antibiotic regimen for treatment of bacterial sepsis in neonates.

Keywords: Antibiotic susceptibility, early-onset, late-onset, neonatal septicemia

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Introduction

Neonatal sepsis is a global health challenge which can be devastating, causing high morbidity and mortality in newborns.[1] The definition of early-onset sepsis is variable from 3 days (American Academy of Pediatrics) to 7 days (Centers for Disease Control based on epidemiological studies).[2,3] Despite recent knowledge, techniques and advances in management of neonatal sepsis, including use of active antibiotics and a range of sophisticated biomarkers to diagnose sepsis, timely identification continues to be a major and challenging problem in management of high-risk neonatal infection in the NICU.[4]

The incidence of neonatal sepsis in developed countries is 1-10/1000 live births, whereas it is roughly three times higher in developing countries like Pakistan.[5]

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A study conducted in Pakistan Institute of Medical Sciences, Islamabad, showed that inadequate antenatal visits significantly increase the risk of neonatal morbidity and mortality even in mothers with low risk.[6] Furthermore, the frequency (up to 25%) of low birth weight deliveries in our country increases the risk of development of sepsis in these neonates.[7]

At present, in developing countries, gram negative organisms remain the major etiology. These organisms have developed multi-drug resistance over the last two decades.[8,9] The reasons for this resistance are indiscriminate and irrational use of antibiotics, over the counter sale of antibiotics and ineffective infection control in maternity centers.[10] The present study was conducted in Pakistan Health Research Council (PHRC), Peshawar, to isolate the bacterial pathogens responsible for neonatal septicemia and to assess their antibiotic susceptibility profile.

Materials and Methods

This descriptive study was carried out at PHRC, Khyber Medical College Peshawar, from 2012 to 2015 (4 years). We included blood samples (n = 2685) from suspected neonates across the district of Peshawar submitted to PHRC for culturing and sensitivity testing. Gender, age and weight of neonates were recorded. Blood was cultured on Tryptic Soy Broth and incubated for 37°C. For sub-culturing, Blood agar, SS-Agar and MacConkey's media were used. Positive growth was identified using standard biochemical tests, colony morphology and Gram staining. Modified Kirby-

Bauer disc diffusion method was used for antibiotic sensitivity testing. A total of 20 antibiotics were used for testing antibiotic sensitivity: Amikacin, Ampicillin, Augmentin, Aztreonam, Cefoperazone, Cefotaxime, Cefpirom, Ceftriaxone, Cephradine, Ciprofloxacin, Clarithromycin, Doxycycline, Enoxacin, Fortum, Gentamicin, Imipenem, Nalidixic Acid, Ofloxacin, Tobramycin and Vancomycin. Clinical Laboratory Standard Institute (CLSI) guidelines were applied to determine antibiotic sensitivity. Statistical analysis was done using SPSS v. 16.0. *P*-value < 0.05 was considered significant.

Results

Among 2,685 blood samples, 1,534 (57.1%) were found culture positive for bacterial growth. Out of 1,534 cases of neonatal septicemia, 71% (n = 1089) had Early Onset Sepsis (EOS) while 29% (n = 445) had Late Onset Sepsis (LOS) (Table 1). Culture positivity in males was significantly higher (55.8%) than females (44.2%) (*P* = 0.02). In low birth weight (< 2500 g), the neonatal incidence of septicemia was found higher (58.3%) than neonates with normal birth weight (41.7 %). The characteristics of neonates are described in Table 1. Incidence of gram positive cocci was 21.4% (n = 328) while that of gram negative bacilli was 78.6% (n = 1206). *E. coli* was the most common isolated bacterium (n = 811 – 52.8 %). *Staph. aureus* was isolated from 19.5% (n = 300) culture positive samples, *Pseudomonas* from 13% (n = 199), *Klebsiella* from 6.7% (n = 102), *Proteus* from 5.7% (n = 87), *Staphylococcus epidermidis* from 1.8% (n = 28) and *Salmonella* spp. from 0.5% (n = 07) (Table 2). Amongst the antibiotics used, Imipenem, Enoxacin, Ofloxacin and Ciprofloxacin showed high activity against all the bacterial isolates. The majority of the organisms showed low sensitivity to all the other antibiotics used. Table 3 shows the antibiotic sensitivity pattern of blood culture isolates (Table 3).

Discussion

Neonatal septicemia is a life-threatening emergency, and rapid treatment with antibiotics is essential for a favorable outcome.[11] In our study, blood culture positivity was found to be 57.1%. Similar high culture positivity was also reported by Rahman *et al.,* 2002[9] in Pakistan (62.8%). Incidence of EOS (71%) was higher than LOS (21%) which is in agreement with previous studies.[12,13] In the present study, males showed higher blood culture positivity (55.8%) than females (44.2%) which is comparable to other studies.[14,15] Low birth weight neonates were found to be more prone to septicemia as compared to normal birth weight neonates (*P*= 0.007). These findings are in tandem with the study by Jeong *et al.,* 2006.[16] In the present study, 78.6% of isolates were Gram negative bacteria while gram positive bacteria accounted for 27.2% of cases of neonatal septicemia (*P* = 0.00). These findings are in accordance with two studies, one from Pakistan[17] and the other from India.[18] The predominance of *E. coli* (52.8%) in the present study was also reported by Shah & Desai in 2011.[19] In developing countries, high prevalence of Gram negative is consistently reported.[20] *E. coli* and other Gram negative bacteria showed low sensitivity to commonly used antibiotics while high sensitivity was observed for fluoroquinolones (ciprofloxacin, Ofloxacin and Enoxacin) and Imipenem. A similar trend of high resistance was also reported by Hassan *et al*.[12] in Gram negative bacteria. Rizwan *et al.*[21] in 2005 and Kayange *et al. in* 2010[22] observed high sensitivity of isolated bacteria, especially *E. coli, Klebsiella* and *Staph. aureus*

Table 1. Characteristics of neonates.

Description	Number of cases (*n* = 2685)	Culture positive (*n* = 1534)	%	*P*-Value
Gender				
Male	1547	856	55.8	0.02
Female	1138	678	44.2	
Birth Weight (g)				
< 2500	1625	895	58.3	0.007
≥ 2500	1060	639	41.7	
Early onset sepsis		1089	71	
Late onset sepsis		445	29	

Table 2. Distribution of organisms in neonates with septicemia.

	N	%
Gram Positive cocci *(Staph. Aureus and epidermidis)*	328	21.4
Gram Negative bacilli	**1206**	**78.6**
E. coli	811	52.8
Proteus	87	5.7
Pseudomonas	199	13
Klebsiella	102	6.7
Salmonella spp.	07	0.5
Total	**1534**	**100%**

Table 3. Antibiotic susceptibility pattern of blood culture isolates (% Sensitive).

Antibiotics tested	*E. coli* (*n* = 811)	*Proteus* (*n* = 87)	*Pseudomonas* (*n* = 199)	*Klebsiella* (*n* = 102)	*Salmonella* spp. (*n* = 07)	*Staph. Aureus* (*n* = 300)	*Staph. Epidermidis* (*n* = 28)
Amikacin	58	54	36.8	58.9	100	73.7	85
Ampicillin	10.2	20.5	11.4	18	16.7	53.2	56
Augmentin	22.5	15	9.3	23.6	20	36.5	81.4
Aztreonam	25.2	20.2	28.4	28.3	28.5	12.6	16.3
Cefoperazone	27.2	NT	30.8	NT	20	50	65
Cefotaxime	18.3	26.2	32.8	16.3	71.4	49.8	59.9
Cefpirom	38	29.4	30.4	34.6	14.3	70.5	86.1
Ceftriaxone	22	24	30.8	20.4	42.9	38.8	41.2
Cephradine	NT	8.3	5	6.6	40	47.5	53.4
Ciprofloxacin	67.3	80	55.7	67.4	66.7	62	28.5
Clarithromycin	9.9	10.1	19.7	13	25	35.2	30.6
Doxycycline	29.1	24	42.3	30.4	20	46.3	51
Enoxacin	83.3	60	77.5	60	66.7	60	55
Fortum	16.7	29.9	43.6	27.3	28.6	30.9	40.2
Gentamicin	26.2	16.9	36.9	16.7	33.3	43.8	50
Imipenem	94.7	87.1	94.4	85.3	66.7	95.5	71.4
Nalidixic Acid	46.5	52	37.9	66.7	50	25.6	32.4
Ofloxacin	77.7	75.6	68.3	71.5	83.3	74.6	86.5
Tobramycin	22.3	16.7	40	27	50	51.7	70
Vancomycin	NT	NT	NT	NT	NT	51.7	86
Sensitive to more than 7 antibiotics	2.7	0	3.3	0.3	4.8	4.9	4.5
Resistance to all antibiotics	4.8	5.9	1.7	3.1	2.1	1.4	1.1
NT = Not Tested							

towards Imipenem and ciprofloxacin. In the present study, *Staph. aureus* showed high resistance to commonly used antibiotics as compared to Imipenem, ciprofloxacin, Amikacin, Enoxacin and Ofloxacin. A similar trend of high resistance and low sensitivity to conventional antibiotics was also reported in other studies.[23,24] Imipenem showed high sensitivity to all the bacteria isolates and remained the prime drug of choice for treatment of neonatal septicemia. This result correlates with the finding of Arrieta, 1997 where Imipenem was recommended.[25]

In conclusion, gram negative bacteria are the leading cause of neonatal septicemia. Imipenem, Enoxacin, Ofloxacin and Ciprofloxacin can be used as an effective antibiotic regimen for treatment of bacterial sepsis. Special attention should be given during treatment of neonatal septicemia to proteus species because of its great emergence of multiple drug resistance.

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Conflict of interest

There is no conflict of interest among authors of the manuscript.

Authors' contributions

Contributions	Authors name
Conception and design	Obaid Ullah, Tasleem Akhtar, Aftab Khan
Collection and assembly of data	Amin jan , Arif Mehmood Khan, Ambreen
Analysis and interpretation of the data	Israr Ahmad, Obaid Ullah
Drafting of the article	Obaid Ullah, Aftab Khan
Critical revision of the article for important intellectual content	Tasleem Akhtar, Israr Ahmad, Amin Jan
Statistical expertise	Obaid Ullah, Ambreen, Arif Mehmood Khan
Final approval and guarantor of the article	Obaid Ullah, Aftab Khan

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