Research Article

Antimicrobial culture sensitivity pattern in neonatal sepsis in a tertiary-care hospital

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Received July 9, 2015. Accepted July 15, 2015

Abstract

Background: Bacterial sepsis is one of the most common causes of mortality and morbidity in neonates. The spectrum of bacteria that cause neonatal sepsis varies, and antibiotic resistance is an increasing problem of these bacteria.

Objective: To determine the bacteriological profile and antibiotic sensitivity pattern of neonatal sepsis in the neonatal intensive-care unit (NICU), so that the empirical antibiotics can be decided to tackle the organisms in the NICU.

Materials and Methods: A prospective study was carried out in the NICU of Pediatric Department of Guru Gobind Singh Government Hospital, Jamnagar, India. During the study duration of one-and-a-half year, 713 neonates with suspected sepsis were investigated. Data such as name, age, sex, birth weight, and gestational age were recorded. Neonates were evaluated for bacterial etiologic agents by blood culture, and their antimicrobial sensitivity was evaluated.

Result: The blood culture was positive in 368 (51%) neonates, of which 145 (39%) were gram positive and 223(61%) gram negative. The common isolates were *Klebisella*, *Staphylococcus aureus*, and coagulase-negative *Staphylococci*. The sensitivity of gram-negative organisms was low to the commonly used antibiotics such as amikacin (15.70%), gentamicin (13.90%), and ampicillin + sulbactam (8.97%). The sensitivity of gram-positive organisms was better to the commonly used drugs such as ampicillin + sulbactam (71.03%), gentamicin (63.45%), and cotrimoxazole (55.86%).

Conclusion: Gram-negative organisms comprised the majority of the neonatal infections, with *Klebsiella* being the most prevalent. Resistance to both gram-positive and gram-negative organisms among the first-line antibiotics is a major concern. Continuing surveillance of infections is still needed in order to choose the most appropriate empirical therapy for neonatal sepsis.

KEY WORDS: Neonatal sepsis, antimicrobial resistance, microorganism

Introduction

Neonatal sepsis is a clinical disorder showing systemic signs of infection along with bacteremia in the first month of life[1]. Neonatal sepsis is one of the common causes for morbidity and mortality among neonates in India affecting 4% of the neonates[2,3]. Standard treatment of neonatal sepsis includes the use of antimicrobial agents. Antibiotics are continued, changed, or discontinued depending on the laboratory test results, extent of clinical suspicion, and cultures.[4]

Empirical antimicrobial treatment of patients with sepsis is usually based on the general principles of antimicrobial drug use and the knowledge gathered from the public, rather than on evidence-based recommendations specific to patients of neonatal sepsis.[5] Currently, no universally accepted guidelines are available for empiric therapy in patients with neonatal sepsis.[6,7] Unnecessary, injudicious, or excessive use of antibiotics has led to an alarming rise in antibiotics resistance, which is a cause of concern. Many studies suggest that resistance is directly associated with the selection of inappropriate antimicrobials, which leads to increased patients' mortality.[8] Improved guidelines for antibiotic treatment in neonatal sepsis

Access this article online	
Website: http://www.ijmsph.com	Quick Response Code:
DOI: 10.5455/ijmsph.2016.09072015106	

should be prepared according to institutional etiology and microbial sensitivity pattern.[9] Use of appropriate antibiotics according to bacterial profile and culture sensitivity results would minimize the risk of severe morbidity and mortality and help in reducing the emergence of multidrug-resistant organisms.[10,11] Thus, blood cultures and sensitivity testing are important for the diagnosis of neonatal sepsis and institution of early empirical antibiotic treatment. As these neonates often reach the health-care facilities late and in a critical condition, institution of early appropriate antibiotic treatment is essential for the optimum outcome. This study was carried out to determine the bacteriological profile and antibiotic sensitivity pattern of neonatal sepsis in our NICU, so that appropriate antimicrobial policy could be made for empirical treatment of neonatal sepsis to tackle the organisms in our NICU.

Materials and Methods

A prospective study was carried out in neonatal intensive-care unit (NICU) of Pediatric Department of Guru Gobind Singh Government Hospital, Jamnagar, India. Prior permissions of the Institutional Ethics Committee, Head of Pediatrics Department, and Head of Microbiology Department were obtained for conducting the study. An appropriate study protocol and pro forma were developed and discussed with the teaching staff members of the Pharmacology Department, Head of Pediatrics Department, and Head of Microbiology Department.

Selection Criteria of Patient

Inclusion Criteria

1. Confirmed or suspected cases of neonatal sepsis in patients aged 0–28 days, admitted to the NICU.

Exclusion Criteria

1. Patient's age more than 28 days of life.
2. Neonates with other serious complications.

Collection of Data

During the study period, neonates (0–28 days of age) admitted with suspected diagnosis of early onset sepsis (0–7 days of age) and late onset sepsis (8–28 days of age) were investigated. Written informed consent was obtained from their parents/guardians. Data of patients matching the inclusion criteria were recorded. Admitted neonates who did not fulfill the abovementioned inclusion criteria and those who met the exclusion criteria were excluded from the study. A total of 713 cases were collected during the study duration of 18 months from January 2012 to June 2013.

Data such as name, age, sex, birth weight, and gestational age were recorded in the previously prepared case record form. Neonates with suspected sepsis were investigated for bacterial etiologic agents. Blood samples were collected with proper antiseptic precautions[12] by a pediatrician before starting any antibiotic therapy and sent to a microbiology laboratory for the identification of isolates by Gram stains and culture growth. Approximately, 2 cc of blood was drawn and inoculated into brain–heart infusion broth, and it was incubated at 37°C for 24 h. Subcultures were made on both blood agar and MacConkey's agar[13] after 24 h and 48 h. Antibiotics sensitivity was performed by Kirby bauer's disc diffusion method by antibiotics shown in Table 1.

Result

During the study period, 713 patients with neonatal sepsis were admitted. Among them, 449 (62.97%) were male and 264 (37.03%) were female subjects. Of the 713 patients, 467 (65.5%) of them showed early onset sepsis and 246 (34.5%) showed late onset neonatal sepsis. Among these neonates, 246 (34.5%) were preterm, 461 (64.66%) were term, and 6 (0.84%) were postterm. The number of patients admitted in NICU with normal birth weight, low birth weight, very low birth weight, and extremely low birth weight was 293 (41.09%), 263 (36.89%), 150 (21.04%), and 7 (0.98%), respectively.

The culture positivity rate was 51% (368/713). From 368 organisms identified by Gram staining, 145 (39%) were gram positive and 223 (61%) were gram negative. The common isolates were *Klebsiella*, *S. aureus*, and coagulase-negative *Staphylococci*. Other pathogens were *Escherichia coli*, *Acinetobacter*, *Streptococcus* species, *Enterococci*, gram-positive Bacilli, *Pseudomonas aeruginosa,* and *Proteus mirabilis* [Table 2]. The sensitivity of gram-negative organisms was low to the commonly used antibiotics such as amikacin (15.70%), gentamicin (13.90%), and ampicillin + sulbactam (8.97%) [Table 3]. The sensitivity of gram-positive organisms was better to the commonly used drugs such as ampicillin + sulbactam (71.03%), gentamicin (63.45%), and cotrimoxazole (55.86%) [Table 4]. Low resistance to quinolones was noted.

Of the total 713 neonates, 657 (92.14%) neonates survived, 41 (5.75%) died, and 15 (2.10%) were discharged against medical advice.

Discussion

Of the 713 patients, the blood culture was positive in 368 (51%) of them. This is comparable with the studies done by Shrestha et al.[14] and Shahian et al.,[15] in which the blood culture positivity rate was 44% and 43%, respectively. The culture yield is higher than the rate obtained in a study done by Jyothi et al. (19.2%).[16]

In this study, 60.6% organisms causing neonatal sepsis were gram negative and 39.4% gram positive. This is in agreement with the studies done by Shrestha et al.[14] and Kayange et al.,[17] which also show that gram-negative organisms are more common causes of neonatal sepsis.

The most common pathogens isolated from the patients of neonatal sepsis were *Klebsiella pneumoniae* (42%), followed by *Staphylococcus aureus* (17%), coagulase-negative *Staphylococcus* (14%), and *Escherichia coli* (7%). *K. pneumoniae* was also the predominant organism for neonatal sepsis in

Table 1: Antibiotics used for culture sensitivity

Antibiotic agent for gram-positive bacteria	Disc content (μg)	Antibiotic agent for gram-negative bacteria	Disc content (μg)
Ampicillin + sulbactam	20	Ampicillin + sulbactam	20
Cotrimoxazole	25	Cotrimoxazole	25
Tetracycline	30	Cefotaxime	30
Cefotaxime	30	Ciprofloxacin	5
Ciprofloxacin	5	Tetracycline	30
Levofloxacin	5	Gentamicin	10
Linezolid	30	Piperacillin	100
Cloxacillin	1	Chloramphenicol	30
Roxithromycin	15	Ceftizoxime	30
Cephalexin	30	Ofloxacin	5
Lincomycin	2	Amikacin	30
Gentamicin	10	Gatifloxacin	10

Table 2: Frequency of organisms isolated by culture

Organism	No. of isolates	Percentage
Klebsiella pneumoniae	154	42
Staphylococcus aureus	62	17
Coagulase-negative *Staphylococcus*	53	14
Escherichia coli	24	7
Acinetobacter	21	6
Streptococcus species	18	5
Enterococci	14	4
Gram-positive bacilli	12	3
Pseudomonas aeruginosa	9	2
Proteus mirabilis	1	0
Total	368	100

Table 3: Culture sensitivity of gram-negative isolates

Drugs	Gram-negative organisms (sensitive) *n* (%)						Total sensitive, *N* (%)	Total resistant, *N* (%)
	Acineto-bacter	*Escherichia coli*	*Enterococci*	*Klebsiella*	*Proteus*	*Pseudomonas*		
Ampicillin/sulbactam	1 (4.75)	4 (16.67)	3 (21.43)	9 (5.84)	0 (0)	3 (33.33)	20 (8.97)	203 (91.03)
Cotrimoxazole	8 (38.1)	8 (33.33)	4 (28.57)	76 (49.35)	0 (0)	3 (33.33)	99 (44.39)	124 (55.61)
Cefotaxime	3 (14.29)	3 (12.5)	1 (7.14)	7 (4.55)	0 (0)	4 (44.44)	18 (8.07)	205 (91.93)
Piperacillin	4 (19.05)	2 (8.33)	0 (0)	9 (5.84)	0 (0)	5 (55.56)	20 (8.97)	203 (91.03)
Chloramphenicol	7 (33.33)	15 (62.5)	1 (7.14)	95 (61.69)	1 (100)	2 (22.22)	121 (54.26)	102 (45.74)
Ciprofloxacin	6 (28.57)	10 (41.67)	1 (7.14)	105 (68.18)	0 (0)	3 (33.33)	125 (56.05)	98 (43.95)
Ceftizoxime	2 (9.52)	3 (12.5)	0 (0)	6 (3.9)	0 (0)	1 (11.11)	12 (5.38)	211 (94.62)
Tetracycline	5 (23.81)	7 (29.17)	0 (0)	74 (48.05)	0 (0)	3 (33.33)	89 (39.91)	134 (60.09)
Ofloxacin	9 (42.86)	7 (29.17)	1 (7.14)	113 (73.38)	1 (100)	3 (33.33)	134 (60.09)	89 (39.91)
Gentamicin	2 (9.52)	4 (16.67)	2 (14.29)	22 (14.29)	0 (0)	1 (11.11)	31 (13.9)	192 (86.1)
Amikacin	2 (9.52)	8 (33.33)	0 (0)	23 (14.94)	0 (0)	2 (22.22)	35 (15.7)	188 (84.3)
Gatifloxacin	18 (85.71)	19 (79.17)	2 (14.29)	123 (79.87)	1 (100)	5 (55.56)	168 (75.34)	55 (24.66)
Total (223)	21	24	14	154	1	9	223	

Table 4: Culture sensitivity of gram-positive isolates

Drugs	Gram-positive organisms (sensitive) *n* (%)				Total sensitive	Total resistant
	Cons	Gpb	*Staphylococcus aureus*	*Streptococcus sp*		
Ampicillin/sulbactam	43 (81.13)	12 (100)	38 (61.29)	10 (55.56)	103 (71.03)	42 (28.97)
Cotrimoxazole	42 (79.25)	12 (100)	19 (30.65)	8 (44.44)	81 (55.86)	64 (44.14)
Cephalexin	41 (77.36)	12 (100)	2 (3.23)	7 (38.89)	62 (42.76)	83 (57.24)
Tetracycline	41 (77.36)	12 (100)	15 (24.19)	11 (61.11)	79 (54.48)	66 (45.52)
Cefotaxime	39 (73.58)	12 (100)	5 (8.06)	7 (38.89)	63 (43.45)	82 (56.55)
Ciprofloxacin	44 (83.02)	12 (100)	14 (22.58)	7 (38.89)	77 (53.10)	68 (46.90)
Levofloxacin	49 (92.45)	12 (100)	33 (53.23)	12 (66.67)	106 (73.10)	39 (26.90)
Linezolid	48 (90.57)	12 (100)	58 (93.55)	17 (94.44)	135 (93.10)	10 (6.90)
Cloxacillin	41 (77.36)	12 (100)	2 (3.23)	7 (38.89)	62 (42.76)	83 (57.24)
Roxithromycin	42 (79.25)	12 (100)	5 (8.06)	10 (55.56)	69 (47.59)	76 (52.41)
Lincomycin	42 (79.25)	12 (100)	9 (14.52)	10 (55.56)	73 (50.34)	72 (49.66)
Gentamicin	45 (84.91)	12 (100)	26 (41.94)	9 (50)	92 (63.45)	53 (36.55)
Total (145)	53	12	62	18	145	145

CONS: Coagulase negative streptococci. GPB: Gram Positive bacilli.

the studies done by Aletayeb et al.,[18] Shrestha et al.,[14] and Jyothi et al.[16] *K. pneumoniae*, *S. aureus*, and coagulase-negative *Staphylococci* were the predominant organisms for neonatal sepsis in the study done by Shrestha et al.[14] and Jyothi et al.[16] *P. aeruginosa* was the predominant organism for neonatal sepsis in the study done by Bhat et al.[19] *S. aureus* was the predominant organism for neonatal sepsis in the study done by Mhada et al.[20] Shahian et al.[15] and Dias et al.[21] reported coagulase-negative *Streptococci* as the major organisms for neonatal sepsis in their studies.

Antibiotic resistance is today a global problem. Reports of multiresistant bacteria causing neonatal sepsis in developing countries are increasing. The wide availability of over the counter antibiotics and the inappropriate use of broad spectrum antibiotics in the community may explain this situation. It is difficult to compare antibiotic resistance between different setup, because the epidemiology of neonatal sepsis is extremely variable.

The analysis of drug resistance pattern showed that, among gram-negative isolates, decreased sensitivity was observed to be against the commonly used antibiotics such as amikacin (15.70%), gentamicin (13.90%), ampicillin + sulbactam (8.97%), and cefotaxime (8.07%). The susceptibility to the aminoglycoside antibiotics was less when compared with the studies done by Jyothi et al.,[16] Shrestha et al.,[14] and Bhat et al.[19] The gram-negative organisms were the most sensitive to ofloxacin (60.09%) and ciprofloxacin (56.05%). There was a decreased sensitivity to even the reserve drugs such as piperacillin (8.97%).

Antibiotics with good susceptibility toward gram-positive organisms are ampicillin + sulbactam (71.03%), gentamicin (63.45%), and cotrimoxazole (55.86%). This is comparable with the studies done by Shrestha et al.[14] and Bhat et al.[19] Gram-positive organisms were the most sensitive to linezolid (93.10%). This is comparable with the study done by Jyothi et al.[16]

On observing the sensitivity pattern of *K. aerogenes*, ciprofloxacin (68.18%) and cotrimoxazole (49.35%) were found to be the most effective drugs with the least resistance. *Klebsiella* were the most sensitive to ciprofloxacin in the studies done by Kayange et al.[17] and Aletayeb et al.[18] Most of the strains showed a low sensitivity to amikacin (14.94%), gentamicin (14.29%), ampicillin + sulbactam (5.84%), piperacillin (5.84%), and cefotaxime (4.55%). There is a low sensitivity to cefotaxime when compared with the other studies.[14,15,17]

For *S. aureus*, cloxacillin (96.77%), cefotaxime (91.94%), and ciprofloxacin (77.42%) were found to be the most effective drugs with the least resistance. Similar results were also observed in the studies done by Shrestha et al.[14] and Rahman et al.[22]

Pseudomonas showed the highest sensitivity to piperacillin (55.56%) and cefotaxime (44.44%). In the studies done by Dias et al.[21] and Rahman et al.,[22] ciprofloxacin was the most sensitive drug. In the studies done by Bhat et al.[19] and Aletayeb et al.,[18] *Pseudomonas* sp.were the most sensitive to aminoglycoside antibiotics such as amikacin and gentamicin.

There is a decreased sensitivity of microorganisms to the commonly used drugs such as ampicillin/sulbactam and aminoglycosides. Sensitivity of gram-negative and gram-positive organisms to cefotaxime was 8.07% and 43.45%, respectively. Thus, resistance is developing even to the third-generation cephalosporins, which is of great concern. In this study, the maximum sensitivity (93.10%) was observed to linezolid (91%). Sensitivity to linezolid was much higher than that to other antibiotics, but it should not be used indiscriminately and be kept as a reserve drug; otherwise, resistance to linezolid may develop, thereby threatening the treatment. Sensitivity pattern of ciprofloxacin and other fluoroquinolones is also promising. In neonatology, the use of ciprofloxacin in life-threatening infections, although rare, is justified by the fact that clinical benefits largely overweight the potential risks.

The various studies indicate a gradual increase in the emergence of antibiotics-resistant organisms. However, many factors play a role in the development of resistance such as no uniformity in the usage of antibiotics, indiscriminate use, and availability of antibiotics. Antibiogram may vary depending on the study group and the hospital setup. So, the trend nowadays is toward comparative studies in the same hospital over the years.

Conclusion

The majority of the organisms causing neonatal sepsis are gram-negative. *K. pneumoniae* is the most predominant organism causing neonatal sepsis. Linezolid, levofloxacin, and ampicillin + sulbactam are the most sensitive antibiotics for gram-positive organisms, whereas flouroquinolones are for the gram-negative organisms causing neonatal sepsis. Most of the organisms have developed resistance to the commonly used antibiotics such as ampicillin + sulbactam, cefotaxime, and aminoglycosides. Resistance developed even to higher antibiotics such as Piperacillin and third-generation cephalosporins owing to injudicious use is of great concern. Therefore, the authors suggest that surveillance of antimicrobial resistance is necessary. Moreover, an antibiotic policy should be formulated in the hospital. Antibiotics should be used depending on the antibiotic sensitivity pattern of the isolates.

Acknowledgments

We acknowledge the help and support provided by the Dean, the Superintendent, and the staffs of the Department of Pediatrics and Department of Microbiology, MP Shah Medical College, Jamnagar, Gujarat, India, for carrying out this study.

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How to cite this article: Vaniya HV, Patel NM, Agrawal JM, Trivedi HR, Dhanani JV, Balat JD. Antimicrobial culture sensitivity pattern in neonatal sepsis in a tertiary-care hospital. Int J Med Sci Public Health 2016;5:661-665

Source of Support: Nil, **Conflict of Interest:** None declared.