Microbiological profile of early-onset neonatal sepsis in a tertiary care hospital in South India

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Received – 12 August 2016 Initial Review – 16 August 2016 Published Online – 28 August 2016

ABSTRACT

Aims: To identify the microbiological profile of neonatal sepsis and antibiotic sensitivity patterns of various isolates in the Newborn Intensive Care Unit at a Tertiary Care Hospital in Karnataka. **Materials and Methods**: In this retrospective study, the laboratory data of primary blood culture reports of 240 neonates admitted with clinical suspicion of early-onset sepsis, from April 2014 to March 2016, were analyzed. **Results**: Total 240 samples were received during the study period, of which 40, (16%) were found to be positive. Out of these, 18 (7.5%), 18 (7.5%), and 4 (1%) had Gram-positive, Gram-negative, and *Candida* infection, respectively. The most common organism noted among Gram-positive isolates was *Staphylococcus aureus* (22.5%), followed by coagulase-negative staphylococci (12.5%) and *Enterococcus* (10%). Among Gram-negative isolates, *Acinetobacter* was the most commonly implicated organism (20%), followed by *Klebsiella pneumoniae* (12.5%), *Escherichia coli* (7.5%), and *Pseudomonas aeruginosa* (5%). **Conclusion**: This study emphasizes the need for close observation to be carried out at regular intervals to describe the various pathogens causing early-onset neonatal sepsis and their ever changing antibiotic sensitivity pattern which will provide useful data to guide practice and policies on rational use of antibiotics.

Key words: *Early-onset neonatal sepsis, Blood culture, Antibiotic sensitivity pattern*

Sepsis has been known to be the predominant cause of neonatal mortality; it is being responsible for causing an estimated 30-50% of the total neonatal deaths in developing nations [1]. It has been estimated that up to 20% of neonates can develop sepsis, and this contributes to approximately 1% of mortality [2]. Sepsis-related mortality must be thought of as largely preventable when considering advances in recognition and treatment of the same.

MATERIALS AND METHODS

Analysis of blood culture reports retrieved from the Newborn Unit of K. S. Hegde Medical College and Hospital, Mangalore, was done. The primary blood culture data of 240 consecutive neonates admitted to the Neonatal Intensive Care Unit, with clinical suspicion of early-onset sepsis, from April 2014 to March 2016, constituted the inclusion criteria. Blood culture reports of babies suspected to have late-onset sepsis were excluded. No secondary blood culture reports were analyzed. The reports were collected and analyzed to determine the microbiological profile and antibiotic susceptibility pattern of isolates. Blood specimens were drawn under aseptic precautions before starting any antibiotic therapy and inoculated in BacT Alert/PF Plus blood culture bottles. The blood was cultured by use of a BacT/Alert three-dimensional (3D) (bioMérieux Inc) automated blood culture system. Each blood culture was composed of a set of three (FA Plus aerobic, Standard Aerobic, and Standard Anaerobic) bottles. The rate of positivity among the blood cultures and the time to detection (TTD) constituted the primary endpoint. TTD was defined as the time duration beginning from insertion of bottles into the instrument, to the detection of pathogens. The bottles that generated a positive signal in the 3D were subjected routinely to Gram-staining and further growth on blood agar plates and MacConkey agar plates. These plates were then incubated overnight at 37°C with 5% carbon dioxide. Vitek 2 system (bioMérieux Inc.) was utilized for identification purposes. A false-positive was defined as the lack of detection or growth of microorganisms and identifiable via a positive signal in the 3D system. Antimicrobial susceptibility testing was established by use of Kirby-Bauer Disc Diffusion Method as per the Clinical and Laboratory Standards Institute recommendations [3].

Data were maintained in Microsoft office Excel and tests of proportions were used for analysis. Results were recorded in percentages.

RESULTS

During the study period, 240 blood samples from clinically suspected cases of early-onset neonatal sepsis were obtained out of which blood culture was positive in 40 cases, the positivity rate being 16%. The most common organism noted among Gram-positive isolates was *Staphylococcus aureus* (22.5%), followed by coagulase-negative staphylococci (CONS) (12.5%) and *Enterococcus* (10%). Among Gram-negative isolates, *Acinetobacter* was the most commonly implicated organism (20%), followed by *Klebsiella pneumoniae* (12.5%), *Escherichia coli* (7.5%), and *Pseudomonas aeruginosa* (5%) (Table 1).

Gram-positive organisms were found to be sensitive to linezolid, vancomycin, and chloramphenicol. Among these, *S. aureus* was most responsive to linezolid (100%) and chloramphenicol (56%). Teicoplanin, vancomycin, and linezolid showed 100% sensitivity patterns to CONS. Ampicillin (75%), vancomycin (50%), and linezolid (50%) were found to be effective against Enterobacteriaceae. 75% of Enterobacteriaceae and 11% of *S. aureus* showed sensitivity to ampicillin while CONS was found to be non-sensitive (Table 2).

Gram-negative organisms were found to be most sensitive to ciprofloxacin, amikacin, and gentamicin. Among these, *K. pneumoniae* showed the highest susceptibility to amikacin (100%), followed by ciprofloxacin (80%) and gentamicin (60%). *P. aeruginosa* revealed a significant (100%) sensitivity to both amikacin and gentamicin followed by ciprofloxacin (50%). *Acinetobacter* had sensitivity to amikacin (75%) followed by ciprofloxacin (63%) and gentamicin (25%). In our study, *E. coli* was determined to have 100% sensitivity to all of the above antibiotics, i.e., amikacin, ciprofloxacin, and gentamicin (Table 3). In our newborn unit, the first line antibiotic of choice is cefotaxime and netilmicin.

DISCUSSION

The estimated incidence of neonatal sepsis has been previously reported to be approximately 4%, where intramural live births are concerned [4]. In India, as per the National Perinatal Database 2002-2003, the incidence of neonatal septicemia has been reported to be 30/1000 live births [5]. Neonatal sepsis can be caused by a large variety of Gram-positive as well as Gram-negative bacteria, and rarely, yeasts as well [6]. The spectrum of organisms that causes neonatal sepsis varies over time and also from region to region, possibly due to the changing patterns of antibiotic utilization [7].

The organisms most often implicated in neonatal sepsis in developing countries are Gram-negative organisms; notably, *Klebsiella, E. coli*, Pseudomonas, and *Salmonella*. Among the Gram-positive isolates, *S. aureus*, CONS, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* have been most commonly noted [8]. Group B streptococcus (GBS) has rarely been described by Kuruvilla et al. [9]. It may not be seen at all [10], although it must be remembered that maternal rectovaginal carriage rates of GBS may be actually similar to those that have been noted in developed countries [11]. Studies on the African perspective reveal a low incidence [12], except in South Africa [13]. In Asia too, GBS infection has been noted to be rare [8].

In a survey in Saudi Arabia, Al-Harthi et al. identified *K. pneumoniae* and *Serratia* sp. as being the prominent pathogens responsible for causing neonatal meningitis. They also reported the high frequency of *Serratia* infection as being unique, as this organism has been rarely implicated in other regions across the globe. However, no GBS was isolated, which was in stark contrast to reports obtained from Europe and America [14]. In a study conducted in Malaysia by Boo and Chor, it was reported that the most common pathogens isolated in 1986 and 1987 were *Streptococcus epidermidis* and *S. aureus*. However, after 1988, *Klebsiella* species emerged as the most commonly implicated organism [15]. It is really not clear as of now, whether these differences in isolated organisms reflect true variations in pathogens across the world, thus reflecting an epidemiological transition in many regions, or whether it is simply reflective of an epidemiological bias, quite possibly linked to the observation that most early-onset sepsis babies die at home, before arriving at the

Table 1: Distribution pattern of microorganisms obtained from blood culture (n=40)

Pathogen profile	Number of isolates (%)
Gram-positive isolates	18 (7.5)
Staphylococcus aureus	9 (22.5)
CONS	5 (12.5)
Enterococci	4 (10)
Gram-negative isolates	18 (7.5)
E. coli	3 (7.5)
K. pneumoniae	5 (12.5)
Acinetobacter	8 (20)
P. aeruginosa	2 (5)
Yeast	4 (1)
Candida sp.	4 (10)

CONS: Coagulase-negative staphylococci, *E. coli*: *Escherichia coli*, *K. pneumonia*: *Klebsiella pneumoniae*, *P. aeruginosa*: *Pseudomonas aeruginosa*

Table 2: Antibiotic sensitivity pattern of Gram-positive isolates

Antibiotics	*S. aureus* (n=9) (%)	CONS (n=5) (%)	*Enterococcus* sp. (n=4) (%)
Erythromycin	2 (22)	0	0
Clindamycin	2 (22)	2 (40)	0
Ciprofloxacin	1 (11)	0	2 (50)
Chloramphenicol	5 (56)	0	0
Teicoplanin	3 (33)	5 (100)	0
Amikacin	2 (22)	0	0
Gentamicin	3 (33)	2 (40)	1 (25)
Ceftazidime	1 (11)	0	0
Cefotaxime	4 (44)	1 (20)	0
Ampicillin	1 (11)	0	3 (75)
Vancomycin	3 (33)	5 (100)	2 (50)
Linezolid	9 (100)	5 (100)	2 (50)

CONS: Coagulase-negative staphylococci, *S. aureus: Staphylococcus aureus*

Table 3: Antibiotic sensitivity pattern of Gram-negative isolates

Antibiotics	*K. pneumonia* (n=5) (%)	*E. coli* (n=3) (%)	*P. aeruginosa* (n=2) (%)	Acinetobacter (n=8) (%)
Amikacin	5 (100)	3 (100)	2 (100)	6 (75)
Gentamicin	3 (60)	3 (100)	2 (100)	2 (25)
Ampicillin	3 (60)	0	0	0
Ciprofloxacin	4 (80)	3 (100)	1 (50)	5 (63)
Levofloxacin	1 (20)	2 (67)	1 (50)	4 (50)
Ceftriaxone	2 (40)	1 (33)	1 (50)	4 (50)
Cefoperazone	3 (60)	1 (33)	0	0
Ceftazidime	2 (40)	1 (33)	1 (50)	5 (63)
Cefotaxime	0	1 (33)	0	3 (38)
Cefepime	2 (40)	1 (33)	1 (50)	2 (25)
Imipenem	2 (40)	3 (100)	2 (100)	4 (50)
Cotrimoxazole	0	2 (67)	1 (50)	2 (25)

health facilities, and they do not appear in the collected data [8].

The Indian scenario is ever changing and poses interesting observations. A study conducted in the Southern Indian state of Karnataka reported that 70.5% neonatal septicemia cases were caused by Gram-negative isolates. *K. pneumoniae* was found to be the primary pathogenic entity followed by *S. aureus* accounting for as much as 35.4% and 22.9% cases, respectively [5]. Gandhi et al. observed that *E. coli* and *S. aureus* were the most common organisms associated with neonatal sepsis [16]. Roy et al. reported an overall predominance of Gram-negative organisms and CONS were more frequently isolated (16.5%); compared to *S. aureus* (14%) [17]. Sarangi et al. reported that both Gram-positive and Gram-negative bacteria were accountable for blood stream infections. They further state that among Gram-positive organisms, most commonly implicated were CONS (88.5%) followed by *S. aureus* (7.3%) [18]. Pandita et al. has described the predominant pathogen to be *Klebsiella* (42.8%), among the Gram-negative isolates, followed by *E. coli* (18.1%). Among Gram-positive isolates, CONS (57.4%) was the predominant organism. Moreover, it was also noted that 6% of these Gram-positive isolates were methicillin-resistant *S. aureus* [19].

Other Gram-negative organisms commonly isolated include *E. coli*, *Acinetobacter* spp., and *Pseudomonas* spp. [5]. *Acinetobacter* has been noted to be an important entity by both Arora and Jaitwani [20] and Vinodkumar and Neelagund [21]. Karthikeyan and Premkumar have previously reported the isolation of *K. pneumoniae* and also described it to be the most common pathogen implicated for early-onset sepsis. They also concluded that *S. aureus* was the second common isolate, accounting for 22.9% of derived blood cultures [22].

Our study revealed certain fascinating observations in that equal involvement of both Gram-positive as well as Gram-negative organisms was noted. This was in contrast to reports by Muley et al. [5] and Roy et al. [17]. It validated the findings of Sarangi et al. [18] by assessing the involvement of CONS and *S. aureus*; although, our study did show a higher percentage of *S. aureus* infections. Another interesting observation was the emergence of *Acinetobacter* as a common organism implicated in neonatal sepsis. The analysis of sensitivity patterns suggests an imminent seismic shift in antibiotic policy, as increasing insensitivity to ampicillin is matched only by the ever increasing sensitivity of Gram-positive organisms to antibiotics such as vancomycin and linezolid. A further notable observation is the increasing utility of ciprofloxacin for Gram-negative coverage.

This study highlights the variable nature of antibiotic sensitivity patterns observed in a variety of geographical zones. The ever evolving nature of organisms encountered in neonatal units' warrants constant review of sensitivity patterns as well. Multiple antibiotic resistance is one of the greatest hurdles in the effective management of infections.

CONCLUSION

This study stresses on the need for longitudinal surveillance at frequent intervals to describe the various pathogens attributable for early neonatal sepsis as well as their ever changing antibiotic sensitivity pattern so as to formulate an acceptable and rational antibiotic protocol. With increasing resistance of various pathogens to commonly used antibiotics, it becomes imperative to formulate appropriate unit specific antibiotic policies.

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Funding: None; Conflict of Interest: None Stated.

How to cite this article: Gupta A, Philip P, Madhava KK. Microbiological profile of early-onset neonatal sepsis in a tertiary care hospital in south India. Indian J Child Health. 2016; 3(4):286-289.