Microbial profile of neonatal sepsis and antibiotic resistance from a tertiary care hospital in South India

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Abstract

Introduction: Neonatal sepsis is a major cause of neonatal mortality, the clinical outcome of which depends on early diagnosis and initiation of appropriate antibiotics. The emergence of multi-drug resistant strains has limited the choice of available antibiotics. Thus, antibiotic resistance pattern of pathogens iscritical for both therapy and infection control.

Objective: To determine the etiological agents of neonatal sepsis and their antibiotic resistance pattern.

Materials and Methods: In this study, all neonates with suspected sepsis, admitted to neonatal intensive care unit during January to December, 2014 were included. Aerobic blood culture was done using BACTEC FX system. Identification and antibiotic susceptibility testing of isolates from positive cultures was carried out.

Results: Out of 522 neonates who developed clinical sepsis, 64 grew pathogens on blood culture. The most common organisms were coagulase negative *Staphylococcus species* (CONS)(32.8%), *Klebsiella pneumoniae* (18.7%), *Escherichia coli* (9.4%), *Enterococcus faecalis* (7.8%) and *Candida species* (7.8%). The gram negative isolates showed high resistance to ampicillin (90%), gentamicin (70%), ceftriaxone (66.7%), cotrimoxazole (56.7%), amikacin (53.3%) and ciprofloxacin (43.3%). In contrast, imipenem (16.7%), piperacillin-tazobactam (26.7%) and cefepime-tazobactam (0%) were effective with lower resistance rates. A large majority of the Enterobactericeae isolates (66.7%) were extended spectrum beta-lactamase producers. Among the gram positive isolates, resistance to penicillin, erythromycin and ciprofloxacin were 93.1%, 62.1%and 51.7% respectively. These strains showed uniform sensitivity to vancomycin, teicoplanin and linezolid.

Conclusion: While CONS were the predominant isolate, gram negative bacilli and *Enterococcus sp* have shown high resistance to commonly used antibiotics. Low resistance was observed with Cefepime-tazobactam, imipenem (for gram-negative isolates) and vancomycin, teicoplanin and linezolid (for gram-positive isolates) in our study.

Keywords: Neonatal sepsis, Bacteraemia, Antibiotic resistance, Blood culture.

Introduction

Neonatal sepsis refers to the presence of blood stream infection during first 28 days of life. It is classified in two categories on the basis of epidemiology and time of onset. The infection which manifests within first 72 hours of life is termed as early-onset neonatal sepsis (EONS), while late-onset neonatal sepsis (LONS) is considered if the features of sepsis appear after 72 hours.[1] Neonatal sepsis continues to be a challenging scenario to paediatricians in developing as well as developed countries. It accounts for a significant proportion of neonatal mortality and morbidity. In India 16.4% neonatal death is associated with neonatal sepsis.[2] Although neonatal sepsis is essentially a systemic infection, the spectrum of sepsis in neonate can vary from subclinical disease to severe systemic infection. The signs of neonatal sepsis viz. Irritability, hyperpyrexia or hypothermia, lethargy, poor feeding, poor perfusion, hypotension, tachycardia, respiratory distress, jaundice are vague and non-specific.[3] Although isolation of bacterial pathogen in culture from bloodis the gold standard, a majority of these patients remain culture-negative. A negative culture is not an adequate criteria to rule out infection. Furthermore, there is a lack of sensitive markers for diagnostic purpose and the diagnosis is often relied on clinical suspicion.[4] The success of therapy is essentially dependent on early diagnosis and initiation of appropriate antibiotics. The antibiotics used for empirical therapy should have spectrum of activity which encompass the common pathogens. Therefore, comprehensive information of pathogens implicated in neonatal sepsis and their antibiotic susceptibility profile in a region is essential to choose appropriate antibiotics for empirical therapy as well as to avoid high antibiotic consumption. The aim of this study was to find out the etiological agents of neonatal sepsis and identify their resistance pattern in our tertiary care hospital.

Materials and Methods

In this study, we retrospectively analysed records of neonates admitted to neonatal intensive care unit (NICU) of our hospital with suspected sepsis during January to December, 2014. The non-NICU cases and cases where blood culture was not carried out were excluded. An aerobic blood culture was done using BACTEC FX system (Becton, Dickinson and Company, USA) before starting the empirical therapy. The antibiotic therapy is further guided and modified based on the blood culture and antibiotic susceptibility report. The gram negative bacteria were tested against an antibiotic panel consisting of cotrimoxazole (1.25/23.75μg), ampicillin (10 μg), gentamicin (10 μg), amikacin (30 μg), ceftriaxone (30 μg), ciprofloxacin (5μg), imipenem (10 μg), piperacillin-tazobactam (100/10 μg) Colistin (10 μg) and cefepime-tazobactam (30/10μg). The gram positive antibiotic panel included penicillin (10 μg), erythromycin (15μg), ciprofloxacin (5 μg), cefoxitin (30

μg), vancomycin (30 μg), teicoplanin (30 μg) and linezolid (30 μg). We have used S. aureus ATCC 25923, E. coli ATCC 25922 and P. aeruginosa ATCC 27853 as controls for antibiotic susceptibility test. Ceftazidime and ceftazidime-clavulunic acid combined disc test was used to detect Extended Spectrum beta-lactamase (ESBL) for gram negative isolates which showed resistance to third generation cephalosporins.[5]

Results

We analysed records of a total of 522 neonates with clinical sepsis who were admitted in our NICU during the study period and had blood culture sent for isolation of microbial agent. Out of these, 64 neonates (36 male and 28 female) grew pathogens on blood culture. Among these culture proven sepsis cases, 53 (82.8%) were early onset neonatal sepsis (EONS) and 11 (17.2%) late onset neonatal sepsis (LONS). The microbial agents in EONS and LONS are listed in Table 1. The resistance pattern of gram negative and gram positive bacteria is detailed in Table 2 and 3.

Discussion

In our study blood culture yield was 12.2%, of which EONS accounted for 82.8% and LONS 17.2% respectively. The yield of blood culture varies widely in different studies because of differences in culture techniques, infection control measures and regional variation. In a study from North India, culture proven sepsis was found in 7.5/1000 live birth where 85% neonates had EONS and 15% had LONS.[6] In a similar study from a secondary level rural hospital in South India, blood culture was positive in 26.2% cases and EONS was more common (94.4%) than LONS (5.6%).[7]

We found coagulase negative *Staphylococcus species* (CONS) (n=21, 32.8%) was the most common organism in both EONS and LONS group, followed by were *Klebsiella pneumoniae* (n=12, 18.7%), *Escherichia coli* (n=6, 9.4%), *Enterococcus faecalis* (n=5, 7.8%), *and Candida spp*. (n=5, 7.8%). Although CONS are common skin commensal and their isolation in blood culture may represent improper asepsis in venepuncture, they are increasingly being recognised as pathogen in neonatal sepsis.[8-10] CONS has been reported as the predominant isolate by several authors while the prevalence varied from 5-15% in EONS and 31-58% in LONS in different studies.[9,11] Laboratory criteria such as growth within 48 hours, isolation of the same strain on multiple blood cultures and absence of polymicrobial growth in addition to clinical signs of sepsis has been used to differentiate blood culture contamination from true bacteraemia. In our study, CDC/NHSN criteria was followed and growth of CONS in at least two blood cultures were considered as significant.[12] Growth of CONS on single blood cultureand growth of commensals such as *Micrococcus sp*., Diphtheroids was reported as contamination. Among the coagulase negative species, *S. epidermidis* and *S. hemolyticus* are well recognised agent of neonatal sepsis, especially in LONS. Low birth weight, presence of central venous catheters (CVC), indwelling devises, mechanical ventilation and parenteral nutrition are important risk factors associated with CONS.[8]

Enterobacteriaceae members such as *K. pneumoniae, E. coli* along with *Candida species* especially *C. tropicalis* (n=4) were major pathogen in EONS in our study (Table 1). Similar to CONS, isolation of *Candida species* from blood culture may result from skin contamination. However, several species of Non-albicans *Candida* such as *C. dubliniensis, C. tropicalis, C. lusitaniaea, C. parapsilosis* and *Candida glabrata*, are emerging as important pathogens in settings of neonatal sepsis accounting for 1.4 to 15.8% in various studies.[9,11,13,14] Tomar et al reported that obtaining two blood cultures from different sites at the same time improved thediscrimination of pathogen from contaminants.[15]

The gram negative isolates showed high resistance to ampicillin (90%), gentamicin (70%), amikacin (53.3%), ceftriaxone (66.7%), cotrimoxazole (56.7%), and ciprofloxacin (43.3%). In contrast, imipenem (16.7%), piperacillin-tazobactam (26.7%), colistin (10%) and cefepime-tazobactam (0%) were effective with lower resistance rates (Table 2). Ampicillin, aminoglycoside and third-generation cephalosporinsare regularly used to treat sepsis in neonate. High resistance to these antibiotics among gram negative bacteria have been reported from India.[7,16] Pavan Kumar et al from Southern India reported 100%, 52.9% and 31.2% resistance to ampicillin, gentamicin and third-generation cephalosporin respectively among Gram-negative bacilli.[7] Bhat et al from South India and Chandel et al in a multi-centric study reported similar findings.[16,17] We identified Extended Spectrum beta-lactamase (ESBL) producing strains by phenotypic combined disc test using ceftazidime and ceftazidime-clavulunic acid discs.[5] ESBL production was found in 16 out of 24 Enterobactericeae isolates (66.7%). This is in accordance with other reports from India. Over one-third of ESBL producer isolates in neonatal sepsis have been found to be *Klebsiella pneumoniae* and *E. coli*.[17] Expression of ESBL enzymes in clinical isolates results in treatment failure and higher mortality.

In this present study, resistance to penicillin, erythromycin and ciprofloxacin among the gram positive isolates were 93.1%, 62.1% and 51.7% respectively. These isolates were uniformly sensitive to vancomycin, teicoplanin and linezolid (Table 3). Methicillin resistance was detected by cefoxitin disc diffusion method for *S. aureus* and CONS isolates. We found methicillin resistance in seven CONS isolates. CONS are known to display higher resistance to methicillin while having lesser virulence potentials, vague signs of sepsis and lower mortality than *S. aureus*.[10] Although vancomycin resistance is not uncommon in *Enterococcus species*, we could not find it in our study.[18]

Table 1: Blood culture isolates from EONS & LONS

Organisms	EONS (n=53)	LONS (n=11)
CONS	16 (30.2%)	5 (45.5%)
K. pneumoniae	11 (20.8%)	1 (9.1%)
E. coli	6(11.3%)	0
E. faecalis	5 (9.4%)	0
C. tropicalis	3 (5.7%)	1 (9.1%)
Non-fermenter GNB	3 (5.7%)	0
A. baumannii	2 (3.8%)	1 (9.1%)
C. freundii	2 (3.8%)	0
S. aureus	2 (3.8%)	0
Enterobacter sp	1 (1.9%)	2 (18.2%)
S. pyogenes	1 (1.9%)	0
P. mirabilis	1 (1.9%)	0
C. albicans	0	1 (9.1%)

Table 2: Antibiotic resistance pattern of gram negative bacterial isolates from neonatal sepsis

	Cotrimoxazole	Ampicillin	Gentamicin	Amikacin	Ceftriaxone	Ciprofloxacin	Imipenem	Piperacillin-Tazobactam	Cefepime-Tazobactam	Colistin
K. pneumoniae (n=12)	9 (75%)	11 (91.7%)	9 (75%)	8 (66.7%)	9 (75%)	5 (41.7%)	3 (25%)	6 (50%)	0 (0%)	1 (8.3%)
E. coli (n=6)	5 (83.3%)	6 (100%)	5 (83.3%)	3(50%)	4 (66.7%)	4 (66.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Non-fermenter GNB (n=3)	0 (0%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	1 (33.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
A. baumannii (n=3)	2 (66.7%)	3 (100%)	2 (66.7%)	2 (66.7%)	3 (100%)	2 (66.7%)	2 (66.7%)	2 (66.7%)	0 (0%)	2 (66.7%)
C. freundii (n=2)	0 (0%)	2 (100%)	2(100%)	0 (0%)	1 (50%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
P. mirabilis (n=1)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Enterobacter sp (n=3)	1 (33.3%)	3 (100%)	2 (66.7%)	2 (66.7%)	1 (33.3%)	2 (66.7%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total (n=30)	17 (56.7%)	27 (90%)	21 (70%)	16 (53.3%)	20 (66.7%)	13 (43.3%)	5 (16.7%)	8 (26.7%)	0 (0%)	3 (10%)

Table 3. Antibiotic resistance pattern of gram positive bacterial isolates from neonatal sepsis

	Penicillin	Erythromycin	Ciprofloxacin	Vancomycin	Teicoplanin	Linezolid
CONS (n=21)	20 (95.2%)	11 (52.3%)	8 (38%)	0 (0%)	0 (0%)	0 (0%)
E. faecalis (n=5)	5 (100%)	5 (100%)	5 (100%)	0 (0%)	0 (0%)	0 (0%)
S. aureus (n=2)	2 (100%)	1 (50%)	2 (100%)	0 (0%)	0 (0%)	0 (0%)
S. pyogenes (n=1)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Total (n=29)	27 (93.1%)	18 (62.1%)	15 (51.7%)	0 (0%)	0 (0%)	0 (0%)

Conclusion

We found a large majority of Enterobactericeae isolates from neonatal sepsis were ESBL producers. Gram negative bacilli displayed high resistance to commonly used antibiotics like ampicillin, gentamicin and ceftriaxone. Second line antibiotics such as vancomycin, teicoplanin and linezolid showed good in-vitro sensitivity against the gram positive isolates, while Cefepime-tazobactam, imipenem were effective against gram-negative isolates. Skin commensals such as CONS and *Candida spices* were found as predominant isolate from neonatal sepsis in our hospital. Despite the low virulence and commensal nature, their potential to cause sepsis in neonates mandates stringent surveillance and infection control.

Conflict of Interest: None.

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How to cite this article: Kali A, Ramakrishnan K, Charles PMV, Srirangaraj S, Seetha KS. Microbial profile of neonatal sepsis and antibiotic resistance from a tertiary care hospital in South India. *Indian J Microbiol Res* 2019;6(1):57-60.