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

Arunava Kali¹, Kalaivani Ramakrishnan²*, Pravin Charles M. V³, Sreenivasan Srirangaraj⁴, Seetha K.S⁵

¹-³Associate Professor, ⁴Professor, Dept. of Microbiology, Mahatma Gandhi Medical College & Research Institute, Sri Balaji Vidyapeeth University, Pondicherry, India

*Corresponding Author: Kalaivani Ramakrishnan
Email: kalaimicro21@gmail.com

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 is critical 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 Enterobacteriaceae 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 blood is 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.³ 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, teicoplanin and linezolid.
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. 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%).

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. 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. 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. 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.

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. lusitaniae, 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. Tomar et al reported that obtaining two blood cultures from different sites at the same time improved thediscrimination of pathogen from contaminants.

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 ceftazidime (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. 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. Bhata et al from South India and Chandel et al in a multi-centric study reported similar findings. We identified Extended Spectrum beta-lactamase (ESBL) producing strains by phenotypic combined disc test using ceftazidime and ceftazidime-clavulanic acid discs. ESBL production was found in 16 out of 24 Enterobacteriaceae 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. 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. Although vancomycin resistance is not uncommon in Enterococcus species, we could not find it in our study.
linezolid showed good in vivo sensitivity against the gram positive isolates, while Cefepime-tazobactam, imipenem were effective against gram-negative isolates. Skin commensals such as CONS and Candida species 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.

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

| Organisms       | EONS (n=53) | LONS (n=11) |
|-----------------|-------------|-------------|
| CONS (n=21)     | 20 (95.2%)  | 0           |
| E. faecalis (n=5) | 5 (100%)    | 0           |
| S. aureus (n=2) | 2 (100%)    | 0           |
| S. pyogenes (n=1) | 0 (0%)      | 0           |
| Total (n=29)    | 27 (93.1%)  | 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 species 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.
References

1. Kristof K, Kocsis E, Nagy K. Clinical microbiology of early-onset and late-onset neonatal sepsis, particularly among preterm babies. *Acta Microbiol Immunol Hung* 2009;56:21-51.
2. Sankar MJ, Neogi SB, Sharma J, Chauhan M, Srivastava R, Prabhakar PK, et al. State of newborn health in India. *J perinatol official J California Perinatal Assoc* 2016;36:S3-S8.
3. Panigrahi P, Chandel DS, Hansen NI, Sharma N, Kandefer S, Parida S, et al. Neonatal sepsis in rural India: timing, microbiology and antibiotic resistance in a population-based prospective study in the community setting. *J Perinatol* 2017;37:911-921.
4. Klingenberg C, Kornelisse RF, Buonocore G, Maier RF, Stocker M. Culture-Negative Early-Onset Neonatal Sepsis - At the Crossroad Between Efficient Sepsis Care and Antimicrobial Stewardship. *Frontiers in Pediatrics*. 208;6:285.
5. CLSI. Performance standards for antimicrobial susceptibility test. Approved Standard. CLSI Document M100-S25. 25th ed. Wayne, Pennsylvani: Clinical and Laboratory Standards Institute; 2015.
6. Marwah P, Chawla D, Chander J, Guglani V, Marwah A. Bacteriological profile of neonatal sepsis in a tertiary-care hospital of Northern India. *Indian Pediatr* 2015;52:158-159.
7. Pavan Kumar DV, Mohan J, Rakesh PS, Prasad J, Joseph L. Bacteriological profile of neonatal sepsis in a secondary care hospital in rural Tamil Nadu, Southern India. *J Family Med Prim Care* 2017;6:735-738.
8. Marchant EA, Boyce GK, Sadarangani M, Lavoie PM. Neonatal sepsis due to coagulase-negative staphylococci. *Clin Dev Immunol* 2013;586076:22.
9. Bizzarro MJ, Shabanova V, Baltimore RS, Dembry LM, Ehrenkranz RA, Gallagher PG. Neonatal sepsis 2004-2013: the rise and fall of coagulase-negative staphylococci. *J Pediatr* 2015;166:1193-1199.
10. Shivanna V, Sunkappa SR, Venkatesha D. The rising trend of coagulase-negative staphylococci in neonatal septicemia. *Indian J Pathol Microbiol* 2016;59:510-512.
11. Ozkan H, Cetinkaya M, Koksal N, Celebi S, Hacimustafaoglu M. Culture-proven neonatal sepsis in preterm infants in a neonatal intensive care unit over a 7 year period: coagulase-negative Staphylococcus as the predominant pathogen. *Pediatr Int* 2014;56:60-66.
12. Horan TC, Andrus M, Dudek MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control* 2008;36:309-332.
13. Juyal D, Sharma M, Pal S, Rathaur VK, Sharma N. Emergence of non-albicans Candida species in neonatal candidiasis. *N Am J Med Sci* 2013;5:541-555.
14. Turhan EE, Gursoy T, ovali F. Factors which affect mortality in neonatal sepsis. *Turk Pediatr Ars* 2015;50:170-175.
15. Tomar P, Garg A, Gupta R, Singh A, Gupta NK, Upadhayay A. Simultaneous Two-site Blood Culture for Diagnosis of Neonatal Sepsis. *Indian Pediatr* 2017;54:199-203.
16. Bhat YR, Lewis LE, K EV. Bacterial isolates of early-onset neonatal sepsis and their antibiotic susceptibility pattern between 1998 and 2004: an audit from a center in India. *Ital J Pediatr* 2011;37:1824-7288.
17. Chandel DS, Johnson JA, Chaudhry R, Sharma N, Shinkre N, Parida S, et al. Extended-spectrum beta-lactamase-producing Gram-negative bacteria causing neonatal sepsis in India in rural and urban settings. *J Med Microbiol* 2011;60:500-507.
18. Kaushal S, Banerjee T, Anupurba S, Kumar A. Vancomycin-resistant enterococci in neonatal stool as a cause of septicemia: Challenges for infection control practices. *Indian J Pathol Microbiol* 2016;59(4):548-550. doi: 10.4103/0377-4929.191802.

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.