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Original Research Article

The changing bacteriological profile of neonatal sepsis in a tertiary care hospital – Emergence of *Citrobacter* septicemia

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ABSTRACT

Introduction: Neonatal Mortality Rate (NMR) is one of the indicators of health status of a nation and neonatal sepsis is one of the commonest causes of NMR contributing to 19% of all neonatal deaths in India. All over India, there is a gradually increasing trend of multi-drug resistant (MDR) pathogens in neonatal intensive care units (NICU) and special newborn care units (SNCUs) in tertiary care hospitals.
Aim: To determine the changing trend in the bacteriological profile of neonatal septicemia and their antibiotic susceptibility pattern (AST).
Materials and Methods: It is a retrospective study conducted on total 452 blood culture samples collected from SNCU over 1-year period from July 2017 to June 2018 in tertiary care government hospital. Organisms were identified by the standard protocol and their antimicrobial susceptibility testing was determined as per latest CLSI guidelines.
Results: out of 452 blood culture of neonatal septicemia patients, 138 cases (30.53%) were culture positive Most common isolated gram negative organism was *Klebsiella* spp. 55/138 (39.85%) followed by *Citrobacter* spp. 23/138 (16.66%). Most common isolated gram positive organism was *Staphylococcus aureus* 25/138 (18.11%) followed by Coagulase Negative Staphylococcus (CONS) (8.69%). Among gram negative isolates most sensitive antimicrobial was Imipenem (87.6%) followed by Levofloxacin (87.4%) and Piperacillin + Tazobactam (46%) and resistant to, Cotrimoxazole, Amoxicillin + clavulanate and 3rd generation Cephalosporins.
Conclusion: *Klebsiella* spp. is the predominant organism of neonatal sepsis in the Indian subcontinent, although significant rise in proportion of *Citrobacter* spp. is occurring. High resistance to cephalosporins is a cause of concern, as they are one of the most common prescribed antibiotic groups.

1. Introduction

Neonatal sepsis is a systemic infection in the first 28 days of life, encompasses with bloodstream infections, pneumonia and meningitis. It is the third most common cause of deaths among neonates.[1,2] In India about one-third of neonatal mortality is due to neonatal sepsis and death occurs in 30% of culture positive. neonates.[3–5] Based on the onset of illness, Neonatal sepsis is classified as early onset sepsis (EOS) (<72 h), occurs due to pathogens present in the genital tract of the mother and other one is late onset sepsis (LOS) (>72 h), occurs due to pathogens acquired either from the hospital or from the community.[3] All over India, there is a gradually increasing trend of multi-drug resistant (MDR) pathogens in neonatal intensive care units (NICU) and special newborn care units (SNCUs) in tertiary care hospitals. As per the Centers for Disease Control and Prevention (CDC) guidelines Multi-drug resistance was

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defined as the acquired resistance to at least one agent in three or more antimicrobial categories. Strict antibiotics stewardship program will enable us to counteract multi-drug resistance patterns of emerging pathogens.[4–6]The prevalence of organism in SNCUs, differ from tertiary care NICUs in our country and also different from that of the Western world. Pathogens such as *Klebsiella pneumoniae* and *Escherichia coli* are the most common cause of neonatal sepsis in India and South Asia.[7,8]

Culture-negative sepsis and MDR neonatal sepsis are challenging conditions in the management of sepsis in newborns. Blood culture is the most important microbiological tool in sepsis diagnosis and treatment. In developing countries, many neonates have been diagnosed with sepsis based on clinical suspicion, referred to as culture-negative sepsis.[9] Recent reports suggest that antibiotic use is up to 16 times greater for culture-negative sepsis therapy than for culture-proven sepsis therapy.[10] The high consumption of antibiotics in culture-negative sepsis cases potentially relates to an increased risk of colonization by antibiotic-resistant bacteria in neonates.[11] In addition, culture-negative sepsis cases treated with antibiotics are ignored in epidemiological studies. Therefore, more studies are needed to obtain a better understanding of culture-negative sepsis, as it also contributes to significant morbidity and mortality.

The majority of studies were done in tertiary care units with SNCU. Gradually increasing trends of *Citrobacter* species in our SNCU prompted us to do this study to evaluate the organism and their antibiogram pattern in neonatal sepsis. This study aimed to review neonatal sepsis epidemiology, including cases of culture-negative and culture-proven neonatal sepsis, and antimicrobial resistance patterns at a tertiary level hospital.

2. Materials and Methods

This was a retrospective study were conducted from July 2017 to July 2018 in the tertiary care government medical college and associated hospital. Data were obtained in the study Performa from case sheet of suspected neonatal septicemia. All laboratory data of blood culture samples with contamination and all laboratory records of bacterial isolates from blood culture samples that were not tested for antimicrobial susceptibility were excluded in this study. Overall, the blood culture records of suspected neonatal septicemia of 452 newborn patients were recorded and further processed.

2.1. Methodology

0.5-1ml of blood samples were collected in 10 ml of brain heart infusion broth under aseptic conditions incubated at 37°C for 7 days under aerobic conditions and further processes manually. The blood culture sample were subcultures on 1,3 and 5th day on blood agar, MacConkey agar& chocolate agar plate. Gram staining was performed from culture growth. In addition, identification of Gram-positive cocci organisms was identified using slide catalase and tube Coagulase tests and further cultured on Mannitol salt agar media for *staphylococcus aureus*. Gram-negative bacilli were identified by colony morphology and additionally biochemical tests were performed, which includes Triple Sugar Iron (TSI), oxidation- fermentation test (O/F), decarboxylase tests, Motility of organisms, Indole ring test, methyl red test, vogues proskauer test, Urease hydrolyze, and citrate utilization tests were performed and differentiate *Enterobacteriaceae* species. The Antibiotic susceptibility testing was performed by the Kirby Bauer disk diffusion method, following laboratory protocol and the interpretation of the diameter of inhibition was done according to latest Clinical and Laboratory Standards Institute (CLSI) guidelines. Blood culture broth that showed no growth after 7 days of incubation at 37°C was reported as no growth. These bottles were further incubated for 2 weeks to check for growth of fastidious organisms.[12]

3. Result

Out of 452 suspected neonatal septicemia newborns patients showed that 138 (30.54%) were positives for blood stream infections. Amongst 138 positive blood culture isolates of neonatal septicemia patients Gram positive and Gram negative bacteria isolates were constituted 39 (39/138=28.26%) and 99 (99/138=71.74%) isolated respectively. Of the 39 Gram positive bacteria blood culture isolates of neonatal septicemia showed *Staphylococcus aureus* (64.10%) were predominant isolated followed by Coagulase-negative Staphylococcus (CONS:30.76%) and Enterococcus faecalis (5.12%). (Figure 1)

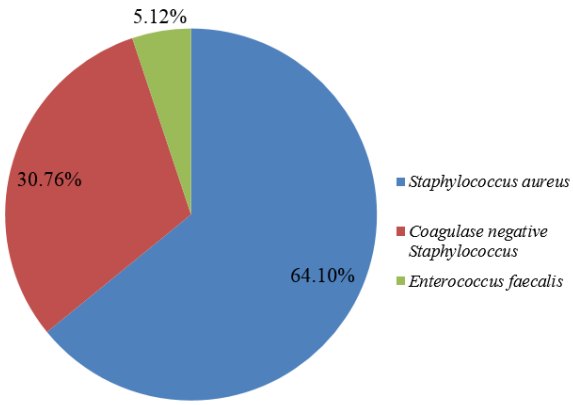

Fig. 1: Gram-positive blood culture isolated bacterial isolates of neonatal septicemia patients. (n=39)

Out of 99 isolated Gram negative bacterial blood culture isolates of neonatal septicemia *Klebsiella pneumoniae* (55.55%), followed by *Citrobacter* freundii (23.23%), *Escherichia coli* (14.14%), Pseudomonas aeruginosa (6.06%) and *Acinetobacter baumannii* (1.01 %) were isolated. (Figure 2)

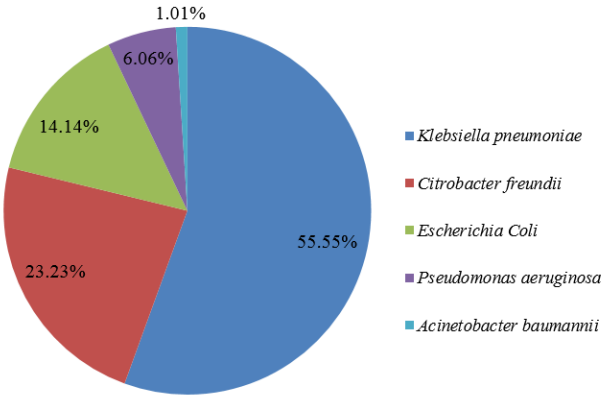

Fig. 2: Gram-negative blood culture isolated bacterial isolates of neonatal septicemia patients. (n=99)

The antimicrobial susceptibility pattern among Gram-negative bacteria blood culture isolates of neonatal septicaemia showed susceptibility to Imipenem (60-100%), Levofloxacin (70-100%) while 80-100% resistant to 3^{rd} generation cephalosporin's. (Table 1)

Out of Gram positive bacteria blood culture isolates of neonatal septicaemia patients were mostly sensitive to vancomycin and Linezolid and resistant to Ampicillin (80-100%). Out of total 25 isolates of *Staphylococcus aureus* 8/25 (32%) were Methicillin resistant *staphylococcus aureus* (MRSA). (Table 2)

The most common risk factor for neonatal mortality amongst neonatal septicaemia patients was prematurity (32%) followed by septicaemia (19%) and birth asphyxia (16%). (Figure 3)

4. Discussion

In this study blood culture positivity rate for bacterial isolates were 30.54% (138/452). Similar rates were also found by Shashi Gandhi et al and MP. Samagaet al in 2013 and 2017 year respectively.[13–15] Difference in a positivity rate of blood culture in different studies might be due to different reasons like the variation in culture methods, study plan, administration of prior antibiotics from the primary center or unsuccessful control of hospital-acquired infection.[16,17] Gram negative bacteria were isolated predominantly 99 (71.73%). Gram positive bacteria isolated were 39 (28.26%). Our finding was correlated with study conducted by Habyarimana et al in 2021, showed most common isolated gram negative bacteria was *Klebsiella pneumoniae* 55 (55.55%) and gram

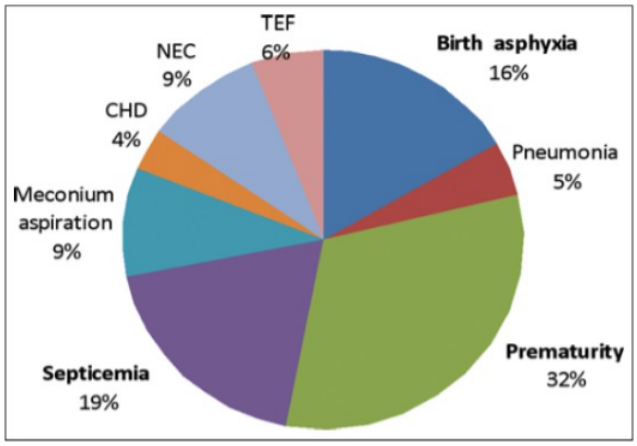

Fig. 3: Causes ofneonatal mortality amongst neonatal septicaemia patients in the study group.(n=138)

positive bacteria was *Staphylococcus aureus* 25 (64.10 %)[18,19] Present study observed that the prevalence of *Citrobacter* freundii was 16.66 % which was considerably high compared to previous studies. *Citrobacter* species was uncommon cause of infection in neonates but may acquire the micro-organism horizontally as nosocomial infection or vertically from the mother at the time of delivery. The magnitude of *Citrobacter* infection has increased over time considering its potential to cause nosocomial infection.

In present study overall sensitivity to gram negative isolates were for Imipenem (87%) and Levofloxacin (87%) and resistant to 3^{rd} generation cephalosporin. Gram positive isolates were mostly sensitive to Vancomycin (94%) and Linezolid (92%) and resistant to Ampicillin (90-100%). Similar reports of resistant and sensitivity were reported by Shashi Gandhi et al (2013), Sharma RS et al (2016), Samagaet al (2017).[13,15] The causative organisms associated with neonatal sepsis vary from place to place and also from hospital to hospital and even in the same hospital at different times.[19,20] Probably due to the unhygienic condition of the vagina during birth and carelessness about the safety precautions of medical personnel who assist the delivery, the baby who delivered by normal vaginal route has a higher rate of sepsis similar to the other different studies.[20–25] Our study findings showed that culture-proven neonatal sepsis cases shared a comparable proportion morbidity and mortality in neonates. The incidence of Neonatal Sepsis declined from 111 per 1000 live births in 1998 to 2001 to 19 per 1000 live births in 2016 to 2019, an 82.9% decrease (P<0.0001), mean 4% decrease per year.[26]

5. Conclusion

Neonatal sepsis is a global public health issue, so we recommend more comprehensive, extensive, and large-scale studies to better understand the magnitude of the disease. We should formulate our antibiotic policy and

Table 1: Antimicrobial susceptibility pattern of Gram-Negative bacteria blood culture isolates of neonatal septicaemia patients. (n=99)

	AK	AMC	COT	CTX	CS	Le	IM	Pt
Klesiella pneumonia (55)	45%	58%	62%	10%	62%	82%	91%	87%
Citrabacter freundii (23)	100%	0%	100%	0%	100%	100%	100%	0%
Escherichia Coli (14)	49%	49%	39%	12%	49%	78%	81%	88%
Pseudomonas aeruginosa (06)	66%	11%	11%	11%	22%	77%	66%	55%
Acinetobacter baumannii	0%	100%	0%	0%	100%	100%	100%	0%

AK-Amikacin, AMC-Amoxycillin + clavulanic acid, CTX-Cefotaxime, COT-Cotrimoxazole, CS-Cefoperazone + sulbactam, LE-Levofloxacin. IM-Imipenam, PT-Piperacillin + tazobactam

Table 2: Antimicrobial susceptibility pattern of Gram-positive bacteria blood culture isolates of neonatal septicaemia patients. (n=99)

	AK	AMC	COT	CTX	CS	Le	IM	Pt
Staphylococcus aureus (55)	12%	28%	20%	48%	36%	88%	90%	68%
Coagulase negative staphyloccoccus (12)	8%	33%	30%	41%	50%	86%	92%	75%
Enterococcus faecalis (02)	0%	0%	0%	50%	50%	100%	100%	–

AMP-Ampicillin, AMC-Amoxycillin + clavulanic acid, AZM- Azithromycin, LE-Levofloxacin, COT- Cotrimoxazole, LZ- Linezolid, VA-Vancomycin, CK- Cefoxitin.

emphasize on rationale antibiotics use and infection control practices. Our findings emphasized the surge in *Citrobacter* species in developing countries and the need for significant actions that will improve efforts to prevent infection in neonates while controlling the use of antibiotics. We also advocate the development of alternative, affordable pathogen identification approaches that can serve as add-ons to traditional microbiological techniques to improve the management of neonatal sepsis and the prevention of antimicrobial resistance.

6. Conflict of Interest

The authors declare no relevant conflict of interest with respect to research, authorship and or publication of this article

7. Source of Funding

None.

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