Section: Microbiology

Original Research Article

PROSPECTIVE STUDY ON ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF LATE ONSET NEONATAL SEPSIS CASES IN A TERTIARY CARE SNCU CAUSED BY BURKHOLDERIA CEPACIA COMPLEX

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Abstract

Background: The purpose of the study was to assess the antimicrobial susceptibility profile of late onset neonatal sepsis cases in a tertiary care SNCU caused by Burkholderia cepacia complex. **Materials and Methods:** Blood specimen collected by vene-puncture aseptically from suspected cases of late onset neonatal sepsis incubated in BacT /ALERT machine. Isolates grown were identified and in-vitro antimicrobial susceptibility testing was performed following standard laboratory protocol using conventional and automated methods. **Result:** Growth was detected in 173 blood specimens from a total of 402 specimens collected from clinically suspected cases of neonatal sepsis. The study highlighted on different clinico-epidemiological aspects .53.76% of the culture positive specimen was from male patients. Median age at onset of symptoms was Day 11 and median birth weight of the baby was 2200 grams. 39.9% of late onset neonatal sepsis was preterm babies. 79 of the 153 GNB isolates detected were Burkholderia cepacia complex. Meropenem was most in-vitro sensitive (91%) against BCC followed by Cotrimoxazole(89%) and Minocycline (81%) respectively. Colistin and Ciprofloxacin were resistant in 43% and 70% isolates respectively. BCC isolates were totally resistant against Imipenem, Ceftriaxone, Cefoperazone – Sulbactam, Cefepime, Aminoglycosides and Piperacillin-Tazobactam. **Conclusion:** Meropenem or Cotrimoxazole remains the best choice for empirical therapy prior to the identification of causative micro-organisms in our set up in severe cases of clinically proven or suspected neonatal sepsis. The emergence of BCC as the most common cause of late onset neonatal sepsis and its resistance to most of the first line antimicrobials used in therapy raises a matter of concern.

INTRODUCTION

Burkholderia cepacia complex (BCC) is a group of saprophytic Gram-negative bacilli that can cause infections ranging from asymptomatic or subclinical ones in healthy adult populations to severe respiratory or blood-stream infections in patients with serious underlying diseases such as Cystic fibrosis.[1] Hospitalized patients and patients with deranged immune response are always at a greater risk to develop severe, life-threatening opportunistic infections caused by B.cepacia complex .BCC is a cluster of approximately ten species including B.cepacia, B.multivorans, B.cenocepacia etc. differentiated by molecular and biochemical methods.[2] Survival of BCC in disinfectants and intrinsic resistance to multiple antibiotics favour its spread in Intensive Care Units(ICU) in patients with prolonged ICU stay or those with indwelling devices making it significant to look for the species level identification among the large group of oxidase positive ,non-fermentative Gram negative bacilli specifically differentiating it from Pseudomonads.[3] Therefore, BCC presents as a rare but potential cause of hospital acquired neonatal sepsis cases in Special Newborn Care Unit(SNCU) where the transmission of the infection is mostly mediated by caregivers coming in contact with indwelling devices and disinfectants.

Neonatal sepsis is the second most common cause of mortality in newborns globally accounting for a staggering 24% of all deaths.[4,5] Neonatal mortality and morbidity therefore throws a major challenge more so across the developing countries and demands prompt diagnosis and initiation of

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appropriate antibiotic therapy for preventing any catastrophe. Persistence of multi-resistant BCC in any SNCU therefore signifies greater challenge to contain the infections in neonates.

A prospective, observational study was therefore undertaken to assess the prevalence of late-onset neonatal sepsis in SNCU in a tertiary care hospital in Eastern India with special emphasis to determine the role of Burkholderia cepacia complex as a probable causative agent highlighting the clinico-epidemiological factors and antimicrobial susceptibility profiles of the said isolates.

MATERIALS AND METHODS

The present study was undertaken in the Department of Microbiology of a tertiary care hospital in Eastern India from March 2023 to August 2023 after obtaining approval from Institutional Ethics Committee. Approximately 1 ml of blood specimen were collected by vene-puncture aseptically maintaining proper precautions in the specified automated paediatric blood culture bottles before starting empirical antibiotic therapy at the SNCU of Paediatric department from suspected cases of late onset neonatal sepsis admitted there. After obtaining the relevant history the specimen was immediately transported to the Bacteriology laboratory for further processing. All the consecutive, non-repetitive samples during the study period with significant clinical history were included in the study after obtaining informed consent. Inclusion criteria involved were- neonate's age >72 hours and within 28 days and presenting with clinical features of sepsis, i.e lethargy, hypothermia, refusal to feed, respiratory distress etc.[6,7] Neonates developing clinical features within 72 hours of birth, having congenital anomalies, severe jaundice, H/O perinatal asphyxia were excluded from the study. Relevant data regarding clinical symptoms, predisposing factors like birth weight and gestational age at delivery and serum CRP level and total leucocyte count were obtained using the study proforma.

All the blood culture bottles were incubated aerobically at 37ºC in the BacT/ALERT machine for 5 days. Once bacterial growth was observed in the bottle as indicated by the machine, the specimen was inoculated on MacConkey agar, 5% sheep blood agar and Chocolate agar plates and incubated at 37ºC overnight aerobically. The observations were recorded as per standard laboratory protocol using conventional methods such as colony morphology of the growth obtained on subculture plates and findings of the Gram-stained smears.Gram negative bacilli showing bipolar staining and oxidase and catalase positive, motile, non-lactose fermenters with musty odour were presumptively identified as BCC and subsequently the identification of the bacterial growth and its antimicrobial susceptibility was performed using automated method VITEK -2. The antibiotic susceptibility profiles were interpreted as per CLSI 2023 guidelines.[8]

RESULTS

Growth was detected in 173 blood specimens from a total of 402 specimens collected from clinically suspected cases of neonatal sepsis[Table 1]. 93 out of the culture positive specimen were from male patients accounting for majority (53.76%) of the positive cases[Table2]. Median age at onset of symptoms was Day 11 and median birth weight of the baby was 2200 grams. 69 of the 173 culture positive cases of late onset neonatal sepsis were preterm babies accounting for 39.9% of all. Gram negative septicaemia was responsible for most of cases (88.44%) of late-onset sepsis cases among neonates [Table3]. Further evaluation revealed that more than half of the gram negative bacillary (GNB) isolates (79 isolates) belonged to the BCC, outnumbering all other Gram-negative isolates taken together [Figure:1]. 45.66% of all late onset neonatal sepsis cases were caused by BCC only [Table 4].

Meropenem was most sensitive (91%) against BCC being closely seconded by Cotrimoxazole(89%). Minocycline was also sensitive against majority (81%) of the isolates. Tigecycline was in-vitro sensitive in 56% isolates and rest 44% were intermediate –sensitive and no isolates were detected to be resistant. But Tigecycline should not be considered for therapy in sepsis patients as it attains poor serum concentration. Colistin and Ciprofloxacin also indicated an increasing trend of in-vitro resistance against the isolates as resistance was observed in 43% and 70% cases respectively. Imipenem, Ceftriaxone, Cefoperazone –Sulbactam and Cefepime were totally ineffective as all the 79 isolates were resistant to these drugs. Aminoglycosides and Piperacillin-Tazobactam were also resistant against 78 out of the 79 isolates of BCC.[Table5&6].

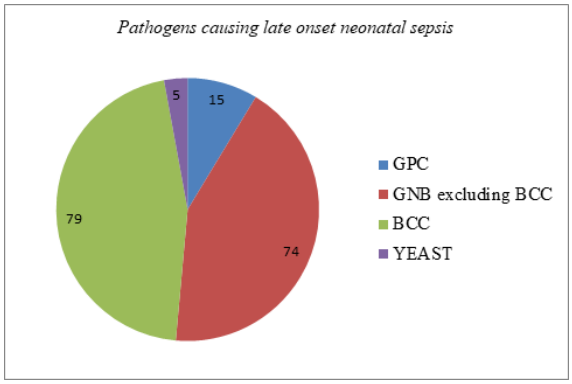

Figure1: Distribution of pathogens causing late onset neonatal sepsis

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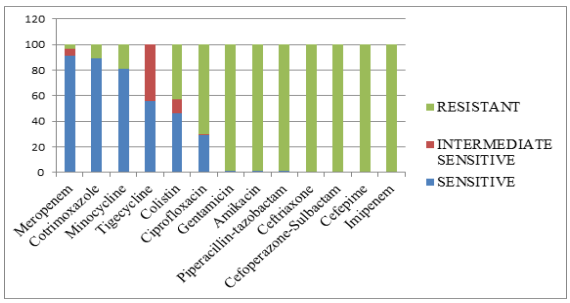

Figure2: Resistance pattern of BCC isolates in percentage to antibiotics used for neonatal sepsis.

Table 1: Incidence of culture-positive paediatric blood specimen

Total number of specimen collected	N-402	Percentage(%)
Growth obtained	173	43.03
No growth	229	56.97

Table 2: Gender wise distribution of culture-positive neonatal sepsis cases

Gender	N=173	Percentage (%)
Male	93	53.76
Female	80	46.24

Table 3: Distribution of pathogen causing sepsis among neonates in a tertiary care hospital

Micro-organism	N=173	Percentage (%)
Gram positive cocci	15	8.67
Gram negative bacilli	153	88.44
Gram positive yeast resembling morphology of Candida	5	2.89

Table 4: Species wise distribution of pathogens causing late onset neonatal sepsis

Micro-organism	N=173	Percentage (%)
Staphylococcus aureus	5	2.89
Coagulase negative Staphylococci	2	1.16
Streptococcus spp	1	0.58
Enterococcus faecalis	5	2.89
Enterococcus faecium	2	1.16
Burkholderia cepacia complex	79	45.66
Klebsiella pneumoniae	22	12.72
Enterobacter cloacae	3	1.73
Escherichia coli	6	3.47
Pseudomonas aeruginosa	13	7.51
Serratia marcescens	2	1.16
Acinetobacter baumannii	17	9.83
Acinetobacter lwoffii	4	2.31
Stenotrophomonas maltophilia	5	2.89
Alcaligenes faecalis	1	0.58
Elizabethkingia meningoseptica	1	0.58
Gram positive yeast resembling morphology of Candida	5	2.89

Table5: In-vitro susceptibility pattern of commonly used antibiotics against BCC isolates

Name of the antibiotic	Sensitive	Intermediate Sensitive	Resistant
Meropenem	72	5	2
Cotrimoxazole	70	0	9
Minocycline	64	0	15
Tigecycline	44	35	0
Colistin	36	9	34
Ciprofloxacin	23	1	55
Gentamicin	1	0	78
Amikacin	1	0	78
Piperacillin-Tazobactam	1	0	78
Ceftriaxone	0	0	79
Cefepime	0	0	79
Cefoperazone-Sulbactam	0	0	79
Imipenem	0	0	79

Table 6: Susceptibility pattern of commonly used antibiotics against BCC isolates in percentage

Name of the antibiotic	Sensitive(%)	Intermediate Sensitive(%)	Resistant(%)
Meropenem	91	6	3
Cotrimoxazole	89	0	11
Minocycline	81	0	19
Tigecycline	56	44	0

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Colistin	46	11	43
Ciprofloxacin	29	1	70
Gentamicin	1	0	99
Amikacin	1	0	99
Piperacillin-Tazobactam	1	0	99
Ceftriaxone	0	0	100
Cefepime	0	0	100
Cefoperazone-Sulbactam	0	0	100
Imipenem	0	0	100

DISCUSSION

Our study showed a culture positivity rate of 43% in samples collected from clinically suspected neonatal sepsis cases in SNCU which was in accordance with studies done in other developing countries. Nyma et al,[9] (2020) study done among 173 sick neonates in Dhaka, Bangladesh reported a prevalence of 69%. Mezgebu et al,[10] also reported an overall prevalence rate of 39.5% of neonatal sepsis cases in NICU in 2022 where poor socio-economic status of unmarried mothers receiving little antenatal care was a major cause of concern in the study population.Golinska et al,[11] study found the prevalence rate of late onset neonatal sepsis cases to be a little less in Europe at 24.5 % in 2016.

The median age of disease onset was 18.5 days and median birth weight was 1490 gm in late onset neonatal sepsis cases in a 5years study in Latin America from 2015 to 2019.[12] The mean age of late onset neonatal sepsis was 12.1 days in an Ethiopian study by Aydiko et al in 2022.Most of the babies were pre-term(63.6%) but had a normal birth weight of more than 2500gms in that study.[13] Our study also revealed a significant number of cases(40%) in pre term and low birth weight babies raising the concern for better antenatal care for pregnant women.

Meta-analysis by Sophi et al,[14] (2021) revealed around 60% of neonatal sepsis to be caused by Gram negative bacteria in Asia & Africa. Moftian et al,[15] reported 53.6% of neonatal sepsis cases to be caused by gram negative bacteria whereas our study found an even higher percentage (88%)of cases caused by GNB.

BCC is still now considered as a rare cause of late–onset neonatal sepsis and there is paucity of data regarding that. Cetin et al,[16] reported 16% cases to be caused by BCC in a recent study in Turkey. Chandrasekaran et al,[17] reported a study of 59 cases of neonatal sepsis mostly of early onset in origin from Southern part of India in 2016. 51.6% of late onset neonatal sepsis cases of gram-negative bacterial origin was caused by BCC in our study indicating a high proportion of infection to be hospital acquired rather than being community acquired in origin.

Sethi et al,[18] study from North India reported a trend of BCC isolates to show the highest susceptibility to co-trimoxazole (89%) followed by levofloxacin (76%) and minocycline (74%).BCC showed maximum susceptibility to ceftazidime (72.1%) and minocycline (55.8%) whereas maximum resistance was seen with β–lactamase inhibitor drugs (83.7%) in another Indian study by Shukla et al.[19] A recent study indicated the isolates to be fully sensitive to Cotrimoxazole and mostly sensitive to meropenem (91%), doxycycline (85%), gatifloxacin (85%), piperacillin+ tazobactam (82%), ceftazidime (79%), and levofloxacin (71%).[20]

Susceptibility pattern of this study revealed Meropenem and Cotrimoxazole to be the drugs of highest efficacy closely seconded by Minocycline though resistance against all of these drugs were noted in small proportion of isolates. No resistant isolates were detected against Tigecycline though 44% isolates were intermediate sensitive against the drug in in-vitro susceptibility testing. Tigecycline attains a poor serum concentration and therefore should not be considered as a therapeutic option in sepsis cases.[21] 100% BCC isolates were resistant to all the other antimicrobials considered for first –line use in patients allergic to Cotrimoxazole, including 3rdand 4thgeneration Cephalosporins, Piperacillin-Tazobactam. Ciprofloxacin was also resistant in 70% of the isolates making it unsuitable for empirical therapy. The high numbers of BCC isolates(43%) emerging to be in –vitro resistant to Colistin raises a grave concern. This multi-drug resistance pattern against those drugs had probably evolved due to frequent and long-term usage in the Intensive Care Units. All the BCC isolates were in-vitro resistant to Imipenem though 91% of them were sensitive to Meropenem. Previous studies had also indicated that Meropenem and Doripenem have greater activity in vitro against B. cepacia complex than Imipenem or Ertapenem.[22] Therefore, Meropenem or Cotrimoxazole remains the best choice for empirical therapy prior to the identification of causative micro-organisms in our set up in severe cases of clinically proven or suspected neonatal sepsis[Figure2]

Strength and limitation of the study: The strength of this study was that it had dealt with a large number of neonatal sepsis cases caused by BCC thereby being able to identify an emerging trend of the antimicrobial resistance pattern of BCC against commonly used drugs as per CLSI guidelines.

The study had its own limitation in lacking molecular confirmation because of economic constraints.

CONCLUSION

The emergence of BCC as the most common cause of late onset neonatal sepsis and its resistance to

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most of the first line antimicrobials used in therapy raises a matter of concern. Meropenem or Cotrimoxazole should be the drug of choice for empirical therapy in our set up for neonatal sepsis more so if suspected to be a hospital acquired one. The dosage and duration of parenteral drugs administered to combat the infection should further be guided by the result of anti-microbial susceptibility testing and must depend on the clinical and microbiological response to therapy. As BCC remains a notorious cause for hospital acquired infection because of its survival in disinfectants and its intrinsic and acquired resistance to multiple drugs used commonly in hospital set up to manage the gram negative bacterial infections, therefore it needs to emphasize upon differentiation between colonization and infection to use the antimicrobials judicially against the bacteria. Rational and judicial use of antibiotics along with early detection and prompt initiation of treatment holds the key. Infection control measures should be stringently followed to prevent transmission of hospital acquired infections especially in ICUs. Institutional antimicrobial policy should be adhered strictly to decide whether monotherapy or combination therapy should be preferred specifically against severe infections and in presence of underlying factors like cystic fibrosis.

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