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RESEARCH ARTICLE

MICROBIAL PROFILE AND ANTIBIOGRAM OF NEONATAL SEPTICEMIA AT A TERTIARY CARE HOSPITAL IN BANGALORE

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ABSTRACT

Introduction: Carbapenem resistance among the Enterobacteriaceae is emerging worldwide. The Carbapenem Resistant Enterobacteriaceae (CRE) are associated with high rates of morbidity and mortality particularly among critically ill patients. Resistance to the most widely used carbapenems can be mediated by a variety of mechanisms, including β-lactamases, porin changes, and changes in penicillin-binding proteins. But the most important mechanism of resistance to carbapenems is carbapenemase production. Hence the present study is conducted to detect the carbapenemase activity in Enterobacteriaceae isolates using different phenotypic methods from clinical specimens.

Objective: To compare chromogenic agar medium, the modified Hodge test, and Ertapenem +EDTA discs for phenotypic detection of carbapenemase activity in Enterobacteriacae.

Materials and methods: A total of 75 ertapenem resistant, characterized Enterobacteriaceae isolates were obtained from various clinical samples like pus(30), urine(21), sputum(11), blood(11), endotracheal tube(1), conjunctival swab(1). Isolates showing intermediate susceptibility or resistant to ertapenem by the disc diffusion method are further tested on chrom agar, by combined disc diffusion test using ertapenem and EDTA(Ethylene Di-amine Tetra acetic acid), and modified Hodge test.

Results: Of the 75 ertapenem resistant isolates tested, 40(53.3%) Klebsiella, 35(46.6%) E.coli, all isolates showed growth on
Chromagar (100%), 62 are positive for MBL production by combined disc diffusion test using ertapenem and EDTA(82.6%), 13 negative for MBL(17.3%), the Modified Hodge test detected 38(50.6%) isolates as carbapenamase producers.

Conclusion: Our findings suggest that there is a need to do surveillance to detect MBL producers. Carbapenems needs to be used judiciously so as to prevent the spread of multi drug resistance and to use effective antibiotics.

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INTRODUCTION

Neonatal septicemia is a major cause of mortality and morbidity amongst neonates in India. In India, neonatal septicemia is responsible for one-fourth to nearly half of the neonatal deaths next to perinatal hypoxia. (Gotoff and Behrman, 1970; Rajiv Aggarwal *et al*., 2001) Because of a weak immunity, neonates are more susceptible to infection. Early diagnosis of this life threatening condition is mandatory for a timely treatment and a favourable outcome (Gotoff and Behrman, 1970; Rajiv Aggarwal *et al*., 2001; Betty Chacko and Inderpreet Sohi, 2005). Neonatal sepsis is a severe clinical syndrome characterized by systemic signs of infection, shock and system organ failure; diagnosis is confirmed on positive blood culture which is the gold standard. Neonatal septicemia is categorized into two groups: *Primary* and *Secondary*, Primary neonatal septicemia is further divided into early onset septicemia (before 72hrs of life) and late onset septicemia (after 72hrs of life) for epidemiological and therapeutic purposes. (Ved Parkash Takkar *et al*., 1974) The pattern of organisms causing neonatal septicemia is constantly changing and is further compounded by the problem of frequent emergence of drug resistant bacteria. Therefore there is a need for regular bacteriological monitoring in neonatal wards.

Objective

The goal of the study was to know the microbial profile of neonatal septicemia, their antimicrobial susceptibility and drug resistance pattern.

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MATERIALS AND METHODS

Sample
Blood samples from neonates in the age group of 0-28 days presenting with one or more clinical features suggestive of septicemia were included in the present study.

Period: Between September 2012 to August 2013.

Protocol:

1)Blood culture

Blood was drawn aseptically and inoculated into a blood culture bottle containing Brain Heart Infusion broth. They were incubated at 37° C under aerobic conditions for 7 days. The first subculture was done after 24 hours of incubation, the second on the third day and a final on the seventh day onto Chocolate agar, 5% sheep blood agar and MacConkey agar plates aerobically at 37° C for 24 hours, and the plates were observed for growth. The growth was identified by colonial characteristics, gram's stain and standard biochemical tests. Cultures which did not yield any growth following three subcultures were reported negative at the end of 7 days.

2) Antibiotic susceptibility and resistance testing

The antimicrobial susceptibility testing was performed by Kirby–Bauer disc diffusion method and zone size interpreted as per CLSI guidelines (2013) -The colonies were also tested with a 30 µg cefoxitin disc in a Mueller Hinton plate for methicilin resistance

I. D-test was done by placing 15-µg erythromycin disk and 2-µg clindamycin disk spaced 15–26 mm apart. Flattening of the zone of inhibition adjacent to the erythromycin disk or any hazy growth within the zone of inhibition around clindamycin was considered MLS-Bi phenotype.
II. Extended spectrum beta lactamase screening and confirmation was done by using Ceftazidime 30µg and Cefotaxime 30µg by disk diffusion method as per CLSI criteria[5]
III. Metallo beta lactamase detection was done by combined disc diffusion test with Meropenem (10 µg) and EDTA (10µL of 0.1 M)

RESULTS

Total of 1129 blood samples studied out of which, 263 (23.29%) were blood culture positive. Among them, 161 (61.2%) were males and 102 (38.7%) were females.182 (69.3%) were preterm. Early onset septicemia was more common, seen in 60.9% of cases than late onset septicemia (39.1% cases). Gram negative organisms were predominant in 58.5% of cases than Gram positive organisms in 28.5%. *Klebsiella species* and *Escherichia coli* were the commonest organisms isolated in 50 % and 14.8% of cases respectively. Staphylococcus aureus was the major gram positive bacteria with 70 (78%). Among them 48 (68%) were MRSA and 22(31.4%) were MSSA. Among MRSA, 14(29.3%) and among MSSA, 4 (18.18%) were MLS-Bi. 18 (37.5%) and 5 (22.7%) showed constitutive resistance among MRSA and MSSA respectively. Among CONS 5 were Methicillin resistant and 14 were methicillin sensitive. 1 isolate showed MLS-Bi phenotype and 2 showed MLSB-c phenotype. 22 (8.3%) were Candida isolates among which 21(95.4%) were non albicans Candida species. Majority of gram negative isolates were susceptible to ceftazidime, piperacillin tazobactum followed by ciprofloxacin and Gram positive isolates were 100% susceptible to vancomycin and showed high resistance to Penicillin, erythromycin, ciprofloxacin. 51 (66.1%) and 32(41.6%) of Klebsiella isolates showed ESBL and MBL production respectively, majority of which were from early onset sepsis cases. Inducible clindamycin resistance was noted in 12.4%

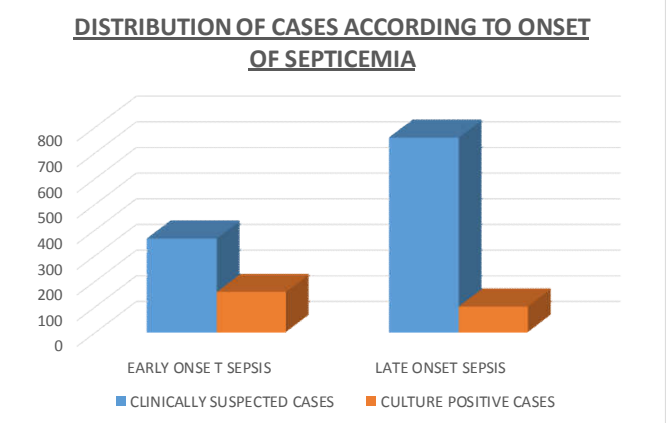

Figure 1.

Table 1. The total spectrum of isolates with EOS and los cases VS clinically suspected and culture positive cases

Diagnosis	Clinically suspected cases no (%)	Culture positive cases no (%)
Early onset septicemia	368(32.6)	160(60.9%)
Late onset septicemia	761(67.4%)	103(39.1%)
Total	1129(100)	263(100)

Table 2. The total positive and negative blood culture cases

Blood culture	Number of patients	%
Positive	263	23.3
Negative	866	76.7

DISCUSSION

Neonatal septicemia is a major cause of mortality and morbidity in neonates in the developing countries. The disease continues to pose a challenge to the pediatricians in making a definitive clinical diagnosis due to the subtle and non-specific signs and symptoms; hence laboratory diagnosis plays a major role. Definitive diagnosis rests on a positive blood culture, to identify the pathogen and determine its antibiotic susceptibility pattern. In the present study maximum culture positive cases were seen in neonates less than 72 hours of age (early onset septicemia) as compared to neonates aged more than 72 hours of life (late onset septicemia). Majority of the cases were preterm.

Table 3. The total spectrum of isolates with Early Onset Sepsis and Late Onset Sepsis cases

Isolates	Culture positive Early Onset Sepsis (%)	Culture positive Late Onset Sepsis (%)	Total No (%)
Klebsiella pneumoniae	66(25.1)	11(4.1)	77(29.2%)
Escheichia coli	14(5.3)	9(3.4)	23 (8.7)
Pseudomonas aeruginosa	---	13 (4.9)	13 (4.9)
Citrobacter species	12(4.7)	6(2.2)	18 (6.9)
Gram negative non fermenters	8(3)	13 (4.9)	21 (7.9)
Staphylococcus aureus	49(18.6)	21(8.2)	70 (26.8)
Coagulase negative Staphylococci	8(3.2)	11(4.2)	19 (7.4)
Non Albicans Candida species	3(1.1)	18(6.8)	21 (7.9)
Candida albicans	---	1(0.3)	1 (0.3)
Total	160 (60.9)	103 (39.1)	263 (100)

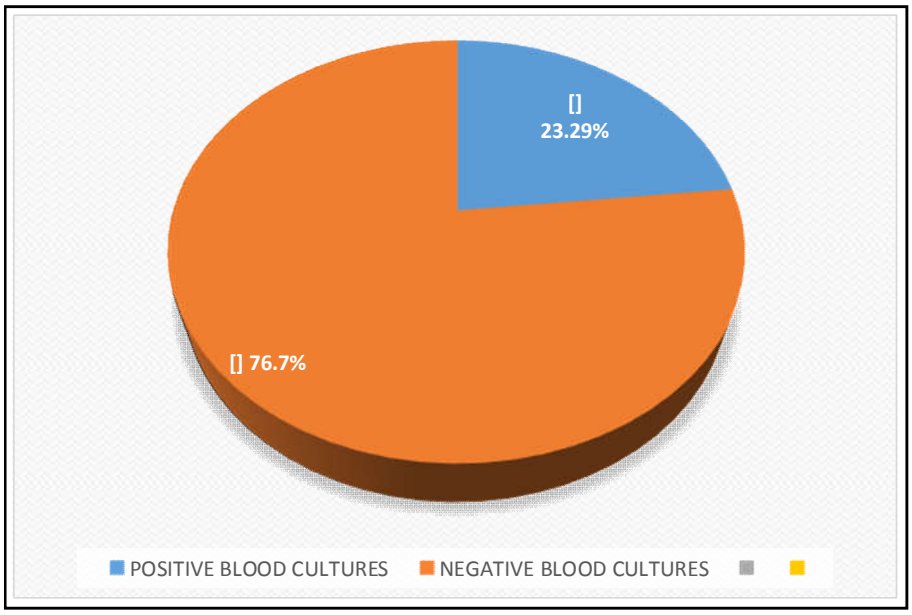

Figure 2. Distribution of positive and negative blood cultures

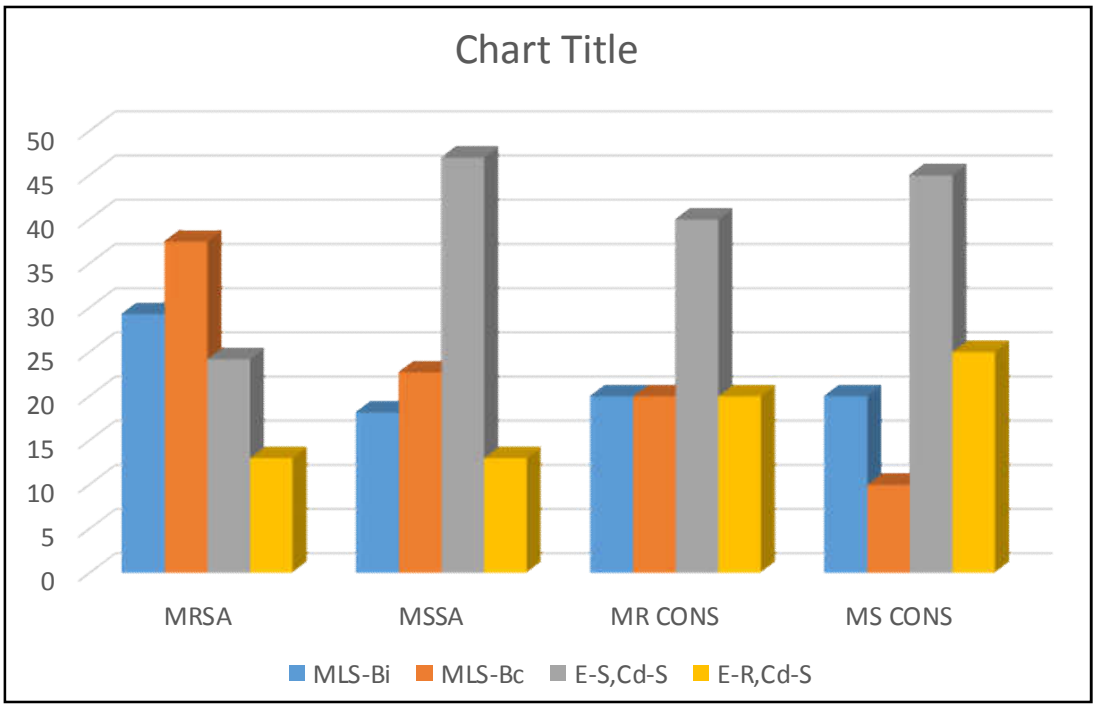

Figure 3. MRSA, MSSA, MR CONS and MS CONS with inducible

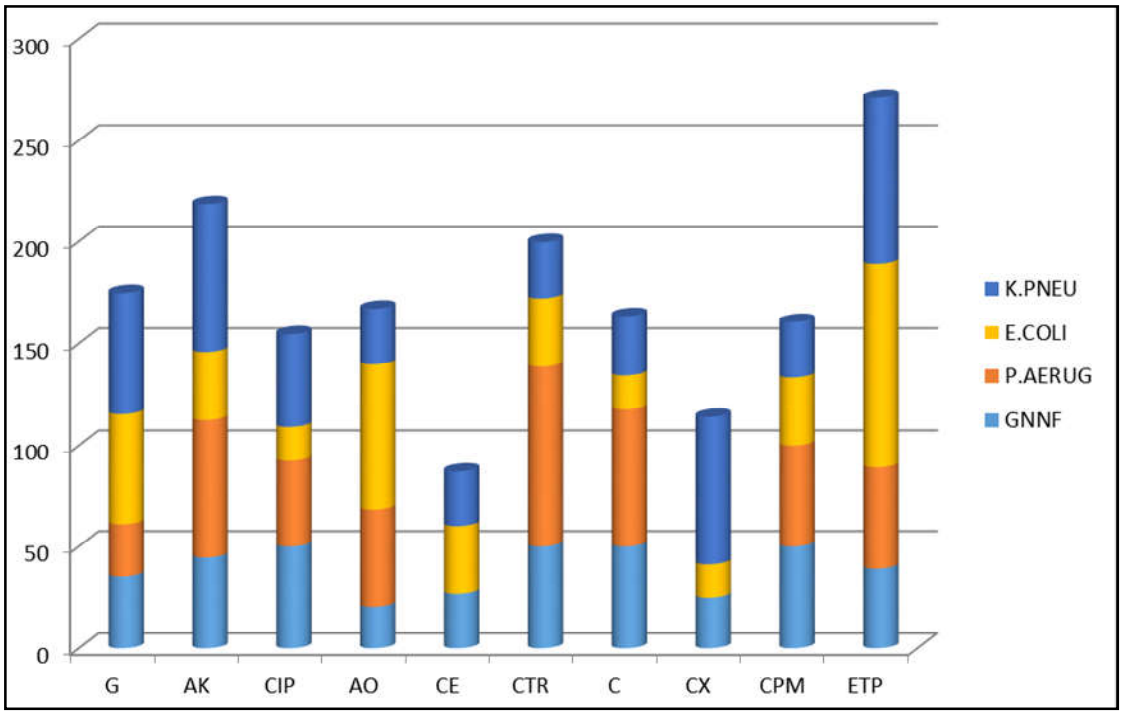

Figure 4. Sensitivity pattern of Gram negative isolates

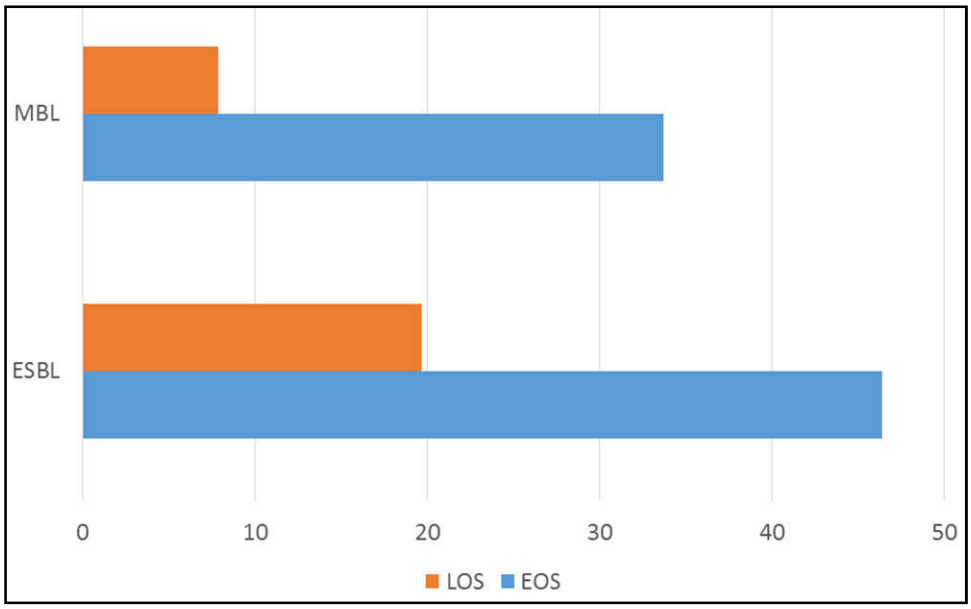

Figure 5. ESBL and MBL detection among Klebsiella isolates in early and late onset sepsis

Gram negative organisms are the predominant causative agents with Klebsiella pneumonia (50%) as leading cause of septicemia. Staphylococcus aureus (14.8%) is the commonest Gram positive organism causing neonatal septicemia. The study highlights that ESBL and MBL incidence is increasing. It also highlights the emergence of non albicans Candida species as a cause of neonatal sepsis.

Conclusion

Spectrum of the causative agents of neonatal septicemia and their antibiotic susceptibility patterns are constantly changing from time to time. Also there is emergence of non Albicans Cnadida species as causative agent for neonatal sepsis. There is also emergence of ESBL and MBL cases due to indiscriminate antibiotic use. Periodic review of cases to assess changing trends in the infecting organisms and their antimicrobial susceptibility is important.

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