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Research Article

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Multiple drug resistance (MDR) and phenotypic detection of extended spectrum β-lactamases (ESBL) and metallo β-lactamases (MBL) producing enterobacteriaceae isolates of neonatal sepsis

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ABSTRACT

Neonatal sepsis is one of the major causes of morbidity and mortality in newborns. In developing countries, multiple drug resistant (MDR) organisms causing neonatal sepsis are threat to current β lactam therapy leading to treatment failure. Emergence of ESBLs and MBLs are a vital factor in the treatment of infections associated with sepsis. This study aims at phenotypic detection of Extended spectrum β-lactamases (ESBL) and Metallo β-lactamases (MBL) producing Enterobacteriaceae isolates of Neonatal Sepsis and to assess the burden of MDR. Total 19 clinical isolates of Enterobacteriaceae were isolated from 115blood samples of suspected cases. Antimicrobial susceptibility was determined by Kirby–Bauer's disk diffusion method. Isolates were screened for ESBL and MBL production by Clinical and Laboratory Standards Institute (CLSI) disk method, and confirmation was done by CLSI phenotypic disk confirmatory test. Out of 115 cases, 19 (17%) were culture positive. Among them, 13(68.4%) were Klebsiella pneumoniae, 4(21%) were Escherichia coli and 2(10.5%) were Citrobacter species. Most of the isolates of Escherichia coli and Klebsiella pneumoniae were resistant to more than two drugs. Citrobacter spp. were resistant to all the drugs except Amikacin. Among 19 isolates, 3(15.7%) of Escherichia coli isolates were ESBL producers and 2(15.3%) of Klebsiella pneumoniae isolates were MBL producers. Continued monitoring of antibiotic susceptibility pattern and routine detection of ESBL and MBL is required in hospitals and private laboratories. The resistance pattern and early detection of ESBL and MBL producing isolates would be important for reducing neonatal morbidity and mortality rates.

Keywords: ESBL, MBL, Enterobacteriaceae, Neonatal sepsis, antibiotic resistance

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INTRODUCTION

Sepsis is the commonest cause of neonatal morbidity and mortality and is responsible for 30-50% of total neonatal deaths each year in developing countries[1].The pattern of pathogens causing neonatal sepsis has been constantly changing. Compounding the problem is the frequent emergence of resistant pathogens in the nurseries[2].

During the past decade, ESBL producers have frequently been implicated in neonatal intensive care units. Recent study involving four different centers in India showed that *Klebsiella species* and *Escherichia coli* are the most common GNB causing neonatal sepsis and one-third are ESBL producers in both community and hospital settings and is associated with very high mortality rate (33%) in these patients[3].

Recently, metallo β-lactamases (MBLs) have emerged as one of the most fearful resistance mechanisms due to their ability to hydrolyze all β-lactam antibiotics including carbapenems. Its spread on highly mobile gene elements limits the therapeutic options[4]. It has thus become essential to be alert about the trend in antibiotic susceptibility patterns

of pathogens to save the therapy. Therefore, this study aims at phenotypic detection of ESBL and MBL producers in Enterobacteriaceae isolates of neonatal sepsis and to assess the burden of multiple drug resistance.

EXPERIMENTAL SECTION

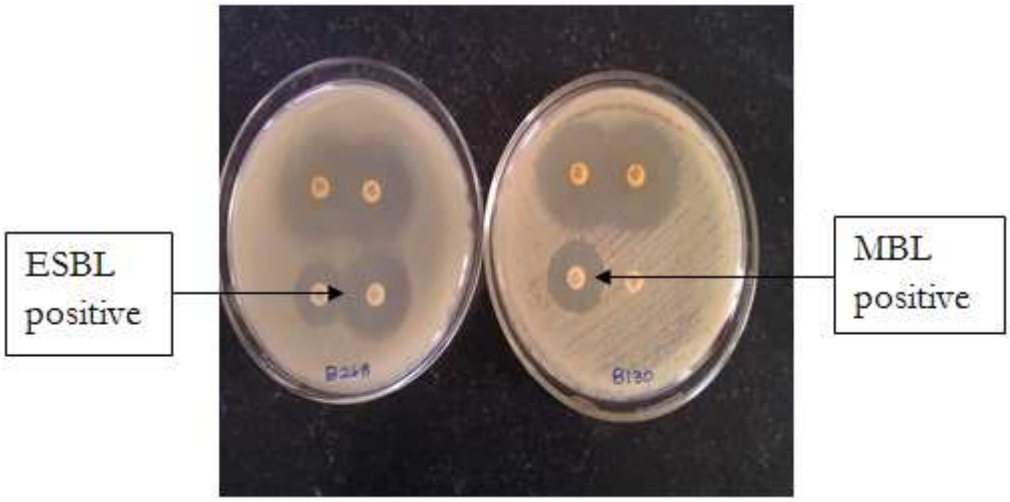
This study was conducted from May 2013 to March 2014 in Microbiology Department of BLDEU's Shri B. M. Patil Medical College, Hospital and Research Center, Bijapur, after obtaining due approval from the institutional ethical committee. A total of 115 blood samples were collected during the study.

Briefly, 1–2 ml of blood was collected for culture into 10 ml of Brain heart infusion broth with sodium polyanetholsulphonate. These broths were incubated at 37°C under aerobic conditions for 7 days and observed for the growth of organisms(turbidity). Any sign of growth after 24 hours was followed by sub-culture on MacConkey's agar and blood agar plates (HiMedia Laboratories, Mumbai) and identified using standard microbiological techniques. If no growth observed then subcultures were repeated on day 4 and 7.Antibiotic susceptibility was determined by Kirby- Bauer disc diffusion method using Clinical and Laboratory Standard Institute (CLSI) guidelines (2011).Antibiotic disc were purchased from HiMedia Laboratories, Mumbai. Quality control was achieved by using standard strain of *E. coli* ATCC 25922. Isolates resistant to the third generation cephalosporins were tested for ESBL production and isolates showing resistance to imipenem were tested for MBL production.

Detection of ESBL: This was performed by phenotypic confirmatory test as per the 91 recommendations of CLSI. The ceftazidime(30 g) discs alone and in combination with clavulanic acid (ceftazidime + clavulanic acid, 30/10 g discs) were used. An increase of 5mm in zone of inhibition of the combination discs in comparison to the ceftazidime disc alone was considered to be ESBL producer (Fig 1).

Detection of MBL: This was performed by Imipenem EDTA combined disc test. Two (10 g) imipenem discs were placed on a plate inoculated with the test organism, and 10 µl of 0.5 MEDTA solution was added to one disc. A zone diameter difference between the imipenem and imipenem + EDTA of 7 mm was interpreted as a positive result for MBL production(Fig 1).

Fig 1 ESBL and MBL producers



RESULTS AND DISCUSSION

Among 115 cases, 19 were culture positive (Table 1). Out of 19 isolates that belonged to Enterobacteraceae, 13(68.4%) were *Klebsiella pneumoniae*, 4 (21%) were *Escherichia coli* and 2 (10.5%) were *Citrobacter species.*

The worldwide emergence of multi-drug resistant bacteria is a growing concern and they are usually found in hospitals where antibiotic use is frequent and the patients are in critical condition[5].

In the present epoch, the emanation and spread of MDR organisms in the community is of great concern. Infections by MDR organisms lead to prolonged hospitalization, increased mortality and morbidity[6]. As per the definition of CDC, isolates that were resistant to two or more of the most commonly used antimicrobial classes for the treatment were placed in MDR category[7].Isolates exhibiting co-resistance to at least any two of the following drugs were considered as MDR and these drugs were: Third generation cephalosporin (cefixime/ceftriaxone/ceftazidime), an

aminoglycoside (amikacin), a fluoroquinolone (ciprofloxacin), and a folate pathway inhibitor (co-trimoxazole). In our study, 3 isolates of *E.coli*, 11 *K. pneumoniae* and 2 *Citrobacter spp* were found to be MDR (Table 2). Various authors have reported high percentage of MDR in their study[3,8,9].Our findings are in concordance with them.

Table 1: Enterobacteraceae isolates

Total cases	Culture positive Enterobacteraceae isolates
115	19(17%)

Table 2: Antimicrobial resistance pattern of the isolates

	E.coli (n=4)	K. pneumoniae (n=13)	Citrobacter sp (n=2)
Ampicillin	3(75%)	13(100%)	2(100%)
Amoxyclav	3 (75%)	13(100%)	2(100%)
Sparfloxacin	4(100%)	10(76.9%)	2(100%)
Cefuroxime	3(75%)	13(100%)	2(100%)
Ceftazidime	3(75%)	10(76.9%)	2(100%)
Gentamicin	2(50%)	12(92.3%)	2(100%)
Cotrimoxazole	2(50%)	5 (38.4%)	2(100%)
Ciprofloxacin	4(100%)	12 (92.3%)	2(100%)
Cloxacillin	4(100%)	13(100%)	2(100%)
Amikacin	1(25%)	11 (84.6%)	0
Lomefloxacin	3(75%)	12 (92.3%)	2(100%)
Ofloxacin	3(75%)	13(100%)	2(100%)

Table 3:ESBL and MBL producers among different isolates

Isolates	No. of isolates	ESBL producers	MBL producers	ESBL + MBL producers
Klebsiella pneumoniae	13	-	02(15.3%)	-
Escherichia coli	04	03(75%)	-	-
Citrobacter species	02	-	-	-
Total	19	03(15.7%)	02(10.5%)	-

Among the 19 Enterobactereceae isolates in the present study, ESBL producers were 3 isolates of *Escherichia coli*(50%)(Table 3) which are similar to that of Nachimuthu Ramesh *et al*[10] and Kumar *et al* [11] who reported a high prevalence of ESBLs among *E.coli*.

Metallo- -lactamases (MBLs) are enzymes belonging to Amblers class B that can hydrolyze a wide variety of -lactams, including penicillins, cephems, and carbapenems except aztreonam[12,13].Although, PCR method though simple, but it has become more difficult with the increased number and types of MBL[14]. Combined disc test is simpler and highly sensitive in detecting MBL isolates[15].Our study showed MBL production in 2 isolates in *Klebsiella* spp. (15.3%) as shown in table 3, which is consistent with the study of Nirav Pandya et al[16].

Coproduction of ESBL and MBL was not present. Citrobacter species were neither ESBL nor MBL producers.

CONCLUSION

Continued monitoring of antibiotic susceptibility pattern and early detection of ESBL and MBL is required for the reduction of neonatal mortality rates and to avoid the intra hospital dissemination of such strains. The rapid and convenient method for their detection is by the phenotypic screening tests, which should be routinely used in all the hospitals and laboratories. Depending on the antibiotic sensitivity pattern of the isolates, antibiotics should be used, and infection control measures should be strictly followed.

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