

Original Research Article

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Prevalence of Metallo Beta Lactamase Production among Bacterial Isolates in Cases of Neonatal Septicemia in HAHC Hospital, Delhi, India

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ABSTRACT

Neonatal septicemia is responsible for nearly half of neonatal deaths. With emerging drug resistance mechanisms like Metallo Beta lactamase (MBL) production, treatment of neonatal sepsis has become a challenge. The present study was done to identify prevalence of bacterial pathogens, their resistance profile and incidence of MBL mediated resistance among Gram negative bacterial isolates in neonatal sepsis. Seventy three cases of suspected neonatal septicemia were included in this prospective study. Blood culture was done by BacT/Alert 3D system, bacterial isolates were identified. All Imipenem-resistant isolates were screened for the production of MBL by the Imipenem-EDTA disc method and by Imipenem with and without EDTA (IPM+EDTA/IPM) Ezy MIC E Strips test. Of 73 blood samples, 42(57.5%) showed bacterial growth. Of those, 23 were Gram positive and 19 were Gram negative. The most common pathogen was *Staphylococcus aureus*. Most common Gram negative isolate was *Acinetobacter spp* (42%). 9(47.3%) Gram negative isolates were resistant to Imipenem. Of these, 8(88.8%) showed expanded growth inhibition zone when subjected to Imipenem-EDTA disk synergy (EDS) test indicating positive MBL production and were also positive for MBL production by Ezy MIC E Strips test. The present study indicated that although Gram-positive species continue to be the predominant causative organisms in neonatal sepsis, Gram negative bacteria have a special significance due to high prevalence of Carbapenem resistance and MBL production. High prevalence of drug resistance is a matter of concern as it leaves the physician no or few options for antimicrobial therapy.

Keywords

Metallo beta lactamase, Neonatal septicemia, Gram negative bacteria

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Introduction

Bacterial sepsis is one of the major causes of morbidity and mortality among neonates. It is characterized as generalized bacterial infection indicated by positive blood culture in the first four weeks of life (Agarwal *et al.*, 2001). The common causative agents for neonatal sepsis are *Staphylococcus spp*, *Enterococci spp*, *E. coli*, *Klebsiella spp*, *Enterobacter spp*, *Pseudomonas aeruginosa* and *Acinetobacter spp* (Roy *et al.*, 2002).

Metallo-beta-lactamase is an enzyme that makes bacteria resistant to a broad range of beta-lactam antibiotics including Carbapenem family. The spread of these enzymes severely limits therapeutic options for infections by pathogens (Peleg *et al.*, 2005).

Evaluation of antibiotic resistance profile and demonstration of evidence of specific resistance like MBL in the prevalent organisms is beneficial for starting early and

appropriate treatment in conditions like neonatal sepsis. The aim of this study was to investigate predisposing factors associated with cases of neonatal septicemia. To identify common organisms and their antibiotic resistant pattern causing septicemia in neonates and to estimate prevalence of carbapenem resistance. Also to establish metallo β -lactamases production by carbapenem resistance isolates.

Materials and Methods

Study design

This prospective study was conducted between 1st June to 31st July 2014 at Department of Microbiology, Hakeem Abdul Hamdard Centenary (HAHC) Hospital, Jamia Hamdard, New Delhi.

Subjects

All clinically suspected cases of neonatal septicemia admitted to HAHC Hospital from 1st June 2014 to 31st July 2014 were included in this study. All information related to birth including weight, sex and any risk factors were collected and tabulated. A duly filled and signed consent form was obtained from the parents of every neonate involved in this study. Ethical clearance was obtained from Institute Ethics Committee, Jamia Hamdard University.

Exclusion criteria

Neonates with any other obvious foci of infection (like culture positive UTI, boils, any other skin infection) were excluded from the study.

Sample collection and procedure

2 ml of blood was collected from each patient using proper aseptic precautions and inoculated immediately into BacT/Alert

culture bottles and incubated for 5 days. Growth on solid medium after subculture was identified on the basis of staining and standard biochemical tests. Antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion method (Bauer *et al.*, 1966) according to CLSI guidelines (Clinical and Laboratory Standards Institute, 2012).

All isolates that showed imipenem resistance were further subjected to species confirmation and MIC detection by VITEK-2 system (Funke *et al.*, 1998). All imipenem-resistant isolates were screened for the production of MBL by the imipenem-EDTA disc method (7) and by Ezy MIC E Strips test.

Statistical tests

Chi square test was used to detect statistically significant correlation among variables. Significance defined as $P \leq 0.05$.

Results and Discussion

Out of the 73 blood samples, 42(57.5%) showed bacterial growth. Out of the 42 culture positive cases, 23 isolates were gram positive and 19 were gram negative (Table 1). In the present study; prematurity (78%), respiratory distress (88%) and obstetric factors (Home delivery, Meconium stained amniotic fluid) were present as risk factors (76-85%) (Table 2).

The most common pathogen was *Staphylococcus aureus*. Most common Gram negative organism isolated was *Acinetobacter spp* (42%). 9(47.3%) Gram negative isolates were found to be resistant to Imipenem. Of these Imipenem resistant isolates, 8(88.8%) showed expanded growth inhibition zone when subjected to Imipenem-EDTA disk synergy (EDS) test (Figure1) indicating positive MBL production and were also positive for MBL production by Ezy MIC E Strips test (Table 3 and Figure 2).

Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteraemia in the first month of life (Sankar *et al.*, 2008). Administration of empirical antibiotics and causes other than bacteria can also result in poor recovery of the bacterial pathogens in culture. In a study done in

neonatal intensive care units of Georgia, 63% of the clinically suspected cases were blood culture positive. In the present study the blood culture positivity rate was 57.5%. However, in other studies from India, the culture positivity rate was 13–22% (Iregbu *et al.*, 2006; Kaistha *et al.*, 2009).

Table.1 Organisms isolated from positive blood cultures

S.No.	Organism isolated	N	Percentage
1	<i>S aureus</i>	17	40.47%
2	Coagulase negative <i>Staphylococcus</i>	5	13.15%
3	<i>Enterococcus</i>	1	2.63%
4	<i>Acinetobacter</i>	8	19.04%
5	<i>Pseudomonas</i>	6	14.28%
6	<i>E coli</i>	3	5.26%
7	<i>KlebsiellaSpp</i>	2	4.76%
Total		42	

Table.2 Predisposing factors and Comorbidity among culture positive neonates and among MBL producers

SNo	Predisposing Factor	Culture positive(n=42)	MBL positive isolates (n=8)
Obstetric Factors			
1.	Preterm Labour	0	0
2.	Fever	0	0
3.	Home delivery	32(76.1%)	8(100%)
4.	Institutional delivery	10(23.8%)	0
5.	Cesarian section	23(54.7%)	8(100%)
6.	Muconium stained amniotic fluid	36 (85.7%)	6(75%)
Neonatal Factors			
1.	Prematurity	33(78.5%)	8(100%)
2.	RDS	6(14.2%)	2(25%)
3.	MAS	12(28.5%)	6(75%)
4.	Fever	25(59.5%)	8(100%)
5.	Respiratory distress	37(88%)	8(100%)

Table.3 Details of MIC E strips of Carbapenem resistant strains

S.no	Organism	MIC Imipenem (µg/ml)	Ezy MIC E Strips test		Calculation	
			MIC -IPM (µg/ml)	MIC-IPM+EDTA (µg/ml)	$\frac{\text{MIC -IPM}}{\text{MIC-IPM+EDTA}}$	Inference-MBL Production
1	<i>Pseudomonas spp</i>	≥ 32(R≥ 8)	48	4	12	+
2	<i>Pseudomonas spp</i>	≥ 32(R≥8)	No zone	4	∞	+
3	<i>E. coli</i>	≥ 16(R≥4)	No zone	4	∞	+
4	<i>Acinetobacter spp</i>	≥ 64(R≥16)	64	3	21.33	+
5	<i>Pseudomonas spp</i>	≥ 16(R≥8)	32	4	8	+
6	<i>Acinetobacter spp</i>	≥ 32(R≥16)	48	2	24	+
7	<i>Acinetobacter spp</i>	≥ 16(R≥16)	No zone	No zone	-	Resistance due to any other mechanism
8	<i>Acinetobacter spp</i>	≥ 32(R≥16)	48	2	24	+
9	<i>Acinetobacter spp</i>	≥ 32(R≥16)	No zone	3	∞	+

Figure.1 Imipenem-EDTA disk synergy (EDS) test

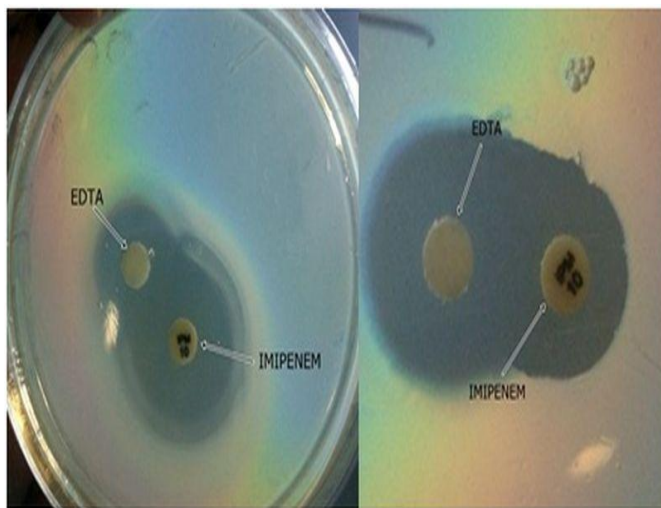
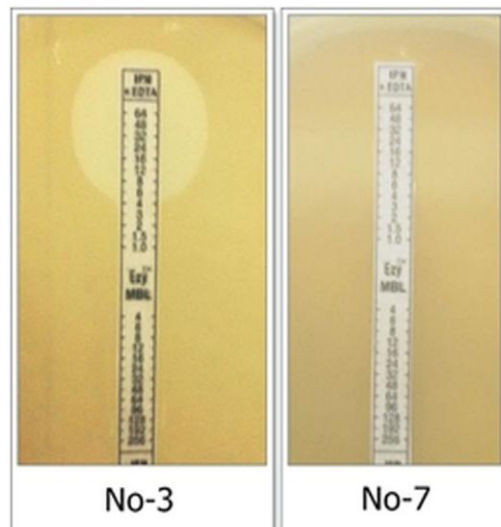


Figure.2 IPM+EDTA/IPM Ezy MIC E Strips test



A male predominance was present in our study. This was in agreement with several other studies (Ahmed *et al.*, 2002; Joshi *et al.*, 2000; Dutta *et al.*, 2010).

Bacterial flora causing neonatal sepsis continues to change with time (Ho, 1992). Significant proportions (50%-37%) of EONS due to gram positive organisms were reported from recent studies (Baltimore *et al.*, 2001). In accordance, 54.7% of isolates were gram positive species in the present study. The rate of *S. aureus* infection in the present study was 40.47%. Reports with rate of infection varying from 3.7%- 7% have been found previously (Aurangzeb *et al.*, 2003; Aurangzeb *et al.*, 2001). However, Karthikeyan (18), in their analysis identified *S. aureus* as a predominant pathogen (50% of EONS). A low rate of *Enterococci* infection of present study (2.63%) is similar to the observations of Dobson and Baker (1990).

Developing countries have identified *E. coli* (Kuruvilla *et al.*, 1998) and *Klebsiella* as the most common causative organism. *E. coli* infection was present in 5.26% and that of *Klebsiella* infection was 4.76%. *Pseudomonas* was higher at 14.28%. Similar observations were reported by Joshi *et al.*, (2000) and Tallur *et al.*, (2000). In contrast, we identified *Acinetobacter* species as the predominant isolate (42.1%) among Gram negative isolates similar to that by Agarwal *et al.*, (2014) who reported a rate of 41.6%.

Over the last decade there have been several articles summarizing the levels of MBLs in the bacterial community (Lee *et al.*, 2003). We observed a high rate of prevalence of Carbapenem resistance (47%); a similar high rate was observed in a study from Tanzania (35%) (Mushi *et al.*, 2014). A slightly lower rate of 24.1% of Carbapenemase production was noted in another study (Mushi *et al.*, 2013).

We found that 88% of the gram negative bacteria that were resistant to carbapenems were attributable to the MBL production. Wattal *et al.*, (2010) reported prevalence of resistance to carbapenems ranging 13 to 15% in *E. coli* and *Klebsiella* spp from ICUs and wards from a tertiary care hospital in Delhi. A similar high rate (68.4%) of MBL production was reported by Vinod *et al.*, (Wattal *et al.*, 2010). MBL production has been reported as high as 70.8% from North India (43). We further confirmed our findings with the help of IPM+EDTA/IPM Ezy MIC E Strip (HiMedia, Mumbai) which showed similar results. The remaining imipenem resistant isolates may have other mechanisms of resistance such as reduced levels of drug accumulation or increased expression of pump efflux; this may be confirmed by genetic analysis.

In the present study; prematurity (78%), respiratory distress (88%) and obstetric factors (Home delivery, Meconium stained amniotic fluid) were present as risk factors (76-85%). Schuchat *et al.*, (2000) found an obstetric risk factor-preterm delivery, intrapartum fever, or membrane rupture \geq 18 hours in 49% of GBS. Tallur *et al.*, (2000) reported association of PROM $>$ 24 hours in 14% and perinatal asphyxia in 22%. Association of meconium stained amniotic fluid with sepsis was identified by Kuruvilla *et al.*, (1998). Agarwal *et al.*, (1990) found EONS more frequently in neonates with perinatal asphyxia.

In the past 3 to 4 years many new transferable types of MBLs have been studied and appear to have rapidly spread. Moreover, given that MBLs will hydrolyze virtually all classes of β -lactams and that we are several years away from the implementation of a therapeutic inhibitor; their continued spread would be a clinical catastrophe.

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