

Metallo-beta-lactamase Producing *Pseudomonas aeruginosa* in Neonatal Septicemia

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ABSTRACT

Gram-negative bacilli are important agents causing neonatal sepsis. The organisms isolated are often resistant to multiple antimicrobials specially which are metallo-beta-lactamases (MβL) producers. Therefore, the present study was conducted with the objective to examine the incidence of MβL producing strains in multidrug resistant (MDR) *Pseudomonas aeruginosa* from cases of neonatal sepsis. Between January-December 2006, 1994 cases of neonatal sepsis were investigated. The isolates obtained were identified and tested for susceptibility to various antimicrobial agents. The multidrug resistant *P. aeruginosa* isolates were screened for the presence of MβL by imipenem-EDTA disc method. Five hundred and ninety three (29.73%) isolates were obtained from culture of neonates. Most frequent offender was *P. aeruginosa* (48.2%). There was an overall predominance of gram-negative organisms. MβL production was seen in 69.5% of imipenem-resistant *P. aeruginosa* isolates. MβL producing *P. aeruginosa* is an emerging threat in neonatal septicemia and a cause of concern for physicians treating such infections.

Keywords: MβL, neonatal sepsis, *Pseudomonas*

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INTRODUCTION

Septicemia is a clinical syndrome associated with considerable morbidity and mortality. In India, neonatal septicemia is responsible for one-fourth to nearly half of the neonatal deaths next to perinatal hypoxia.^[1] Infections with *Pseudomonas aeruginosa* is usually late in onset, nosocomial in origin and epidemic in pattern. *P. aeruginosa*, exhibit intrinsic resistance to various antimicrobial agents including β-lactam antibiotics. In recent years, there has been an increase in carbapenem resistance which is acquired metallo-beta-lactamases (MβL) and reported mainly for *P. aeruginosa* and *Acinetobacter* spp. in several countries. Therefore, *P. aeruginosa* must be considered in all neonatal infections, regardless of the age of onset, so that early, appropriate and often life-saving antibiotic therapy may be instituted.^[2] Thus, the present study was designed to know the incidence of neonatal septicemia, sepsis caused by *P. aeruginosa*, their antibiotic susceptibility pattern and to detect the presence of MβL among the imipenem-resistant isolates of *P. aeruginosa*.

The present study was undertaken during January–December 2006 at Pt.B.D.S, PGIMS, Rohtak

(Haryana). A total of 1994 blood cultures were received in the Microbiology Department., from clinically suspected cases of neonatal septicemia. The blood culture bottles were incubated at 37°C for 7 days. Subcultures were done first at 24 h, then at 48 h, 72 h and on the seventh day onto the blood agar and MacConkey's agar plates. Organisms isolated were identified by standard methods of identification.^[3] *P. aeruginosa* were Gram-negative bacilli, strictly aerobic, oxidase positive, motile, with positive arginine dihydrolase reaction, growth at 42°C and able to reduce nitrates to nitrogen gas. Antibiotic sensitivity tests of the isolates were performed by the Kirby Bauer disc diffusion method for commonly used antibiotics.^[4] All the multi-drug-resistant(MDR) isolates of *P. aeruginosa* were then tested for the sensitivity to imipenem (10μg, HiMedia) and the imipenem-resistant isolates were screened for the production of MβL by the imipenem-EDTA disc method as described by Yong *et al.*^[5] The inhibition zone with imipenem-EDTA disc were <14 mm for MβL-negative isolates and >17 mm for MβL-positive isolates considering the inhibition zone with imipenem to be 6-16 mm.

Of the 1994 blood cultures from neonates, 593(29.73%) showed bacterial growth. Most cases were detected in the first 10 days of life (77.90%). Gram-negative septicemia was encountered in 81.28% cases; Gram-positive cocci were isolated in 18.71% cases. *P. aeruginosa* (48.22%) was the predominant pathogen [Table 1] in Gram-negative isolates, whereas coagulase negative Staphylococci (CoNS) was the most common among Gram-positive cocci. *P. aeruginosa* was mostly resistant to gentamicin(92.3%), amikacin(91.2%), and piperacillin (88.8%). One hundred and eighty two (63.6%) isolates of *P. aeruginosa* were found to be MDR. Of these, 113(62%) were imipenem-sensitive and 69(37.9%) were imipenem resistant. MβL production was observed in 69.5% of imipenem-resistant isolates.

Blood culture is the most important investigation to confirm the diagnosis of neonatal septicemia. In the present study, the incidence of septicemia was 29.73%. This was comparable to 30-75% positivity reported in earlier studies.^[6,7] Most of the cases detected by blood culture occurred in the first 10 days of life (77.90%), a fact that has been reported previously and probably relates to immaturity of the immune system. This warrants the need for close monitoring of the newborns.

A rising incidence of Gram-negative bacteremia has been reported in recent years in neonates.^[6,7] The present study isolated 81.28% of Gram-negative bacilli with *P. aeruginosa* (48.22%) as the predominant pathogen. *P. aeruginosa* has also been reported to be the most common etiological agent of neonatal septicemia by other workers.^[8-10]

Almost all Gram-negative organisms showed resistance to chloramphenicol (70-95%) and gentamicin (50-95%). Reduced chloramphenicol sensitivity (20-44%)^[11,12] and gentamicin sensitivity (23-30%)^[13] have been documented by other workers. The overall resistance rate of *P. aeruginosa* to all antimicrobial agents in our study was also significant and accounted for 62% MDR isolates which correlates with the study by Moniri and colleagues (73.9%).^[10] Multi-drug resistance caused by a variety of resistance mechanisms implies that there are few therapeutic options. Carbapenems are often used as antibiotics of last resort against this organism. Our study showed 37.9% imipenem resistance which is in concordance with Sarkar *et al* (36.36%).^[14]

With increasing the use of carbapenems in hospital settings, the problem of MβL production is also increasing. In our study, 69.5% isolates were found to be MβL producers. This is an emerging threat and a matter of concern for treating physicians. The remaining imipenem resistant isolates may have other mechanisms of resistance such as reduced levels of drug accumulation or increased expression of pump efflux.

To conclude, *P. aeruginosa* is an important Gram-negative bacilli which cause neonatal septicemia and should be borne in mind while dealing with such cases. The MDR strains can cause considerable morbidity and mortality. The study also highlights that MβL incidence is increasing in our region. The wide spread occurrence of MβL producing *P. aeruginosa* isolates poses a great therapeutic problem. The resistance may spread rapidly to various species of Gram-negative

Table 1: Antimicrobial resistance pattern of various organisms

Drugs	<i>Pseudomonas</i> (n-286)	<i>Acinetobacter</i> (n-61)	<i>Enterobacter</i> (n-58)	<i>Citrobacter</i> (n-32)	<i>Klebsiella</i> (n-31)	<i>E.coli</i> (n-14)	CoNS (n-43)	<i>S.aureus</i> (n-35)	<i>Enterococci</i> (n-33)
Chloramphenicol	–	82.0	88.0	93.7	71.0	78.6	48.8	34.2	60.0
Gentamicin	92.3	77.1	88.0	93.7	80.7	50.0	25.6	51.4	68.5
Norfloxacin	–	77.1	77.6	75.0	77.5	64.3	–	–	–
Amikacin	91.2	82.0	63.8	68.8	64.6	14.3	–	–	–
Cefotaxime	–	46.0	77.6	62.5	80.7	35.8	–	–	–
Ciprofloxacin	76.2	55.8	62.1	65.7	64.6	57.2	–	–	–
Ceftizoxime	33.6	41.0	46.6	28.2	38.8	7.2	–	–	–
Ceftriaxone	–	65.6	88.0	62.5	90.4	42.9	–	–	–
Ceftazidime	59	–	–	–	–	–	–	–	–
Cefazolin	–	–	–	–	–	–	30.2	45.7	88.5
Cefuroxime	–	–	–	–	–	–	16.2	40.0	62.8
Erythromycin	–	–	–	–	–	–	39.5	51.4	42.8
Clindamycin	–	–	–	–	–	–	6.9	45.7	34.2
Piperacillin	88.8	–	–	–	–	–	–	–	–
Penicillin	–	–	–	–	–	–	76.7	85.7	82.8

All values are in percentage

bacilli; therefore, to prevent the further spread of MβL producers, it is essential to rapidly detect MβL-positive isolates to aid infection control.

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