

**METALLO-BETA-LACTAMASE PRODUCING PSEUDOMONAS AERUGINOSA IN NEONATAL SEPTICEMIA**R. Murthy<sup>1</sup>, M. Murthy<sup>2</sup>, S. Pradhan<sup>3</sup>, R. Barapatre<sup>4</sup>**HOW TO CITE THIS ARTICLE:**

R. Murthy, M. Murthy, S. Pradhan, R. Barapatre "Metallo-Beta-Lactamase Producing Pseudomonas Aeruginosa in Neonatal Septicemia". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 21, May 26; Page: 5711-5716, DOI: 10.14260/jemds/2014/2654

**ABSTRACT:** The emergence, selective multiplication & dissemination of antibacterial resistance is a serious global problem. This study was conducted with the objective to examine the incidence of metallo-beta-lactamase (M $\beta$ L) producing strains among multidrug resistant (MDR) Pseudomonas aeruginosa from the suspected cases of neonatal sepsis between January 2011 – December 2013. A total of 994 cases admitted with the suspicion of neonatal sepsis were investigated. 295 (29.7%) isolates were obtained from the blood cultures of neonates. The isolates were identified and tested for the susceptibility to various antimicrobial agents. Pseudomonas aeruginosa with 116 (48.3%) isolation among 240 Gram negative isolates, was the predominant pathogen in our study. All the 74 (63.8%) multidrug resistant P. aeruginosa isolates were screened initially for Imipenem resistance, which were further tested for the presence of M $\beta$ L by Imipenem-ethylene diamine tetraacetic acid (EDTA) disc method. M $\beta$ L production was seen in 20 (71.4%) of the 28 Imipenem-resistant Pseudomonas aeruginosa isolates. M $\beta$ L producing Pseudomonas aeruginosa has emerged as a potential threat in cases of neonatal septicemia and poses great therapeutic challenge for physicians treating such infections.

**KEYWORDS:** M $\beta$ L, neonatal sepsis, Pseudomonas aeruginosa.

**INTRODUCTION:** The increase in antibiotic resistance among Gram-negative bacteria has been attributed to the efficient methods of transfer of genetic information among bacteria that can confer resistance to one or multiple antibiotics.<sup>1</sup>

Not only is the increase in resistance of gram negative bacteria faster than gram positive bacteria, but also, there are few newer antibiotics active against gram negative bacteria.<sup>2-6</sup> The beta-lactamase resistance is increasing in the members of the family Enterobacteriaceae and non-fermenters like Acinetobacter spp. and treatment regimens for eradication of Pseudomonas aeruginosa infection are becoming increasingly limited.<sup>7</sup> Neonatal septicemia is responsible for one-fourth to nearly half of the neonatal deaths next to perinatal hypoxia.<sup>8</sup> Infections with Pseudomonas aeruginosa are usually late in onset, nosocomial in origin and epidemic in pattern. P. aeruginosa, exhibits intrinsic resistance to various antimicrobial agents including  $\beta$ -lactam antibiotics.

In recent years, there has been an increase in carbapenem resistance which is acquired through metallo-beta-lactamases (M $\beta$ L) and reported mainly for Pseudomonas aeruginosa and Acinetobacter spp. 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.<sup>9</sup>

Thus, the present study was designed to know the incidence of neonatal septicemia, caused by P. aeruginosa, their antibiotic susceptibility pattern and to detect the presence of M $\beta$ L among the imipenem-resistant isolates of Pseudomonas aeruginosa.

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**MATERIAL & METHODS:** The present study was undertaken during January 2011 to December 2013 at Chhattisgarh Institute of Medical Sciences, Bilaspur, Chhattisgarh. A total of 994 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 72h and on the seventh day onto the blood agar and Mac Conkey's agar plates. Organisms isolated were identified by the standard methods of identification.<sup>10</sup>

Antibiotic sensitivity tests of the isolates were performed by the Kirby Bauer disc diffusion method for commonly used antibiotics.<sup>11</sup> All the multi-drug-resistant(MDR) isolates of *P. aeruginosa* were then tested for the sensitivity to imipenem (10µg, Hi Media) and the imipenem-resistant isolates were screened for the production of MβL by the imipenem-EDTA disc method.<sup>12</sup> 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.

**RESULTS:** Of the 994 blood cultures from neonates, 295 (29.7%) showed bacterial growth. The growth was detected in the 230 (77.9 %) cultures in the first 10 days of life. Gram-negative septicemia was encountered in 240 (81.3%) cases: Gram-positive cocci were isolated in remaining 55 (18.7 %) cases. (Table-1) *Pseudomonas aeruginosa* isolated in 116 (48.3%) was the predominant pathogen among Gram-negative isolates, whereas coagulase negative Staphylococci (CoNS) were the most common Gram-positive pathogens.

*P. aeruginosa* was mostly resistant to Gentamicin (92.3%), Amikacin (91.21%), and Piperacillin (88.8%). In this study, out of 116 *P. aeruginosa* isolates 74 (63.6%) were found to be MDR. Of these, 46 (62.1%) were imipenem-sensitive and 28 (37.9%) were Imipenem resistant. MβL production was observed in the 20 (71.4 %) of Imipenem-resistant *Pseudomonas aeruginosa* isolates (Table-2).

**DISCUSSION:** Blood culture is the most important investigation to confirm the diagnosis of neonatal septicemia. However, blood culture positivity varies considerably from centre to centre and by the time suspected cases from village-level practitioners, primary or community health centres get referred to tertiary care set-up like a Medical College, the isolation rates are awfully low, often to the dismay of clinicians, and occurs most commonly due to indiscriminate and irrational use of antibiotics and poor compliance, obscuring the efficacy of standard microbiological work-up. In the present study, the incidence of septicemia confirmed by the growth and isolation of pathogens was 29.7%. This is comparable to 30-75% positivity reported in earlier studies.<sup>13,14</sup>

Most of the cases detected by blood culture occurred in the first 10 days of life (77.9%), 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. With the advent of newer and automated blood culture systems, isolation rates of blood cultures have definitely gone up, but, mis-reporting of contaminants picked-up at the time of collection should be kept in mind and a co-relation with clinical condition should always be ascertained.

A rising incidence of Gram-negative bacteremia has been reported in recent years in neonates.<sup>13,14</sup> The present study documents the isolation of 240(81.3%) Gram-negative bacilli with *P. aeruginosa* in 116(48.3%) of the 240 isolates as the predominant pathogen. *P. aeruginosa* has also

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been reported to be the most common etiological agent of neonatal septicemia by other researchers.<sup>15,16</sup>

Almost all Gram-negative organisms showed resistance to Chloramphenicol (70-95%) and Gentamicin (50-95%). Reduced chloramphenicol sensitivity (20-44%)<sup>17,18</sup> and Gentamicin sensitivity (23-30%)<sup>19</sup> 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 63.6% MDR isolates which correlates with the study by Moniri and colleagues (73.9%).<sup>20</sup> 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 38% Imipenem resistance which is in concordance with Sarkar et al (36.36%).<sup>21</sup>

With increase in the use of Carbapenems in hospital settings, the problem of M $\beta$ L production is also increasing. In our study, 20 (71.4%) of Imipenem-resistant isolates were found to be M $\beta$ L producers. This is an emerging threat and a matter of concern for the 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, *Pseudomonas aeruginosa* is an important Gram-negative bacilli which causes neonatal septicemia. The MDR strains can cause considerable morbidity and mortality. The study also highlights that incidence of M $\beta$ L production by various pathogens, is a reality in this tribal-dominated region in the state of Chhattisgarh, where such studies are almost non-existent and appropriate antibiotic usage is seldom followed.<sup>22</sup>

The wide spread occurrence of M $\beta$ L producing *Pseudomonas aeruginosa* isolates pose a great therapeutic problem. The resistance may spread rapidly to various species of Gram-negative bacilli. Therefore, to prevent the further spread of M $\beta$ L producers, it is essential to rapidly detect M $\beta$ L-positive isolates. The situation also warrants an immediate implementation of infection control strategies, an effective antibiotic policy including antibiotic re-cycling and stringent antibiotic resistance surveillance measures.

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Drugs	Pseudomonas aeruginosas	Acinetobacter spp.	Enterobacter spp.	Citrobacter spp.	Klebsiella spp.	Escherichia coli	Co NS	S.aureus	Enterococci
	(n = 116)	(n = 30)	(n = 38)	(n = 14)	(n = 15)	(n =27)	(n = 32)	(n = 18)	(n =05)
Amikacin	91.2	82.0	63.8	68.8	64.5	24.3	-	-	-
Cefotaxime	-	46.0	77.6	62.5	80.7	35.8	-	-	-
Cefazolin	-	-	-	-	-	-	30.2	45.7	88.5
Cefuroxime	-	-	-	-	-	-	26.2	40.0	62.8
Ceftizoxime	33.6	42.0	46.6	28.2	38.8	7.2	-	-	-
Ceftriaxone	-	65.6	88.0	62.5	94.4	42.9	-	-	-
Cetazidime	59	-	-	-	-	-	-	-	-
Clindamycin	-	-	-	-	-	-	6.9	45.7	34.2
Ciprofloxacin	76.2	55.8	62.2	65.7	64.6	57.2	-	-	-
Chloramphenicol	-	82.0	88.0	93.7	71.0	78.6	48.8	34.2	60.0
Erythromycin	-	-	-	-	-	-	39.5	51.4	42.8
Gentamicin	92.3	77.1	88.0	93.7	80.7	90.0	25.6	51.4	68.5
Norfloxacin	-	77.1	77.6	75.0	77.5	64.3	-	-	-
Piperacillin	88.8	-	-	-	-	-	-	-	-
Penicillin	-	-	-	-	-	-	76.7	85.7	82.8

**Table 1: Antimicrobial resistance pattern of organisms isolated from cases of neonatal septicemia**

	MDR Pseudomonas aeruginosa (n = 74)	
Imipenem resistant	28	20 MβL + ve 08 MβL - ve
Imipenem sensitive	46	-

**Table 2: Frequency of MβL production, Imipenem resistance among MDR P. aeruginosa isolates.**

**AUTHORS:**

1. R. Murthy
2. M. Murthy
3. S. Pradhan
4. R. Barapatre

**PARTICULARS OF CONTRIBUTORS:**

1. Professor, Department of Microbiology, Chhattisgarh Institute of Medical Sciences, Bilaspur, (C. G.) State.
2. Associate Professor, Department of Anesthesiology, Chhattisgarh Institute of Medical Sciences, Bilaspur, (C. G.) State.
3. Assistant Professor, Department of Microbiology, Chhattisgarh Institute of Medical Sciences, Bilaspur, (C. G.) State.

4. Demonstrator, Department of Microbiology, Chhattisgarh Institute of Medical Sciences, Bilaspur, (C. G.) State.

**NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:**

Dr. R. Murthy,  
#31, Guru Vihar Colony,  
Sarkanda, Bilaspur (C. G.)- 495001.  
Email: ramanesh.murthy@gmail.com

Date of Submission: 02/05/2014.

Date of Peer Review: 03/05/2014.

Date of Acceptance: 17/05/2014.

Date of Publishing: 22/05/2014.