METALLO-BETA-LACTAMASE PRODUCING PSEUDOMONAS AERUGINOSA IN NEONATAL SEPTICEMIA
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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 (MβL) 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 MβL by Imipenem-ethylene diamine tetraacetic acid (EDTA) disc method. MβL production was seen in 20 (71.4%) of the 28 Imipenem-resistant Pseudomonas aeruginosa isolates. MβL 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: MβL, 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.¹

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.²⁻⁶ 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.⁷ Neonatal septicemia is responsible for one-fourth to nearly half of the neonatal deaths next to perinatal hypoxia.⁸ 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 β-lactam antibiotics.

In recent years, there has been an increase in carbapenem resistance which is acquired through metallo-beta-lactamases (MβL) 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.⁹

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 MβL among the imipenem-resistant isolates of Pseudomonas aeruginosa.
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. Antibiotic sensitivity tests of the isolates were performed by the Kirby Bauer disc diffusion method for commonly used antibiotics. 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. 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.

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. 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
been reported to be the most common etiological agent of neonatal septicemia by other researchers.\textsuperscript{15,16}

Almost all Gram-negative organisms showed resistance to Chloramphenicol (70-95%) and Gentamicin (50-95%). Reduced chloramphenicol sensitivity (20-44%)\textsuperscript{17,18} and Gentamicin sensitivity (23-30%)\textsuperscript{19} 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\%).\textsuperscript{20} 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\%).\textsuperscript{21}

With increase in the use of Carbapenems in hospital settings, the problem of MβL production is also increasing. In our study, 20 (71.4\%) of Imipenem-resistant isolates were found to be MβL 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 MβL 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.\textsuperscript{22}

The wide spread occurrence of MβL 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 MβL producers, it is essential to rapidly detect MβL-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.

REFERENCES:
1. Poole K. Overcoming multidrug resistance in gram negative bacteria. Curr Opin Investg Drugs 2003; 4: 128-39.
2. Cornelia G. Fighting infections due to multidrug-resistant Gram positive pathogen. Clin Microbiol Infect 2009; 15: 209-11.
3. Tan TT. “Future” threat of gram-negative resistance in Singapore. Ann Acad Med Singapore 2008; 37: 884-90.
4. Baiden F, Owusu-Agyei S, Webstar J, Chandramohan D. The need for new antibiotics. Lancet 2010; 375: 637-38.
5. Vento S, Cainelli F. The need for new antibiotics. Lancet 2010; 375: 637.
6. Wise R, Piddock L. The need for new antibiotics. Lancet 2010; 375: 638.
7. Neuhauser, MM Weinstin R A, Rydman R, Danziger LH, Karam G, Quinn JP. Antibiotic Resistance among gramm negative bacilli in US intensive care units implications for fluoroquinoloneuse. JAMA 2003; 289: 885-88.
8. Sharma A, Kutty KC, Sabharwal U, Rathee S, Mohan H. Evaluation of sepsis screen for diagnosis of neonatal septicemia. Indian J Pediatr. 1993;60: 559-63.
9. Barton LL, Lustig RH, Fong CT, Walentic CA. Neonatal septicemia due to Pseudomonas aeruginosa. Am Fam Physician. 1986;33: 147-51.
10. Collee JG, Duguid JP, Fraser AG, Marmion BP, Simmons A. Laboratory strategy in diagnosis of infective syndromes. In: Collee JG, Fraser AG, Marmion BP, Simmons AC, editors. Mackie and McCartney Practical Medical Microbiology. Singapore: Churchill Livingstone; 1996.pp.53-94.
11. Bauer AW, Kirby WM, Shermans JC, Truck M. Antibiotic susceptibility testing by a standard single disk method. Am J Clin Pathol. 1966; 45: 493-6.
12. Yong D, Lee K, Yum HJ, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-β-lactamases producing clinical isolates of Pseudomonas spp. And Acinetobacter spp. J Clin Microbiol. 2002; 40: 3798-801.
13. De A, Saraswathi K, Gogate A, Fernandes AR. Bacteremia in hospitalized children: A one year prospective study. Indian J Med Microbiol. 1995;13: 72-5.
14. Sharma M, Goel N, Chaudhary U, Aggarwal R, Arora DR. Bacteremia in children. Indian J Pediatr. 2002;69: 1029-32.
15. Kulkarni A, Vigneswaran R. Acquired neonatal sepsis: Are surveillance cultures helpful? Asian J Pediatr Pract. 2000;4: 11-3.
16. Bhattacharjee A, Sen MR, Prakash P, Gaur A, Anupurba S. Increased prevalence of extended spectrum beta lactamase producers in neonatal septicemic cases at a tertiary referral hospital. Indian J Med Microbiol. 2008; 26: 356-60.
17. Moniri R, Mosayebi Z, Movahedian AH, Mousavi GA. Emergence of multidrug resistant Pseudomonas aeruginosa isolates in neonatal septicemia. J Infect Dis Antimicrob Agents. 2005; 22: 39-44.
18. Kuciikates E, Kocazeybek B. High resistance rate against 15 different antibiotics in aerobic Gram-negative bacterial isolates of cardiology intensive care unit patients. Indian J Med Microbiol. 2002; 20: 208-10.
19. Okesola AO, Oni AA. Antimicrobial resistance among common bacterial pathogens in South-west Nigeria. American Eurasian J Agri Env Sci. 2009;5: 327-30.
20. De A, Deodhar LP. Sensitivity of common bacterial isolates to netilmicin: A potent aminoglycoside. Indian Practitioner. 1992; 45: 599.
21. Sarkar B, Biswas D, Prasad R. A. clinic microbiological study on the importance of Pseudomonas in nosocomially infected ICU patients with special reference to metallo-β-lactamase production. Indian J Pathol Microbiol. 2006;49: 44-6.
22. Murthy M, Patel S, Patel KK, Murthy R. Emergence of Carbapenem resistance among isolates of Pseudomonas aeruginosa in diabetes patients. J. Microb. World.2010; 12 (1):87-91.
Table 1: Antimicrobial resistance pattern of organisms isolated from cases of neonatal septicemia

| Drugs               | Pseudomonas aeruginosa (n = 116) | Acinetobacter spp. (n = 30) | Enterobacter spp. (n = 38) | Citrobacter spp. (n = 14) | Klebsiella spp. (n = 15) | Escherichia coli (n = 27) | CoNS (n = 32) | SAureus (n = 18) | Enterococci (n = 5) |
|---------------------|-----------------------------------|-----------------------------|---------------------------|---------------------------|--------------------------|---------------------------|---------------|-----------------|-------------------|
| 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              |
| Cefturoxime         | -                                 | -                           | -                         | -                         | -                        | -                         | 26.2          | 40.0            | 62.8              |
| Ceftriazone         | -                                 | 65.6                        | 88.0                      | 62.5                      | 94.4                     | 42.9                      | -             | -               | -                 |
| Cefazidime          | 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          | 514             | 42.8              |
| Gentamicin          | 92.3                              | 77.1                        | 88.0                      | 93.7                      | 80.7                     | 90.0                      | 25.6          | 514             | 68.5              |
| Norfloxacin         | -                                 | 77.1                        | 77.6                      | 75.0                      | 77.5                     | 64.3                      | -             | -               | -                 |
| Piperacillin        | 88.8                              | -                           | -                         | -                         | -                        | -                         | -             | -               | -                 |
| Penicillin          | -                                 | -                           | -                         | -                         | -                        | -                         | 76.7          | 85.7            | 82.8              |

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

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

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