Microbiological study of neonatal septicemia with special reference to metallo-beta-lactamase producing pseudomonas aeruginosa

Dwivedi V¹, Murthy R², Pradhan S³, Agrawal E⁴

¹Varun Dwivedi, Phd Research Scholer, Dr. C.V. Raman University, Bilaspur, C.G., ²Dr. Ramanesh Murthy Professor and HOD, Department Of Microbiology, CIMS, Bilaspur (C.G.), ³Dr. Sagarika Pradhan, Assistant. Professor Department Of Microbiology, CIMS, Bilaspur (C.G.), ⁴Dr. Ekta Agrawal, Assistant Professor, Department Of Microbiology, CIMS, Bilaspur, C.G., India

Address for correspondence: Varun Dwivedi, Email: varun.setwin@gmail.com

Abstract

Introduction: Metallo-beta-lactamase (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. The emergence, selective multiplication & dissemination of antibacterial resistance are a serious global problem. Methods: This study was conducted with the objective to know the microbiological profile of neonatal septicemia cases and to examine the incidence of MβL producing strains among multidrug resistant (MDR) Pseudomonas aeruginosa from the suspected cases of neonatal sepsis between January 2012 – December 2014. 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.

Results: 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. Conclusion: It creates a great therapeutic problem as it may spread rapidly to various other species of Gram-negative bacilli. Therefore, to prevent the further spread of MβL producers, it is essential to rapidly detect MβL-positive isolates.

Keywords: Metallo-beta-lactamase, Neonatal Sepsis, Pseudomonas Aeruginosa.
Material & Methods

The present study was undertaken during January 2012 to December 2014 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 [10]. Blood was collected with full aseptic measures according to standard guideline [11]. The blood culture bottles (HiSafe™ Blood Culturing System, Paediatric use – Hi-media, Mumbai) were incubated at 37°C for 7 days. Subcultures were done first at 24 hours, then at 72 hours and on the fifth day onto the blood agar and Mac Conkey’s agar plates. Organisms isolated were identified by the standard methods of identification [12].

Antibiotic sensitivity tests of the isolates were performed by the Kirby Bauer disc diffusion method on Muller Hinton Agar (Hi-media, Mumbai) for antibiotics according to CLSI (Control Laboratory Standard Institute) [13]. 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 MBL by the imipenem-EDTA disc method [14]. An increase in zone diameter of >4 mm around the IPM-EDTA disk compared to that of the IPM disk alone was considered positive for an MBL [15].

Results

Out of 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 while Gram-positive cocci were isolated in remaining 55 (18.7 %) cases (Table-1). P. 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.

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

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

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. Out of 28 imipenem resistant P. aeruginosa, MBL production was observed in the 20 (71.4 %) (Table-2).
Table 2: Frequency of MβL production, Imipenem resistance among MDR *P. aeruginosa* isolates.

| MDR *Pseudomonas aeruginosa* (n = 74) | Imipenem resistant | Imipenem sensitive |
|---------------------------------------|--------------------|--------------------|
|                                       | 28 (37.9%)         | 46 (62.1%)         |
| Imipenem resistant                    | 20 (71.4%) MβL + ve| 08 (28.6%) MβL – ve|
| Imipenem sensitive                   | -                  | -                  |

**Discussion**

Blood culture is the most important investigation to confirm the diagnosis of neonatal septicemia. However, positivity of blood culture varies considerably from centre to centre and by the time suspected cases from rural health practitioners, primary or community health centres get referred to tertiary care set-up, the isolation rates are considerably low, often to the dismay of clinicians. It occurs most commonly due to indiscriminate, inappropriate and irrational use of antibiotics and poor compliance, obscuring the efficacy of standard microbiological work-up. In the present study, the incidence of neonatal septicemia confirmed was 29.7%. This is comparable to 30-75% positivity reported in earlier studies [16, 17].

In our study, most of the cases of neonatal septicemia occurred in the first 10 days of life (77.9%), a fact that has been reported previously. It 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 misreporting 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 [16, 17]. The present study documents the isolation of 240(81.3%) gram-negative bacilli with *P. aeruginosa* in 116(48.3%) as the predominant pathogen. *P. aeruginosa* has also been reported to be the most common etiological agent of neonatal septicemia by other researchers [18, 19].

Almost all Gram-negative organisms showed resistance to Chloramphenicol (70-95%) and Gentamicin (50-95%). Reduced chloramphenicol sensitivity (20-44%) and Gentamicin sensitivity (23-30%) have been documented by other workers [20, 21, 22]. 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%) [23]. Multi-drug resistance caused by a variety of resistance mechanisms leads to 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%) [24].

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.

In conclusion, incidence of neonatal septicaemia caused by gram negative organism (81.3%) was more than that of gram positive organism (28.7%). Among gram negative organisms *P. aeruginosa* was the most common pathogen isolated (48.3%). The MDR strains can cause considerable morbidity and mortality. The study also highlights the emergence of MβL producing by *P. aeruginosa* in imipenam resistance isolates (71.4%).

The increasing occurrence of MβL producing *P. aeruginosa* isolates is a reality in this tribal-dominated region of Chhattisgarh State, where such studies are almost non-existent and appropriate antibiotic usage is seldom followed [25].

**Conclusion**

It creates a great therapeutic problem as it may spread rapidly to various other species of Gram-negative bacilli. Therefore, to prevent the further spread of MβL producers, it is essential to rapidly detect MβL-positive isolates

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