Metallo-B-Lactamase Producing Clinical Isolates Of Acinetobacter Baumannii And Pseudomonas Aeruginosa In A Teaching Hospital Of Rural Gujarat-India.

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Abstract: Background: Production of metallo-β-lactamase, an enzyme that hydrolyze a variety of β-lactams including carbapenems leaving little therapeutic option is increasing. To manage patients effectively, it is important to know the local prevalence of MBLs in the hospital. Present study was undertaken to determine prevalence of metallo-β-lactamase production along with the clinical profile of Acinetobacter baumannii and Pseudomonas aeruginosa. Methods: Prospective cross sectional study was carried out during December-2010 to November-2011. Relevant demographic and clinical details were collected. Acinetobacter baumannii and Pseudomonas aeruginosa were subjected to antimicrobial susceptibility testing by Kirby-bauer disc diffusion method and mini API system, (bioMerieux-France). Imipenem/meropenem resistant isolates were tested for metallo-β-lactamase production by imipenem-EDTA combined disc test. Results: 81 strains of Acinetobacter baumannii and 28 strains of Pseudomonas aeruginosa were isolated. Most common specimens from which Acinetobacter baumanni and Pseudomonas aeruginosa isolated were from respiratory tract i.e. 47(58%) and 12(42.9%) respectively. Majority of the strains of Acinetobacter baumannii 44 (54.3%) and Pseudomonas aeruginosa 17 (60.7%) were isolated from non critical areas. Both organisms showed high prevalence of multidrug resistance with MBLs production of 29.6% in Acinetobacter baumannii and 42.9% in Pseudomonas aeruginosa. Conclusion: Metallo-β-lactamase-mediated carbapenem resistance is a significant threat in hospitalized patients. It should be addressed with rapid detection and stringent infection control measures. [Yagnesh P NJIRM 2016; 7(6): 29-33]

Key Words: Multi drug resistance, Acinetobacter baumannii, Pseudomonas aeruginosa, Metallo-β-lactamase

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Introduction: Acinetobacter baumannii and Pseudomonas aeruginosa are most common gram-negative non-fermenter opportunistic bacterial organisms causing nosocomial infections, especially in immune-compromised patients. Among the β-lactams, carbapenems are potent agents for treatment of life threatening infections caused by them because of their broad spectrum activity and resistance to hydrolysis by most β-lactamases, including the extended-spectrum β-lactamases (ESBL)[1]. Resistance to carbapenems is due to multiple reasons e.g. production of carbapenem hydrolyzing enzymes like metallo-β-lactamases, increase efflux system, decrease outer membrane permeability and alteration of penicillin-binding proteins[2]. Emergence of acquired carbapenemases in Enterobacteriaceae, Pseudomonas species and Acinetobacter species, particularly Ambler class B metallo-β-lactamases (MBLs), has resulted in high-level resistance to all β-lactams except aztreonam. As acquired MBL genes are located on integron structure that resides on mobile genetic elements like plasmids and transposons, widespread dissemination is a problem as is evident from increase in nosocomial outbreaks by MBLs producing Acinetobacter species and Pseudomonas aeruginosa[3]. With the world wide increase in such events, it is important to know the local prevalence of such strains in a healthcare setup.

Early detection of MBL strains is critical for better clinical outcome and to prevent dissemination in hospital environment but there is lack of a standardized method that can be used in routine laboratory confirmation. Several phenotypic methods based on enzyme's zinc dependence and use of chelating agents like EDTA or thiol-based compound have been studied and reported but no single method has been found to be perfect[4, 5]. Molecular methods in spite of being sensitive, specific and reliable are restricted to reference laboratories. During routine laboratory diagnosis, MBL producers are suspected if found resistant to carbapenems and/or ceftazidime that can be confirmed by MBL E test. However, due to high cost many laboratories use other alternative methods like double-disc synergy test and combined disc potentiation test[6].

The present study was undertaken with the objectives of determining the prevalence and clinical profile of metallo-β-lactamase production in clinical isolates of Acinetobacter baumannii and Pseudomonas

aeruginosa in the multidisciplinary tertiary care teaching hospital of rural Gujarat-India.

Methods: This prospective cross sectional study was conducted in a 520-beded tertiary care teaching hospital during the period of December 2010 to November 2011 after approval of the Institutional Human Research Ethics Committee (HREC). Acinetobacter baumannii and/or Pseudomonas aeruginosa isolated from various clinical specimens submitted for bacteriological cultures from admitted patients of all age groups and both sexes were included in the study. Relevant demographic and clinical data of each patient was collected in a pre-designed proforma. The specimens comprised sputum, endotracheal secretion, bronco alveolar lawage, ascitic fluid, pus, urine, blood, CVP tip, urinary catheter tip, drain, etc. Samples were processed as per the standard microbiological techniques. The isolates were identified by using ID 32 GN card in mini API system, (bioMerieux-France).

Antimicrobial susceptibility testing: Antimicrobial susceptibility of all the isolates was performed by using Kirby-Bauer disc diffusion method on Muller-Hinton agar medium (Hi-media Laboratories, Mumbai) as well as mini API system (bioMerieux-France), by using ATB PSE 5 strip. The results were interpreted according to the clinical laboratory standards institute guidelines (CLSI, 2011)[7]. The antibiotics tested for Acinetobacter baumannii by disc diffusion method, were cefotaxime (30µg), piperacillin (100µg), piperacillin/tazobactam (100µg/10µg), ticarcillin/clavulinic acid (75/10µg), ceftazidime (30µg), cefepime (30µg), ceftriaxone (30µg), amikacin (30µg), levofloxacin (5µg), ciprofloxacin (5µg), imipenem (10µg), meropenem (10µg), ampicillin/sulbactum (10/10µg), gentamicin (10µg), tobramycin (10µg), tetracycline (30µg), colistin (10µg) and for Pseudomonas aeruginosa were ceftazidime (30 µg), ciprofloxacin (5 µg), imipenem (10 µg), gentamicin (10 µg), amikacin (30 µg), cefepime (30 µg), piperacillin (100 µg), piperacillin-tazobactam (100/10µg), tobramycin (10 µg), aztreonam (30 µg), meropenem (10 µg), ticarcillin (75 µg), levofloxacin (5µg), and colistin (10µg)

Detection of Metallo-β-lactamase

Isolates showing resistance to either imipenem or meropenem were suspected to be MBL producer and were further tested for confirmation by IPM-EDTA-combined disk test as per the technique given by Yong D et.al.(2002)[8].

An imipenem (10-µg) and an imipenem plus EDTA disk (10-µg + 750 µg) were placed on Mueller Hinton agar. After overnight incubation, the inhibition zone diameter difference of ≥ 7 mm between imipenem and imipenem plus EDTA disk was interpreted as MBL test positive[3, 8].

Result: During the study period a total 5,196 samples were received for culture and antimicrobial susceptibility test from which 81 (1.56%) strains of Acinetobacter baumannii and 28 (0.54%) strains of Pseudomonas aeruginosa were isolated from various non-duplicates clinical specimens. Maximum isolates of Acinetobacter baumannii i.e. 26 (32.1%) were from endotracheal secretion followed by pus 16 (19.8%), tracheostomy secretion 12 (14.8 %), blood 06 (7.4%), sputum 06 (7.4%), urine 06 (7.4%), broncho-alveolar lawage 03 (3.7%), CVP tip 02 (2.5%), ascitic fluid 01 (1.2%), catheter tip 01 (1.2%), drain 01 (1.2%) and pleural fluid 01 (1.2%). Majority of the isolates i.e. 44 (54.3%) were from clinical specimens received from various wards followed by various intensive care units i.e. 37 (45.7%). Of the 28 isolates of Pseudomonas aeruginosa, 08 (28.6%) were from pus and remaining from urine 06 (21.4%), endotracheal secretion 04 (14.3%), sputum 04 (14.3%), tracheostomy secretion 04 (14.3%), CVP tip 01 (3.6%) and ascitic fluid 01(3.6%). 17 (60.7%) isolates were from various wards and 11 (39.3%) from different intensive care units. Acinetobacter baumannii and Pseudomonas aeruginosa were predominantly isolated from male patients i.e. 57(70.4%) and 20 (71.4%) respectively.

Imipenem resistance by Kirby-Bauer disc diffusion method was found in 93.8% (76 out of 81) isolates of Acinetobacter baumannii and 100% isolates of Pseudomonas aeruginosa. Meropenem resistance was found in 98.8% (80 out of 81) isolates of Acinetobacter baumannii, and 100% isolates of Pseudomonas aeruginosa. High prevalence of MDR at our centre is seen in resistance pattern of Acinetobacter baumannii and Pseudomonas aeruginosa. [Table: 1] Metallo-β-lactamase production was confirmed in 29.6% of Acinetobacter baumannii and 42.9% of Pseudomonas aeruginosa.

Overall mortality in patients infected with Acinetobacter baumannii and Pseudomonas aeruginosa, was 27.2% and 28.6%. 17.3% and 14.3% patients were discharged against medical advice while

clinical improvement was seen in 55.5% and 57.2% respectively.

Table: 1 Antimicrobial resistant pattern of A. baumannii and P. aeruginosa

No. of strains resistant /strain tested (%) resistance to		
Antimicrobial agent	**A. baumannii (n=81)**	**P. aeruginosa (n=28)**
Cefotaxime	77/77 (100)	NA*
Ampicillin/sulbactum	81/81 (100)	NA*
Ceftazidime	81/81 (100)	26/28 (92.9)
Ciprofloxacin	81/81 (100)	22/22 (100)
Levofloxacin	72/81 (88.9)	28/28 (100)
Imipenem	76/81 (93.8)	28/28 (100)
Meropenem	80/81 (98.8)	28/28 (100)
Gentamicin	81/81 (100)	27/28 (96.4)
Tobramycin	54/81 (66.7)	26/28 (92.9)
Amikacin	79/81 (97.5)	25/28 (89.3)
Ticarcillin/clavulinic acid	81/81 (100)	NA*
Piperacillin/tazobactum	80/81 (98.8)	13/28 (46.4)
Cefepime	79/80 (98.8)	28/28 (100)
Ceftriaxone	81/81 (100)	NA*
Tetracycline	56/80 (70)	NA*
Piperacillin	81/81 (100)	18/28 (64.3)
Aztreonam	NA*	23/23(100)
Ticarcillin	NA*	26/26 (100)
Colistin	01/81 (1.2)	00/28 (00)

NA* Not tested

Discussion: Carbapenems are used for treating serious and life threatening infections caused by multidrug resistant gram-negative bacilli such as Enterobacteriaceae, Pseudomonas species and Acinetobacter species. Production of metallo-β-lactamase by Acinetobacter baumannii, Pseudomonas aeruginosa and other gram negative bacteria has remarkable therapeutic consequences, since these organisms also carry other multidrug resistance genes and the only possible treatment option remains is the potentially toxic polymyxin B and colistin[9]. In the absence of novel agents in the near future, the spread of metallo-β-lactamase producer may lead to therapeutic dead end[10]. The occurrence of metallo-β-lactamase positive isolate create not only a therapeutic crisis but also serious concern for infection control measures which makes it important to rapidly detect and monitor prevalence of such isolates in a hospital environment[10].

In our study, 81 (1.56%) strains of Acinetobacter baumannii and 28 (0.54%) strains of Pseudomonas aeruginosa were isolated from various non-duplicates clinical specimens during the study period.

We found imipenem resistance in 93.8% Acinetobacter baumannii and 100% Pseudomonas aeruginosa with MBL positivity of 29.6% and 42.9% respectively. MBL positivity rates have varied greatly from 6.5 to 100% in various studies done in different geographical area[3, 5, 11, 13-17]. The MBL positivity in our study is low when compared with findings of 96.6% and 100% MBL positivity in Acinetobacter baumannii and Pseudomonas aeruginosa respectively by Irfan et al. (2008) in Pakistan[3]. High MBL rates have also been reported by many researcher e. g. Muneeza Anwar et. al. (2016, 95.5% in Acinetobacter baumannii) [16], N. Ozkalay et al. (2014, 71.05% in Pseudomonas aeruginosa)[15], Noori et al. (2014, 86.6% in Acinetobacter baumannii)[11] and Pandya et. al. (2011, 96.30% in Pseudomonas aeruginosa) [17]. Similarly very low MBL positivity has also been reported e.g. Noyal et. al. (2009, 6.5% in Acinetobacter baumanni) [13], Purohit et. al. (2012, 9.3% Acinetobacter baumannii)[5]. Our finding of 29.6% MBL positivity in Acinetobacter baumannii is similar to 21% MBL positivity as reported by Kumar et. al. (2011) from Kochi, Kerala[14].

Acinetobacter baumannii and Pseudomonas aeruginosa most frequent infected respiratory tract (58.02%, 42.9%) followed by skin and soft tissue (19.8%, 28.6%) respectively. Maximum MBL producing Acinetobacter baumannii and Pseudomonas aeruginosa were also from respiratory tract specimen i.e.16 (66.7%) and 05(41.7%) respectively. This is similar to findings by Noori et. al. (2014), who reported respiratory tract infections as the most common site of infection along with highest MBL positivity in Acinetobacter baumannii i.e. 52.8%[11]. A study by de Carvalho et. al. (2013) has also found tracheal secretion i.e. 56.3% followed by catheter tip 16.9%, blood 7% and urine 7% as positive for Acinetobacter baumannii [12]. The site of infection can vary in various patients and this could be associated with the type of interventional procedure[11].

Acinetobacter baumannii and Pseudomonas aeruginosa, whether MBL producing or not, are known for their antimicrobial resistance as is also seen in our study where both these isolates have shown very high resistance to antimicrobials including aminoglycosides,

quinolones and third generation cephalosporins[18]. Maximum drug sensitivity in our study was seen against tobramycin (66.7%) in Acinetobacter baumannii and piperacillin/tazobactum (46.4%) in Pseudomonas aeruginosa. 100% resistance was observed in Acinetobacter baumannii against cefotaxime, ampicillin/sulbactum, ceftazidime, ciprofloxacin, gentamicin, ticarcillin/clavulinic acid, ceftriaxone and piperacillin. Islahi et.al. (2014) reported resistant pattern of Acinetobacter baumanni in which 86.9% isolates were resistant to gentamicin, 84.7% to amikacin, 86.4% to ciprofloxacin, 89.1% to ceftriaxone and cefotaxime[19]. Noori et.al. (2014) reported resistant pattern of Acinetobacter baumanni in which 95.4% to piperacillin/tazobactam, 80.6% to amikacin, 92.6% to ciprofloxacin, 40.7% to gentamicin, and 95.4% to ceftazidime[11].

We found 100% resistance to ciprofloxacin, levofloxacin, imipenem, meropenem, cefepime, aztreonam, and ticarcillin in Pseudomonas aeruginosa. A. Varaiya et.al. (2008) reported resistance pattern of Pseudomonas aeruginosa in which amikacin 77%, gatifloxacin 72%, piperacillin/tazobactum 82%, piperacillin 85%, and aztreonam 90% were resistant. [2] Sensitivity to colistin has been observed as an important life saving measure in infection by these strains but studies have started reporting resistance to this drug as well.[2,11,16] In our study, all the isolates of Pseudomonas aeruginosa and 98.8% Acinetobacter baumannii were susceptible to colistin. Colistin resistant Acinetobacter baumannii has been reported to be 1.8% - 15.2% in various studies[11,16,19]. Whereas A. Varaiya et.al. (2008) has reported 52% isolates of Pseudomonas aeruginosa resistant to colistin, which is quite alarming. [2]

Combined mortality of patients infected with Acinetobacter baumannii or Pseudomonas aeruginosa in our study was 27.5%, which is similar to 26% and 16.6% reported by Sunenshine et al. (2007) and Kumar et al. (2011) respectively[14, 20]. Mortality was seen in both MBL positive and negative strains thus MBL positivity may not be associated with increased mortality.

Conclusion: The percentage of multidrug resistant Acinetobacter baumannii and Pseudomonas aeruginosa is significant in our institution. MBL producers are on rise and more difficult to treat which is disturbing and strengthens need for prompt infection control measures which must be well-organized and continued. An active infection control programme, with periodic surveillance of infection by these strains, can effectively prevent the spread of these nosocomial pathogens to improve patient safety.

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