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PREVALENCE OF METALLO BETA LACTAMASE (MBL) PRODUCING PSEUDOMONAS SPECIES AMONG CLINICAL SAMPLES FROM S.V.R.R.G.G.HOSPITAL, A TERTIARY REFERRAL HOSPITAL OF S.V. MEDICAL COLLEGE, TIRUPATI.

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ABSTRACT

Hospital-acquired infections are a major challenge to patient safety. Carbapenems are one of the best for the treatment of critically ill patients. Patients treated with routine antibiotics for Metallo-beta-lactamases (MBL) producing Pseudomonas aeruginosa strains resulted several nosocomial outbreaks in tertiary referral hospitals and poor patient outcome. Early detection of metallo-beta lactamase (MBL) producing Pseudomonas aeruginosa among critically ill hospitalized patients is crucial for optimal treatment and to prevent the spread of resistance. The present study was done over a period of one year from January 2011 to December 2011, in the Department of Microbiology, S.V. Medical College, Tirupati. 200 non repetitive Pseudomonas species were isolated from clinical specimens based on standard tests. Antimicrobial sensitivity was performed by Kirby-Bauer disk diffusion method and evaluated for multidrug resistance and screened for MBL production by combined disk diffusion test (CDDT) using imipenem (IMP), and imipenem (IMP) with EDTA. Among 200 Pseudomonas isolates 22 (11 %) were identified to

be MBL producers and 75 (37.5 %) were multi drug resistant (MDR). All the MBL positive strains were belonging to this MDR group constituting 29.33%. All MBL strains were resistant to ceftazidime and imipenem and 95.45% of strains of MBL were resistant to carbenicillin. Early detection of MBL-production among Pseudomonas isolates may help to initiate appropriate antimicrobial therapy and to prevent the development and dissemination of these multi drug resistant strains.

KEYWORDS: Pseudomonas aeruginosa, Metallo-beta-lactamase (MBL) Imipenem-EDTA combined disc diffusion test (CDDT).

INTRODUCTION

Hospital-acquired infections are a major challenge to patient safety. P. aeruginosa are motile, non-spore forming Gram negative rods that are oxidase positive and lactose nonfermenters. Infections associated with this bacterium are nosocomial respiratory tract infections including ventilator-associated pneumonia (VAP),dermatitis, soft tissue infections, surgical site infections , bacteremia, bone and joint infections, gastrointestinal infections and a variety of systemic infections particularly in critically ill patients. Pseudomonas aeruginosa is responsible for 3-7% bloodstream infections and high mortality rates (27-48%) in critically ill patients.[1] The National Nosocomial Infections Surveillance (NNIS) System reports P. aeruginosa to be the second most common organism isolated in nosocomial pneumonia (17% of cases), the third most common organism isolated in both urinary tract infection (UTI) and surgical site infection (11% of cases), and the fifth most common organism isolated from all sites of nosocomial infection (9% of cases).[2] P. aeruginosa presents a serious therapeutic challenge for treatment of both community-acquired and nosocomial infections, and selection of the appropriate antibiotic to initiate therapy is essential to optimizing the clinical outcome.[3] Even more problematic is the development of resistance during the course of therapy, a complication which has been shown to double the length of hospitalization and overall cost of patient care.[4] Carbapenems, mainly imipenem and meropenem, are useful agents for the treatment of infections due to multi-resistant pseudomonas as these drugs are stable against extended-spectrum and AmpC-ß-lactamases. Unfortunately, the resistance of these organisms to antibiotics, particularly to carbapenems, has posed important therapeutic challenges. In a recent survey, 26.4% of 679 P. aeruginosa isolates that caused ventilator-associated pneumonia were resistant to carbapenems.[5] The common form of P.aeruginosa resistance is either through impaired drug penetration through the porins, activation of efflux

pumps, enzymatic modification due to hyper production of an Amp C-type beta lactamases or carbapenems hydrolyzing beta lactamases like Metallo beta lactamases(MBLs). P.aeruginosa show resistance to imipenem by producing Metallo beta lactamases (MBLs).Therefore, detection of MBL producing P. aeruginosa plays crucial role in the treatment of critically ill and hospitalized patients and also in controlling the spread of resistance among clinical isolates in hospital. The present study was conducted to know the prevalence of MBL producing Pseudomonas species, as the early detection of MBL-producing Pseudomonas isolates may help in appropriate antimicrobial therapy and prevent the development and dissemination of these multi drug resistant strains.

MATERIAL AND METHODS

This study was carried out over a period of one year from January 2011 to December 2011, in the Department of Microbiology, S.V. Medical College, Tirupati. 200 non repetitive Pseudomonas species were isolated and identified by standard microbiological tests from clinical specimens like urine, blood, wounds swabs, pus and sputum. These Pseudomonas species were evaluated for multidrug resistance and screened for MBL production by combination disk diffusion test (CDDT) using imipenem (IMP) and imipenem (IMP) with EDTA. The routine antimicrobial susceptibility pattern of these Pseudomonas species was done by Kirby-Bauer disc diffusion method on Mueller-Hinton (MH) agar plates. Antibiogram of these Pseudomonas species for amikacin (30 µg), ceftazidime (30 µg), ciprofloxacin(5µg),imipenem(10µg), carbenicillin and piperacillin/tazobactam (100/10µg) was performed and interpreted as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Pseudomonas aeruginosa ATCC 27853 was used as a control. Isolates were considered to be carbapenem resistant when the zone of inhibition size around imipenem was ≤13 mm, intermediate 14-15 mm and sensitive ≥16 mm. The isolate was considered as Multidrug resistant (MDR) Pseudomonas if resistant to Ceftazidime, Carbencillin, Piperacilli/ tazobactam and to Imipenem. Metallo- beta- lactamases (MBL) -producing Pseudomonas was suspected when isolates were multidrug resistant, being non-susceptible to at least three different antipseudomonal antibiotic groups (amikacin, ceftazidime, ciprofloxacin, and piperacillin/tazobactam) or if all isolates were resistant to carbapenems (imipenem) or if isolates were resistant to imipenem and additionally at least for two more antipseudomonal antibiotic groups like aminoglycosides, fluoroquinolones, and cephalosporins. Such strains were processed for MBL detection by combined disc diffusion test (CDDT). IMP-EDTA combined disc diffusion test (CDDT) was recommended

[6,7,8,9,10,11,12,13] as a method for screening for MBL production in P. aeruginosa strains as it was easy, cheaper, rapid, reliable method for detection of MBLs which will be crucial for patient management and appropriate infection control procedures.

Combined-disk diffusion test method: Two 10-µg imipenem discs were placed on a Mueller- Hinton agar plates and appropriate amount of(5µl) of EDTA solution was added to one of the discs to obtain a desired concentration(750µg). These plates were incubated over night at 35°C - 37°C and the inhibition zones of the imipenem and imipenem-EDTA discs were compared. An increase in the zone of inhibition size of at least 7 mm around the imipenem-EDTA disc when compared to imipenem disc was recorded as MBL-positive strain. Increase in the zone of inhibition size around the imipenem-EDTA disc when compared to imipenem disc on Muller hinton agar.

RESULTS

In the present study combined disk diffusion test (CDDT) method was used for detection of MBL producing Pseudomonas. The study results were shown in the tables 1to 4.A total of 200 Pseudomonas strains, 191 P. aeruginosa and 9 P. putida, were isolated over a study period of one year (January 2011- December 2011). 22 Pseudomonas isolates (11%) out of 200 were resistant to imipenem and all 22 (11%) were found to be MBL producers by imipenem and imipenem-EDTA combined disc diffusion test (CDDT). Among 191 P. aeruginosa 19 were MBL producers and out of 9 P. putida 3 were MBL producers. More number of MDR(68%) isolates and MBL (51.5%) producers were isolated from samples collected from males. Highest numbers of MBL producing Pseudomonas isolates were isolated from the persons aged above 50 years (36.36 %). Among various samples pus and wound swabs (40.90%) were predominant sources for MBL producers followed by urine samples(31.81%).MBL preponderance was high among patients from surgical wards. 5 out of 7 samples from ICU were MBL producers, which signifies that debilitation and high antibiotic usage favors MBL production.Our findings are similar to the results of many studies conducted in different settings. Studies from Kashmir [14] reported 11.6% MBL producers and others[15] from India reported that 16% of Pseudomonas isolates were resistant to imipenem and 14% were positive for MBL production by combined disk test. Studies from China[16] reported 24.1% MDR and 7.83% MBL production. In this study MBL producers showed very high resistance to all antimicrobials compared to non-producers. All isolates of MBL producing Pseudomonas isolates were resistant to ceftazidime and imipenem. Whereas

44.38% non MBL producers were resistant to ceftazidime and no resistance was seen for imipenem among them. Also high percentage of resistance was observed to ciprofloxacin, amikacin, piperacillin /tazobactam, carbenicillin when compared to non MBL producers. Other studies[14,17]also observed that all imipenem resistant isolates were MBL producers. Imipenem resistance among MBL positive isolates was 78.25% and 14 % in MBL negative isolates in some studies.[18] 100 %resistance to ceftazidime was noticed in some studies[14,18,19] with high percentage of resistance for ceftazidime among MBL producers.[20]

Table 1: Antibiotic Sensitivity of Pseudomonas isolates

Total Samples	Drug Sensitive Pseudomonas isolates	MDR Pseudomonas isolates	MBL Pseudomonas isolates
200	125	75	22

Table 2: MBL and MDR isolates among Pseudomonas species:

	Total no. of Pseudomonas isolates(n=200)	MDR Pseudomonas isolates(n=75)	MBL Pseudomonas isolates(n=22)
P.aeruginosa	191	68	19
P.putida	9	7	3

Table 3: Sample wise distribution of MDR and MBL Pseudomonas isolates

Types of Samples	Total no. of Pseudomonas isolates (n=200)	MDR Pseudomonas isolates (n=75)	MBL Pseudomonas isolates (n=22)
Wound, SSI	81	38(50.64 %)	9(40.90 %)
Urine	59	24(32 %)	7(31.81 %)
Body fluids	22	8(10.64 %)	3(13.63 %)
Blood	17	3(4 %)	1(4.54 %)
Sputum	17	1(1.33 %)	1(4.54 %)
CSF	4	1(1.33 %)	1(4.54 %)

Table 4 : Ward wise distribution of MDR and MBL Pseudomonas isolates

Wards	Total no. of Pseudomonas isolates (n=200)	MDR Pseudomonas isolates(n=178)	MBL Pseudomonas isolates(n=22)
ICU	7	6	5(22.72%)
Paediatrics	32	9	4(18.18%)
Surgical	61	32	6(27.27%)
OBG	26	8	3(13.63%)
Orthopaedics	19	6	2(9.09%)
TBCD	25	4	1(4.54%)
Medical	30	10	1(4.54%)

DISCUSSION

Metallo beta lactamase (MBL) production is a significant problem in hospital isolates of Pseudomonas species as they are associated with a higher morbidity and mortality. With increased use of carbapenems against ESBL-producing isolates the problem of MBL production is also increasing. The development of simple, easy, rapid screening tests designed to detect acquired MBL production plays a crucial step towards large scale monitoring of these emerging resistant isolates. The early detection of MBL-producing Pseudomonas isolates may help in appropriate antimicrobial therapy and prevent the development and dissemination of these multi drug resistant strains. MBLs will hydrolyze all classes of beta lactams and their continued spread may lead to clinical disaster due to therapeutic failures and thus will pose serious challenges for the infection control team. Therefore, detection of MBL producing P. aeruginosa plays crucial role in proper treatment of patients' particularly in critically ill and hospitalized patients and also in controlling the spread of resistance among clinical isolates in hospital. The present study was conducted with the above perspective in view to know the prevalence of MBL producing Pseudomonas isolates to find the possible treatment alternatives. Our findings are very much consistent with many studies reported in literature. In this study out of 200 clinical samples, 129 (64.5%) were from male patients and 71 (35.5%) were from female patients. MDR (68%) and MBL producers (51.5%) were predominant among the samples from males. Predominance of MBL producers among Pseudomonas species isolated from male patients was also reported from other studies.[1, 11, 21, 22, 23] Also, in this study highest number of MBL producing Pseudomonas isolates were isolated from the persons aged above 50 years (36.36 %). Similar high incidence among elderly people was observed among other studies as well.[1, 18, 19] Out of 200 Pseudomonas isolates, 32% were from urine samples, with the incidence of UTI was greater in women than men in this study with nearing results being observed in other studies.[21] In this study pus was the major source for both MDR and MBL positive strains with 37 (49.33%) and 9(40.90%) respectively, followed by urine from suspected cases of urinary tract infections 24(32%) and 7 (31.81%). Other studies with wounds as the major source for MDR and MBL was also noticed. [1,6,14,21,23]Some studies [14]have noticed surgery as a risk factor for acquisition of MBL producers and in our study also majority patients infected with MBL producers had surgical site infections (SSI). Of the 22 MBL positive Pseudomonas isolates, 6 (27.27%) from surgery, 4(18.18%) from Pediatrics. Also out of 7 isolates from ICU 5 (22.72%) were MBLs. This signifies that high antibiotic pressure and debilitated condition in patients of ICU wards favor MBL production, [6] as the use of indwelling medical devices

was common in these areas, which also contributed for their spread.These findings were consistent with the other studies.[1,22] This study reinforces results of other studies [19,21, 24] that MBL isolates from samples like urine, pus and blood are more prone to contaminate the hospital equipment and more chance of spread among the patients. Therefore, detection of MBL producing P. aeruginosa plays crucial role in proper treatment of patients' particularly in critically ill and hospitalized patients and also in controlling the spread of resistance among clinical isolates in hospital.

CONCLUSION

Metallo beta lactamase (MBL) producing Pseudomonas isolates cause serious problem in treating critically ill patients with regular antibiotics. In our study multidrug resistance was observed among MBL producing P. aeruginosa. All imipenem resistant isolates were identified as MBL producers by CDDT. In the absence of novel agents the spread of MBL producers may lead to therapeutic failures. The early detection of MBL producing Pseudomonas species by using simple phenotypic methods like imipenem (IMP)-EDTA combined disc diffusion test (CDDT) may avoid the further spread of these multi-drug resistant strains.

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