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ANALYSIS

# Prevalence of Metallo-β-lactamase in Gram negative uropathogens from a Tertiary Care rural Hospital

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## ABSTRACT

Metallo-β-lactamases (MBLs) production in Gram negative bacilli is a major cause of concern among hospital associated urinary tract infection and is being increasingly reported worldwide. The bacteria having MBL has the potential to spread rapidly within the hospital environment and also across continents posing both therapeutic and control management problem. In recent years, MBL genes have spread from *P.aeruginosa* to members of the Enterobacteriaceae. So the present study was carried out with the aim to know the prevalence of MBLs in Gram negative bacteria from uropathogens and the antimicrobial resistant pattern of MBL. Total of 144 Gram negative bacteria were randomly selected. This isolates were tested for carbapenem resistance and MBL production by phenotypic method. Of 144 Gram negative bacilli isolated from urine, Carbapenem resistance was observed in 20(13.89%). Of these 12(8.33%) were from Enterobacteriaceae and 8(38.09%) from *P.aeruginosa*. A total of 7(4.86%) isolates were metallo-β-lactamases producer in Gram negative bacilli, 6(4.17%) were from *P.aeruginosa* and 1(0.69%) from Enterobacteriaceae. None of the isolates of *E.coli*, *Proteus sps* and *Citrobacter sps* were metallo-β-lactamases producer. Out of 6 MBL producing *P.aeruginosa*, 4(66.67%) were from ICU and 2(33.33%) from postoperative patient. One isolate of *K.pneumonaie* was from ICU. Statistical significance was seen in MBL producers from females (57.14%) and males (42.86%) respectively. MBL isolates showed higher antimicrobial resistance as compared to non-MBL. Conclusion: The prevalence of 4.86% MBL in Gram negative bacilli is a major cause of concern. Timely detection and judicious use of antibiotic can help in confronting the menace of antibiotic resistance.

**Keywords:** uropathogens, metallo-β-lactamases producer, antimicrobial resistant pattern.

## 1. INTRODUCTION

Urinary tract infection (UTI) is the commonest bacterial infectious disease in community practice with a high rate of morbidity and financial cost. It has been estimated that 150 million people were infected with UTI per annum worldwide which costing global economy more than 6 billion US dollars (C. M. Gonzalez et al., 1999).

The spectrum of bacteria causing complicated UTI is much broader than of those causing uncomplicated UTI. However, the most commonly encountered microorganisms are Gram negative bacteria including *Escherichia coli*, *Citrobacter spp*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* and *Proteus vulgaris* whereas *Klebsiella spp*, *Staphylococcus aureus* and *Salmonella spp.* are found rarely (B. Foxman et al., 2003).

The increase in multidrug resistance in bacterial uropathogens is an important and emerging public health problem in both non-fermenting bacilli and isolates of the Enterobacteriaceae family. Carbapenem (imipenem and meropenem) are broad-spectrum antibiotics used for the treatment of serious infections caused by β-lactam resistant Gram negative bacilli. Due to the extensive misuse of antibiotic, the bacteria have developed resistance to carbapenem. Carbapenem resistance arises from two main mechanisms: (i) acquisition of carbapenemase genes that encode for enzymes capable of degrading carbapenems, or (ii) a decrease in the uptake of antibiotics by a qualitative or/and quantitative deficiency of porin expression in association with overexpression of β-lactamases that possess very weak affinity for carbapenems. The most important carbapenemases are categorized as three types of enzymes: (i) the KPC type enzymes first described in the US but now found worldwide; (ii) the VIM, IMP, and NDM metallo-β-lactamases; (iii) the OXA-48 type enzymes circulating among Mediterranean countries & progressively disseminating to other geographical areas (Patrice Nordmann et al., 2012). The emergence of carbapenem resistance may jeopardize or stop the development of modern techniques in medicine.

Metallo-β-lactamases (MBLs) belongs to group of β-lactamases that requires divalent cations, usually zinc, as metal co-factor for their enzymatic activity and no therapeutic option is known to be available to control MBLs.Acquired metallo-β-lactamases (MBL) have the capacity to hydrolyze all β-lactams, including carbapenems. Five different types of MBLs whose prevalence are increasing rapidly are IMP, VIM, SPM, GIM and SIM (Luzzaro F et al., 2004). Among these, IMP and VIM are the most predominant (Senda K et al., 1996). The bacteria having MBL has the potential to spread rapidly (horizontal MBL gene transfer) within the hospital environment and also across continents posing both therapeutic and control management problem. In recent years, MBL genes have spread from *P. aeruginosa* to members of the Enterobacteriaceae (Peleg AY, 2005; Nordmann P, 2002). Invasive infections with MBL-producing isolates are also associated with a higher morbidity and mortality (Walsh TR et al, 2005). Several studies have reported global increase in the prevalence of MBL-producing non-fermenting bacilli and Enterobacteriaceae (Walsh TR et al.,2005; (Garza-Ramos U, 2008; Toleman MA, 2002; Moayednia R, 2014; Bhattacharya D, 2013; Saha R, 2010; D G Deshmukh, 2011;A. Varaiya, 2008). Information on the prevalence of carbapenem resistance and Metallo β lactamases (MBLs) in uropathogens from our area is not known. So the present study was carried out with the aim

- To know the prevalence of Metallo β lactamases (MBLs) in Gram negative bacteria
- To know the antimicrobial resistant pattern of Metallo β lactamases and non- Metallo β lactamases Gram negative bacteria

## 2. MATERIALS & METHODS

The Prospective study was carried out in the department of Microbiology of tertiary care rural hospital during the period of July 2012 to December 2013. A total of 144 Gram negative bacteria isolated from patients suspected of having UTIs were randomly selected.

Gram negative bacilli (n=144) was subsequently tested for carbapenem resistance by using meropenem disc [10µg]. The Isolates was considered resistant if the zone of inhibition was 19mm or less.

Phenotypic detection of MBLs among the uropathogen isolates was carried out using imipenem (10µg) and imipenem (10µg) +EDTA (750 µg) discs as described earlier. (D. Yong et al, 2002). The MBL producing isolates showed a greater than 7mm variation between the inhibition zone around imipenem discs alone and the inhibition zone around imipenem + EDTA discs.

The Isolates were tested for Antibiotic susceptibility testing by Kirby-Bauer disc diffusion method on Mueller Hinton agar as per CLSI Approved Standard M100-S17), (CLSI 2007). The antibiotics tested for urinary tract infection were ciprofloxacin (5µg), amikacin (30µg), gentamicin (10µg), netilmycin (30µg), levofloxacin (5µg), norfloxacin(10µg), cefoperazone (75unit), piperacillin (100µg), ticarcillin (75µg), ceptazidime (30µg). Antibiotic disc was obtained from Hi-media Laboratories Pvt. Ltd, Mumbai, India.

The results were statistically analyzed by standard normal test (z test).

## 3. RESULT

Of 144 Gram negative bacilli isolated from urine, Carbapenem resistance was observed in 20(13.89%). Of these 12(8.33%) were from Enterobacteriaceae and 8(38.09%) from *Pseudomonas aeruginosa*. (Table I) A total of 7(4.86%) isolates were metallo-β-lactamases producer in Gram negative bacilli, 6(4.17%) were from *Ps. aeruginosa* and 1(0.69%) from Enterobacteriaceae. (Table 2)

## 4. DISCUSSION

The emergence of antibiotic resistance is a major concern for both developed and developing countries. Carbapenemases have been prevalent in non-fermentative Gram-negative bacteria since the early 1990s and contribute to carbapenem resistance rates ~ 50% for *Acinetobacter baumanni* and *Pseudomonas aeruginosa.* In contrast, carbapenemase resistance was rare in Enterobacteriaceae throughout the 1990s. Hospital outbreaks with carbapenemase-producing *K.pneumonaie* were first reported in the early 2000s. Since then, carbapenem resistance

rates in Enterobacteriaceae have increased, particularly in *K. pneumonia* (Miyakis, S, 2011, Anonymous. 2010, Gales, A.C., 2010; Davies, T.A, 2011). Resistance to imipenem and meropenem in E. coli is rare worldwide (Anonymous. 2010, Gales, A.C., 2010; Davies, T.A, 2011, Hsueh, P.R, 2011).

Metallo-β-lactamases production in Gram negative bacilli is a major cause of concern among hospital associated urinary tract infection and is being increasingly reported worldwide. In our study, metallo-β-lactamases production in Gram negative bacilli revealed none of the isolates of *E.coli* , *Proteus sps* and *Citrobacter sps* were positive but 6(28.57%) isolates from *Ps. Aeruginosa* and 1(7.69%) of *Klebsiella sps* were metallo-β-lactamases producers. In India, the prevalence of MBLs ranges from 7.5% to 71%. (De AS et al, 2010).

Carbapenem resistance is detected reliably with phenotypic testing methods recommended by the Clinical and Laboratory Standards Institute including disk diffusion testing, though the sensitivity and specificity of the test for detecting low-level metallo-β-lactamase not known. (CLSI.,2007). According to Yong *et al,* the imipenem(IMP) 10 µg-EDTA 750 µg combined disc test has 95.7% sensitivity and 91.0% specificity for detection of Metallo-β-lactamases in MBL-producing *Ps. aeruginosa* and *Acinetobacter spp*. (D. Yong et al., 2002) Galan *et al* 2008) have reported the utility of the same combination for Enterobacteriaceae, with 80% sensitivity and 100% specificity for the detection of MBL. The limitation of our study is not comparing the results with a molecular method due to unavailability.

*Pseudomonas aeruginosa* is a common nosocomial pathogen, notorious for its multidrug resistance (MDR) and life threatening infections in critically ill patients. Lately, carbapenems are being used as the last resort antimicrobial to treat serious infections due to MDR *P. aeruginosa.* (Bijayini B et al., 2008) In a few Indian studies, the rate of carbapenem resistance in *P. aeruginosa* has been reported to vary from 12-37 %. (Gupta E et al., 2006)

Study by Lucchetti et al showed that *Pseudomonas aeruginosa* was the main isolated agent causing infections in the urinary tract, and according to epidemiologic data, 35.0% to 45.0% of all acquired nosocomial infections are urinary and 80.0% are related to catheter use (G.Lucchetti et al., 2005). In our study, statistical significance was seen in MBL producers from females (57.14%) and males (42.86%) respectively.

Out of 21 isolates of *Pseudomonas aeruginosa* from urinary tract infection, meropenem resistance was observed in 8(38.09%) and 6(75%) of these 8 isolates were MBL producers. Our findings are similar to Saha et al who reported thirty-one isolates (35.6%) were resistant to imipenem and 61% of these 31 isolates, were MBL producers by combined disc diffusion test, while 48% were detected by E-test method. (Saha R et al., 2010)

Out of 6 MBL producing *Pseudomonas aeruginosa*, 4(66.67%) were from ICU and 2(33.33%) from postoperative patient. One isolate of *K.pneumonaie* was from ICU. Similar were the findings of Deshmukh et al 2011.

The high incidence of this bacterium in the ICU is probably due to the fact that *P. aeruginosa* is an opportunist pathogen that causes bacteremia in immunocompromised patients, burn victims, patients with urinary infections related to catheters use and nosocomial pneumonia, related to mechanical ventilation, especially in this unit. A remarkable feature in infections by *P. aeruginosa* acquired in the ICU is multiresistance. (Javiya VA et al., 2008)

In a study carried out by Varaiya, et al on incidence of Metallo-β-lactamases producing *Pseudomonas aeruginosa* in ICU patients they found 25% of *Pseudomonas aeruginosa* were found to be carbapenem resistant and 20.8% were found to be MBL producers (A. Varaiya et al., 2008).

In our study we found none of the isolates of *E. coli, Proteus sps, Citrobacter sps* were MBL producers except for *Klebsiella sps* where one isolate was MBL producers. Moayednia R et al observed the prevalence of MBLs producing in hospital *E. coli* and *Klebsiella* isolates in patients with urinary tract infection were 0.3% (2/720) and 2.6% (10/384), and for KPC data were 1.4% (10/720) and 48.4% (186/384), respectively. No MBLs and KPC producing isolate was seen in non-hospital *E. coli* and *Klebsiella* isolates except for one non-hospital KPC producing *Klebsiella* isolate (Moayednia R et al., 2014).

Bhatacharya et al 2013)in their study on Emergence of New Delhi metallo-β-lactamase 1 (NDM-1) producing and multidrug resistant uropathogens causing urinary tract infections in Andaman Islands, India reported for the first time the presence of the New Delhi metallo-β-lactamase (NDM-1) gene in two isolates of *P. mirabilis* in these islands. Whereas in our study we found none of our *Proteus sps* were MBL producers.

The extent of antimicrobial resistance shown by the pathogens towards the commonly employed drugs is an issue of global concern.The indiscriminate, inadequate and the irrational usage of antimicrobials has further contributed to the emergence of resistant strains, which may turn out to be a leading cause for the morbidity and mortality in the developing countries.

In our study, metallo-β-lactamase showed higher antimicrobial resistance to other groups of antibiotics. (Table 3), which is a unique problem with MBLs that show a broad-spectrum resistance profile. The genes encoding MBLs are often procured by class 1 (sometimes class 3) integrons. Other gene cassettes within the integrons confer resistance to other antibiotics such as fluoroquinolones, aminoglycosides and co-trimoxazole. Integrons are, in turn, embedded in transposons, resulting in a highly transmissible genetic apparatus that can be transferred between bacteria. (Walsh TR et al 2005) MBLs inactivate all beta-lactam antibiotics except for aztreonam and strains harboring these enzymes are considered a major clinical threat because coresistance to multiple antibiotic subclasses is frequent and severely limits therapeutic options (Yong, D. et al., 2009).

## 5. CONCLUSION

The prevalence of 4.86% metallo-β-lactamases producer in Gram negative bacilli is a major cause of concern among hospital associated urinary tract infection. Timely detection and judicious use of antibiotic can help in confronting the menace of antibiotic resistance.

## DISCLOSURE STATEMENT

There is no conflict.

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**Table 1**
Prevalence of Carbapenem resistance in Gram negative bacilli

| Isolates | No (%) | CR |
|---|---|---|
| E .coli | 93 | 9(9.68%) |
| Pseudomonas aeruginosa | 21 | 8(38.09%) |
| Klebsiella sps | 13 | 2(15.38%) |
| Citrobacter sps | 13 | 1(7.69%) |
| Proteus sps | 4 | 0 |
| Total | 144 | 20(13.89%) |

The above table depicts higher Carbapenem resistance in *Pseudomonas aeruginosa* 8(38.09%)

**Table 2**
Metallo-β-lactamases production in Gram negative bacilli

| Isolates | Total No | No (%) |
|---|---|---|
| E .coli | 93 | 0 |
| Pseudomonas aeruginosa | 21 | 6(28.57%) |
| Klebsiella sps | 13 | 1(7.69%) |
| Citobacter sps | 13 | 0 |
| Proteus sps | 4 | 0 |
| Total | 144 | 7(4.86%) |

In the above table it is noted that none of the isolates of *E.coli* and *Citrobacter sps* were positive but 6(28.57%) from *Ps. aeruginosa* and 1(7.69%) of *Klebsiella sps* were metallo-β-lactamases producers

**Table 3**
Antimicrobial resistance pattern of MBL and non- MBL producing gram negative isolates

| Antibiotic tested | MBL producers (n=7) % | Non-MBL producers (n=137)% | Z value |
|---|---|---|---|
| Ciprofloxacin | 6(85.71) | 115(83.94) | 3.27* |
| Amikacin | 5(71.43) | 59(43.07) | 1.61 |
| Gentamicin | 4(57.14) | 60(43.80) | 0.70 |
| Netilmycin | 5(71.43) | 65(47.44) | 1.36 |
| Levofloxacin | 5(71.43) | 80(58.39) | 7.3* |
| Norfloxacin | 7(100) | 90(65.69) | 6.19* |
| Cefoperazone | 7(100) | 85(62.04) | 6.76* |
| Piperacillin | 7(100) | 80(58.39) | 7.3* |
| Ticarcillin | 7(100) | 82(59.85) | 7.12* |
| Ceptazidime | 7(100) | 82(59.85) | 7.12* |

* Z >1.98 for difference between MBL and non- MBL producing gram negative isolates
Z >1.98 significant MBL isolates showed higher antimicrobial resistance was noted to antimicrobial agents