# Metallo-β- lactamase production by Pseudomonas aeruginosa of septicemic patients

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*Summary:*

**Background:** P. aeruginosa remains an important cause of life threatening bloodstream infection in immunocompromised patients, particularly those with hematologic malignancies complicated by neutropenia. Metallo-β-lactamase(MBL) belongs to β-lactamases, which requires divalent cations of zinc as cofactors for enzyme activity. They have potent hydrolyzing activity not only against carbapenem but also against other β – lactam antibiotics. MBL determinants are encoded by transferable plasmids and can rapidly spread to other bacteria. Thus, MBL-producing P. aeruginosa strains have been reported to be important causes of nosocomial infections associated with clonal spread.

**Objective:** this study was designed to detect the production of MBL by p. aeruginosa in septicemic patients and show the plasmid profile of such bacteria.

Patients and methods: This study included 53 Pseudomonas aeruginosa isolated from blood of patients their ages ranging from two days to 73 years,28 males and 25 females. Some of the isolates were isolated from acute, 15(28.3%), and chronic, seven (13.2%), leukemic patients, five (9.4%) from each lymphoma and gastrointestinal neoplasms patients. Nine (17%), three (5.7%), six (11.3%) and three (5.7%) from urogenital neoplasms, breast cancer patients, septicemic patients due to burn infections and neonatal septicemia respectively. Disc diffusion method, disc potentiation method and rapid boiling procedure were used for detection of antibiotic susceptibility,MBL production and plasmid profiling.

**Results:** 98.1% of p.aeruginosa were resistant to cefixime,90.6% were resistant to carbenicillin, but all of them were susceptible to impenem. 68% of the isolates were MBL producers , while 32% were non producers. Plasmid profile reveals that all of the tested isolates harbor plasmids with different molecular weight.

**Conclusion:** P.aeruginosa can cause septicemia in cancer patients and other immunocompromised patients, like patients suffering from extensive burns and neonatal infants. All of SPA isolates were susceptible to impenem, and can be used as a drug of choice in treatment of such infections. 90.6% of these isolates were resistant to carbenicilin, and all except one were resistant to cefixime. About two-third on them were MBL producers suggesting the role of such enzyme in their resistance.

**Keyword:** septicemic P.aeruginosa, metallo- β-lactamase.

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**Introduction**:

Microorganism presence in circulating blood are a threat to every organ in the body. Microbial invasion of the bloodstream can have serious consequences , including shock, multiple organ failure, disseminated intravascular coagulation, and death ,with mortality rates ranging from 30% to 50% . Positive blood cultures may help provide a clinical diagnosis as well as a specific etiological diagnosis (1,2). P. aeruginosa remains an important cause of life threatening bloodstream infection in immunocompromised patients, particularly those with hematologic malignancies complicated by neutropenia (3). Metallo-β-lactamase (MBL) belongs to β-lactamases, which requires divalent cations of zinc as cofactors for enzyme activity. They have potent hydrolyzing activity not only against carbapenem but also against other β – lactam antibiotics. The genes that responsible for MBL production are horizontally transferable via plasmids and can rapidly spread to other bacteria. Thus, MBL-producing P. aeruginosa strains have been reported to be important causes of nosocomial infections associated with clonal spread (4). Several phenotypic methods are available for the detection of MBL producing bacteria. All these methods are based on the ability of metal chelators such as ethylene diamine tetra-acetic acid (EDTA ) and thiol – based compounds to inhibit the activity of MBLs. These tests include the double disc synergy tests using EDTA with β- lactam antibiotics (5). Screening and confirmation for the detection of MBL was done by disc potentiation test with EDTA - impregnated β- lactam antibiotic discs (6).

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**Patients, materials and methods:**

This study was include 53 Pseudomonas aeruginosa isolates isolated from blood of patients their ages ranging from two days to 73 years,28 males and 25 females. Some of the isolates were isolated from acute. 15(28.3%), and chronic. seven (13.2%). leukemic patients. five (9.4%) from each lymphoma and gastrointestinal neoplasms (gastric cancer and colon cancer) patients. Nine (17%). three (5.7%). six (11.3%) and three (5.7%) from urogenital neoplasms. breast cancer patients. septicemic patients due to burn infections and neonatal septicemia. respectively.

**Table 1: Causes of Pseudomonas aerugonosa septicemia:**

| Cause of septicemia | No.of isolates | Percentage of isolates(%) |
|---|---|---|
| Acute leukemia | 15 | 28.3 |
| Chronic leukemia | 7 | 13.2 |
| Lymphoma | 5 | 9.4 |
| Urogenital neoplasms | 9 | 17.0 |
| Gastrointestinal neoplasms | 5 | 9.4 |
| Breast cancer | 3 | 5.7 |
| Burn infections | 6 | 11.3 |
| Neonatal septicemia | 3 | 5.7 |
| Total | 53 | 100 |

All of these isolates were collected in a period from February to December 2010 and were reidentified using biochemical tests and according to Forbes et al.2002[7]. Disc diffusion method was used to perform antibiotic susceptibility test[8].Bacterial culture of $10^8$ cell/ml was prepared using BHI broth(Mast diagnostic- Germany) and McFarland's standard tube No. 0.5. Mueller - Hinton agars (Oxoid-UK) were prepared according to the manufacturer instructions and poured at depth of 4mm. Hundred microliter of BHI broth culture was speeded on the surface of Mueller-Hinton agar plates. Antibiotic susceptibility discs (table-2 )were placed on the surface of the implanted agars and then all the plates were incubated at 37 °C overnight. Inhibitory zone for each disc was measured and interpreted according to Vandepitte et al. 2003[8].

**Table 2: Antibiotic discs used in the study and their potency and Manufacturer:**

| Antibiotics | Disc potency/ µg | Manufacturer |
|---|---|---|
| Cefixime | 30 | Bioanalyse-UK |
| Carbenicillin | 100 | Bioanalyse-UK |
| Imipenem | 10 | Bioanalyse-UK |

Each isolates showed resistance to β-lactam antibiotics (carbinicillin and or cefixime and or impenem) was subjected to disc potentiation test to investigate the production of MBL. The test was performed as follows: Test organism culture of $10^8$ cell/ml was prepared using BHI broth and was inoculated onto plates of Mueller-Hinton agar plate (opacity adjusted to 0.5 McFarland opacity standards). A 0.5 M EDTA solution was prepared. Two carbinicillin discs and cefixime discs were placed on the plate: 5 µl of EDTA solution was added to one of the disc each. The inhibition zones of the carbinicillin and carbinicillin -EDTA discs and cefixime and cefixime - EDTA discs were compared after 16-18 hrs of incubation at 35 °C. An increase in the zone size of at least 7 mm around the carbinicillin -EDTA disc and cefixime -EDTA discs was recorded as an MBL-positive strain[9]. Plasmid contents of P. aeruginosa was investigated hence MBL determinants are plasmid encoded using rapid boiling procedure (Reischl et al.2000). In brief. Ampicillin containing Luria broth media were inoculated with SPA isolates. One milliliter of an overnight culture was added to a microcentrifuge tube. centrifuged at 15,000 g for two minutes to pellet the cells. Two hundred of lysis buffer ( 1% Triton X-100 (Fluka-Switzerland) 0.5%Tween-20 (BDH-UK) .10mM Tris-HCl (PH=8)- (BDH-UK) and 1mM EDTA (BDH-UK) was added to the pellet. the mixture incubated at boiling water bath for 10 minutes. Microcentrifuge tube was centrifuged at 15,000 g for two minutes to remove the cell remainders and chromosomal DNA. The supernatant was used for agarose gel electrophoresis.

**Result:**

Antibiotic susceptibility test revealed that. only one (1.9%)out of 53 SPA isolate was susceptible to cefixime. and 52(98.1%) were resistance. while all(90.6%) of them were resistance to carbenicillin. In contrast all(100%) of isolates were susceptible to impenem(table 3).

**Table 3: Numbers and percentages of susceptible and resistant septicemic Pseudomonas aeruginosa isolates to different antibiotics.**

| Antibiotic | No. of susceptible isolates | Percentage of susceptible isolates(%) | No. of resistant isolates | Percentage of resistant isolates(%) |
|---|---|---|---|---|
| Cefixime | 1 | 1.9 | 52 | 98.1 |
| Carbenicillin | 5 | 9.4 | 48 | 90.6 |
| Imipenem | 53 | 100.0 | 0 | 0.0 |

As shown in table (4). the number and percentage of metallo-β lactamase producers were 36(68%) and about 17(32%) were non producers. Figure (1) show inhibition of metallo-β lactamase activity of Pseudomonas aeruginosa by 0.5 M EDTA.

**Table 4: Number and percentage of SPA metallo-β lactamase producers and non producers.**

| No of SPA metallo-β lactamase producers | percentage of SPA metallo-β lactamase producers | No of SPA metallo-β lactamase non-producers | percentage of SPA metallo-β lactamase non producers |
|---|---|---|---|
| 36 | 68% | 17 | 32% |

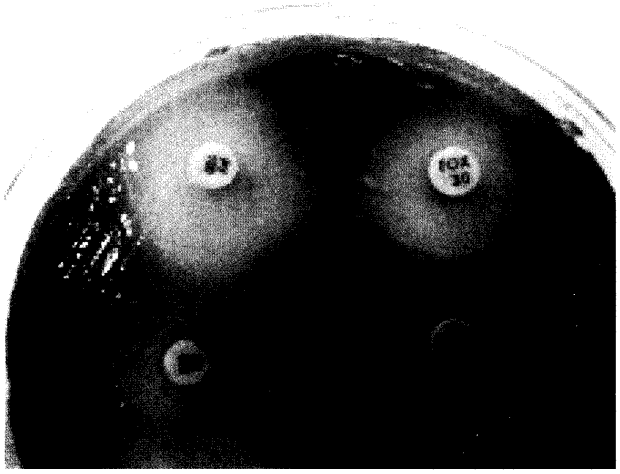

**Figure 1: Inhibition of metallo-β lactamase activity of septicemic Pseudomonas aeruginosa by 0.5 M EDTA. Susceptibility test of isolate: Two lower discs are carbenicillin and cefixime. The two upper are the discs of the same antibiotics potentiated with 0.5M EDTA.**

Plasmid DNA of isolates, were extracted and then subjected to agarose gel electrophoresis. Most of isolates have relatively large plasmids, their molecular size more than 10kb.One out of eight SPA isolates have one megaplasmid only( more than 10kb). All poses small plasmids, the size of small plasmids were about 250bp or slightly more or less. Three had two plasmids, their molecular size between 750bp- 1kb (figure 2).

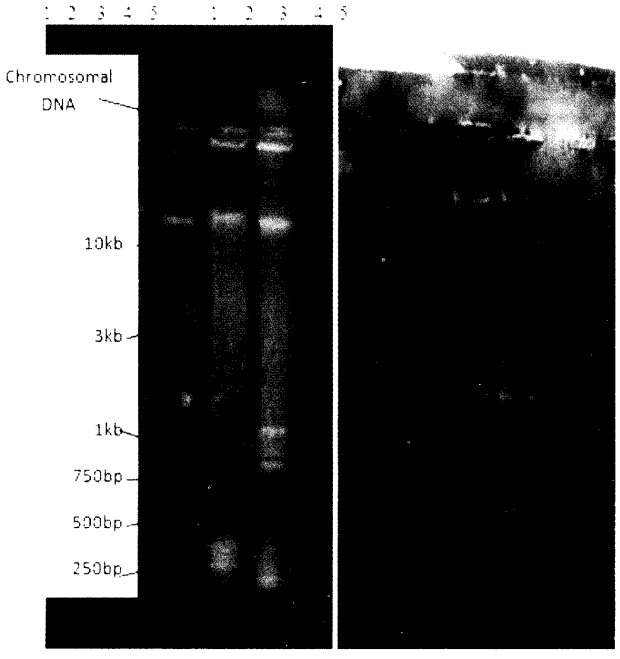


**Figure 2: Plasmid profile of P. aeruginosa**
**Lane 1 is 1kb molecular ladder; other lanes represent plasmid profile of P. aeruginosa.**

**Discussion:**

P. aeruginosa is a common Gram-negative bacillus associated with hospital infections and is often difficult to eradicate due to its resistant drug profile. Therefore, detection of MBL producing Gram-negative bacilli especially P. aeruginosa is crucial for the optimal treatment of patients particularly in critically ill and hospitalized patients and to control the spread of resistance isolated from septicemic patients (9). There is not much information available on MBL producing P. aeruginosa isolates from Iraq. Therefore, we undertook this study to detect the MBL in P. aeruginosa isolates obtained from compromised patients. The occurrence of an MBL-positive isolate in a hospital setting poses a therapeutic problem, as well as a serious concern for infection control management. In this study, we observed a resistance of 98.1% and 90.6% to cefixime and carbenicillin respectively among the P. aeruginosa. Meanwhile,68% of screened bacteria were MBL-positive. No resistance was observed to imipenem . Clearly there is no relationship between the resistance to impenem and MBL production as the resistance to carbapenems was independent of resistance to other beta-lactam antibiotics, indicating a different mechanism of resistance, probably due to the loss of the oprD porin (10) .Obviously, there is astrong relationship between the production of MBL and cefixime and carbenicillin resistance, since all of resistance were MBL producers. Some of them which were MBL non producers were also resistance to cefixime and carbenicillin. This may be due to presence of other mechanisms of resistance, like penicillin binding protein alteration. MBL determinants are plasmid encoded (4). All of the tested isolates harbor different molecular size plasmids. Future studies are needed to characterize MBL encoded plasmid. As conclusion, we found that, P.aeruginosa can cause septicemia in cancer patients and other compromised patients, like patients suffering from extensive burns and neonatal infants. All of isolates were susceptible to impenem, and can be used as a drug of choice in treatment of such infections. 90.6% of these isolates were resistant to carbenicilin, and all except one were resistant to cefixime. About two-third on them were MBL producers suggesting the role of such enzyme in their resistance.

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