

Nosocomial Infection Caused By Mdr-*P. Aeruginosa* Producing Metallo B-Lactamase

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Abstract

Metallo- β -lactamases (MBLs) is an enzyme which digests the most of the β -lactams class of antibiotics such as carbapenems and leads to a broad-spectrum antibiotic resistance for this class of antibiotics in the *Pseudomonas aeruginosa* and a number of another gram-negative bacteria. One of the important characteristic features of these resistance is that it cannot be reversed by any conventional β -lactamase inhibitors. This work was aimed to find out the nosocomial infections caused by MDR *P. aeruginosa* produced by the enzyme Metallo- β -lactamases. This offers findings that can be applied in practice as well as an equation for the bedside clinician and also reflects on the controversy regarding diagnostic tools and techniques, treatment plans, and strategies for prevention. A well oriented observation and study was conducted in Medical Intensive Care Unit (MICU), High Dependency Unit (HDU), Surgical Intensive Care Unit (SICU), and other wards for time period of 4 months. *P. aeruginosa* isolated from the various samples of patients with different infections was established as MDR, if it was found to be resistant to three antibiotics from these groups of antibiotics such as β -lactams, aminoglycosides, carbapenems, and fluoroquinolones. The MBL producing MDR *P. aeruginosa* were diagnosed on the basis of clinical and microbiological aspects. We had used the guidelines given by Clinical and Laboratory Standard Institute (CLSI) 2013 for this. Out of all the 50 isolates of *P. aeruginosa* from 40+patient's sample, the nosocomial infections caused due MBL producing MDR *P. aeruginosa* was 40% whereas 60% incidence was due to non MDR group. The current report shows that the one of the important risk factors for the infection with MDR *P. aeruginosa* is long-term hospitalization and use of immunosuppressive agents like corticosteroids and some others.

Keywords: *antibiotics, aeruginosa, multidrug resistance, carbapenem, lactamase*

Introduction

Pseudomonas aeruginosa is a gram negative, obligate aerobic bacterium with unipolar motility which can cause infection to animals as well as human. It is a major pathogen responsible for wide range of nosocomial infections [1]. *P. aeruginosa* secretes variety of pigments such as pyocyanin, pyoverdine, pyorubin and fluorescen. *P. aeruginosa* is usually preliminarily known by its iridescent look and griddlecake like odor. *In vitro*, *P. aeruginosa* can grow on various ordinary medium. This organism can grow even in diesel, jet fuel and phenol as well, where it is called “*hydrocarbon using microorganism*” or “*HUM bug*” leads to microbial corrosion [2-3]. Its low antibiotic susceptibility is one of the most worrisome characteristics, which is mainly due to the multidrug efflux pumps expression, antibiotic resistant genes present on the main chromosome and reduced permeability of bacterial cellular envelop [4]. The microorganism acquires the antibiotic resistance characteristics mostly mutation in the gene coded by main chromosome or via the horizontal gene transfer from any other resistant bacteria [5].

MDR *P. aeruginosa* is defined as isolates that are intermediate or resistant to minimum of three classes of antibiotics viz. carbapenem, beta lactam, fluoroquinolones etc. On the basis of geographic locations and category of surveillance report, the rate of MDR *P. aeruginosa* reporting rate lies in between 0.6 – 32%. This antibiotic resistance in *P. aeruginosa* is a very serious issue and it grows everyday [6]. The most important reason for the emergence of antibiotic resistance is the exposure for specific antibiotic regularly for more duration [7]. Antibiotic resistance can also be spreaded from person to person transfer of antibiotic resistance organism [8]. The resistance gained through various processes like exposure of antibiotics or crossing of resistance ultimately accumulated and leads to a multidrug-resistant MDR *P. aeruginosa*. Conversion of *P. aeruginosa* to multi drug resistance

P. aeruginosa occurs more commonly in the cases of cystic fibrosis patients. In such patients, a chronic *P. aeruginosa* infection results in concurrent resistance to antibiotics of different classes. This multi drug resistance *P. aeruginosa* has been spread from one patient to another, leading to infections among patients with cystic fibrosis visiting the same hospital [9]. At Apollo Hospital (Bangalore) we have observed a moderate rate of incidence for MDR *P. aeruginosa* (40%).

Metallo β -lactamase (MBL), an enzyme of β -lactamase group, requires zinc for its biological activity. This enzyme shows a hydrolytic action against most of the beta lactam class of antibiotics including carbapenem [10-11]. The gene responsible for the MBL enzyme is IMP and VIM genes which is transferred horizontally among bacteria and present either in chromosome or plasmid of the organism [10-12]. This is the reason why Metallo β -lactamase enzyme, MBL producing *P. aeruginosa* is considered as one of the big threats to cause clonal spread associated nosocomial infection [13]. Scientific reports say that the MBL producing *P. aeruginosa* causes 20-42% of all the incidence of nosocomial infections [14-15]. And hence MBL producing gene and its clonal spread techniques can be one of the best possible targets for the development of antimicrobial therapeutic agents. We don't have sufficient number of antibiotics available in the market which can manage or treat the MBL producing *P. aeruginosa* infection. In current times, the incidence of nosocomial infection caused due to MBL *P. aeruginosa* has been increasing in many countries [14].

More than 10% of the hospital induced pathogenic infections is caused due to *P. aeruginosa*. Day by day, the severity of the *P. aeruginosa* infection becomes worsen and the condition becomes serious as the antibiotic sensitivity is suppressed and the emergence of antibiotics resistance [7].

P. aeruginosa have some specific characteristics of predilection in moist environment and this organism results in a serious problematic in hospital-based environment. A number of

different types of aqueous solutions is being used in the hospital, such as eye drop and eye wash, saline, dripping solutions, irrigation fluids, and dialysis fluids which can get contaminated with the *P. aeruginosa*[6-7]. This microorganism can also present in the equipments used for respiratory tract therapy, traps of sinks, showerheads taps [16-18]. It can contaminate the bronchoscope during the treatment of a patients and transmitted to a fresh host in further process.

Material and Methods:

This work was performed at the Apollo Hospitals Bangalore for four months. The study was aimed to find out the risk factors for causing MDR *P. aeruginosa* infection. Among 50 *P. aeruginosa* isolated from more than 40 patients, 20 strain was MDR producing metallo β -lactamase. The pathogen *P. aeruginosa* were cultured, isolated and characterised in the microbiology laboratory from the various clinical isolate samples with the help of gram-negative identification panel kit (installed vitek2C systems version:06.01). Resistance to antibiotics was tested by automated method (installed vitek2C systems version:06.01) as well as manually (Kirby-Bauer disk diffusion). The protocols and methodologies used during the study was according to the guidelines given by Clinical Laboratory Standards Institute (CLSI) 2013. The sample collection their transportation and also storage is one of the important prerequisites for dependable microbiological processing and better results. The requisition and specimen should be labeled with name of the patient, his date of birth or age, type of sample collected, and time and date of collection of samples. Different sampling methods has been used for the study:

- (i) **Sputum:** An early morning 50 ml sputum sample has been collected in a sterile container.

- (ii) **Tracheal secretion:** Process by which tracheal specimen collected is called tracheostomy. Tracheostomy suctioning has been done in the morning as well as night just before sleeping.
- (iii) **Pus:** A sterile needle is inserted into the site of infection, and a sample of fluid is withdrawn.
- (iv) **Urine:** Morning mid-stream urine had been collected in a sterile container.
- (v) **Bile:** Bile sample had been collected with the help of huber needle.
- (vi) **Wound swab:** Clean the skin around wound gently before sample collection to reduce contamination of normal flora of skin and take deep wound specimens, keep into swab container and send it for microbiological process.
- (vii) **Broncho Alveolar Lavage (BAL):** This process requires a preoxygenation by a fraction of inspired oxygen (FIO₂) for almost 10 minutes to the patient. The bronchoscope moved to the segmental bronchus and followed by lavage with 10 to 100 mL of sterile saline.
- (viii) **Blood:** 10ml of blood sample had been collected from each patient in the blood culture bottle. Sufficient blood amount per culture should withdrawn to optimize recovery of organisms from blood. For various age group patient and for aerobic as well as anaerobic culture, there are different blood culture bottles.
- (ix) **Throat swab:** Swab from the tonsillar and posterior pharynx had been collected.

Microbial processing

- (i) **Direct microscopy:** For direct microscopic observation, gram smear is the most preferable technique in the microbiology laboratory. Direct smear was made from all the sample received for culture and sensitivity (except CVP tip). Blood sample was processed after indicating positive by BacT Alert 3D system. The smear was

then stained with gram stains and observed under microscope. *P. aeruginosa* under gram smear observed as pink colour bacilli.

- (ii) **Culture of specimen:** *P. aeruginosa* are not too much specific in nutritional requirements. They can grow on very simple media. Even though, there are some selective media which identify the biochemical properties of *P. aeruginosa*. Selective *Pseudomonas* media contains some of the important components and these components are antibiotic penicillin-G, dye malachite green, pimaricin, nalidixic acid, ceftrimide, cephaloridine, and other growth inhibitory agents.

Three common media has been used to culture the specimens during this project work, HiChrome UTI agar, MacConkey agar (MA) and Blood agar (BA), but for few samples, like blood, sputum, throat swab and tracheal secretion, Chocolate agar was additional. After culture, the medium plates were incubated at 37°C for overnight. Sometimes it was required to incubate for 48hours.

Observation: *P. aeruginosa* produce colorless to light yellow-green translucent colonies on UTI agar plate which may have a slight iridescence with crinkled edges. On MacConkey agar plate, typical spreading pale-yellow colonies of *P. aeruginosa* is observed because of non-lactose fermentation, and it showstypically display beta hemolysis, a metallic sheen, and blue or green pigment on blood agar plate (fig. 1).



Fig:1 growth of *P.aeruginosa* on UTI agar, MacConkey agar & Blood agar plate.

(iii) **Biochemical reactions:** The most important biochemical characteristics of *P. aeruginosa* is oxidase and catalase test, which is strongly positive. *P. aeruginosa* produces proteolytic exoenzyme called gelatinase which hydrolyze protein to aminoacid and show gelatin liquefaction test positive.

Identification of MDR *P. aeruginosa*: To differentiate & identify MDR *P. aeruginosa* from Non MDR group of *P. aeruginosa*, antibiotic sensitivity test were performed by Vitek2C automated system and confirmed by Kirby Bauer disk diffusion test with all the isolated strains of *P. aeruginosa*. Generally, Non MDR strain was sensitive against all the antibiotic used for *Pseudomonas* but MDR *P. aeruginosa* was resistant against all the antibiotic except Colistin. The antibiotics panel used for *P. aeruginosa* for AST were Beta- Lactams, such as Ceftazidime, Piperacillin, Cefepime, Ticarcillin, Piperacillin&Tazobactam, Ticarcillin&Clavulanate, Carbapenems, such as Imipenem, Dorepenem, Meropenem; Aminoglycosides, such as Tobramycin, Gentamicin, Amikacin; and Fluoroquinolones such as Ciprofloxacin.

The antibiotic susceptibility results were analyzed according to the CLSI-2013 guidelines. The *aeruginosa* strain ATCC 27853 was used as control for the evaluation of antibiotic susceptibility.

Results and Discussion

Detection of Metallo B-Lactamase (MBL): The test will be done through the Modified and improved hodge test (MHT). The Metallo B-Lactamase production by a *P. aeruginosa* is evaluated by the MHT where the isolated organism releases lactamase enzyme and introduces the drug resistance to against carbapenem disk. The cloverleaf-like growth indicates MHT positive result. 0.5 McFarland dilution of the *E.coli* ATCC 25922 in 5 ml of saline was

prepared, and it was diluted 1:10 by adding McFarland and saline in 1:9 ratio. Ten times dilution of *E.coli* ATCC 25922 has been done in normal saline and then it was spreaded on the Mueller Hinton (MH) agar plate and the plate was left for 5 minutes to dry. Antibiotic disks of meropenem which consist of 10 µg were kept in the center of petri plate consisting *E. coli* culture. The test organism was streaked on the straight line from disk edge to plate edge. And then the plate were incubated for overnight at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in ambient air for 16–24 hours and result were interpreted [19-20].

EDTA disk synergy test (EDST):The Muller Hinton agar plate was inoculated with overnight culture suspension of MDR *P. aeruginosa*. After plate drying, a 10µg IMP disk and a sterilized a disk of filter paper were placed at 10mm distance from one edge to another, and 10 µl of EDTA solution (0.5 M) was also put on to the blank disk which is equivalent to almost 1.5 mg/disk. These culture plate along with disks were incubated overnight at 37°C , the appearance of a synergistic inhibition zone were indicated as EDST positive [21].

Imipenem EDTA combined disc test (CDT): An overnight suspension culture of MDR *P. aeruginosa*, which is adjusted to one tenth turbidity of 0.5 McFarland standard, were evenly inoculated on the petri plate containing MH agar with the help of cotton swab. After drying, two antibiotic disk containing imipenem (IMP; 10µg) antibiotics were kept on the dried plate, at 10mm distance from the border to border and 5 µl of EDTA solution (0.5M) was then put on one of the filter papersdisks to get an equivalent concentration of 0.75mg/disk. After 24 hours incubation at 37°C , the inhibition zones of IMP and IMP- EDTA disks were compared. For MBL producing organisms, the zone of inhibition has been found to increased in the plate containing IMP-EDTA by 8 to 15 mm (average 10.5 mm), while the increase of such zones for MBL-negative isolates was 1 to 5 mm (average 3.8 mm) [22].

Data collection: The samples and data were taken from the 42 inward patients infected with *P. aeruginosa* during the study period. Case patients were identified in 7 different wards. All 50 strain of *P. aeruginosa* collected were then processed by various microbiological techniques to separate MDR *Pseudomonas* species from non MDR.

Table: 1. The MDR isolated were tested for the production of Metallo - β Lactamase enzyme from different isolates

S.No	Sample	Total	MDR	Non-MDR
1	Sputum	8	1	7
2	Tracheal Secretion	5	4	1
3	Pus	4	2	2
4	Urine	15	6	9
5	Bile	1	1	-
6	Wound swab	2	2	-
7	Tissue	1	1	-
8	BAL	3	1	2
9	Blood	4	1	3
10	Peritoneal fluid	1	-	1
11	Throat swab	1	-	1
12	ET- secretion	4	-	4
13	CVP tip	1	1	-

Total sample = 50

Total MDR = 20

Total non-MDR = 30

All the MDR *P. aeruginosa* were first tested with Vitek2C (automated method), for MBL production. Then the test was confirmed and compared with manual method like, Modified

Hodge Test (MHT), EDTA disk synergy test (EDST) and Imipenem EDTA combined disk test (CDT).

After these all experiment, it was observed that all the MDR strain isolated were MBL producing.

Results

$$\begin{aligned}\text{MDR } P. aeruginosa \% &= (\text{MDR strain/Total strain isolated}) * 100 \\ &= (20/50) * 100 \\ &= 40\%\end{aligned}$$

From 50 strains of *P. aeruginosa*, 20 strain (40%) were MDR and all those MDR strain were capable to produce Metallo β -lactamase, whereas remaining 30 strain (60%) were from non-MDR group and were unable to produce MBL.

Resistance to antimicrobial agents is becoming major cause for public health threat [8]. Due to the antibiotic resistance ability of pathogen, therapeutic options are becoming limit and results in the high mortality[23]. This study has been conducted to understand the risk factors which leads to cause MDR *P. aeruginosa* infection.

Since metallo- β -lactamases induces antibiotic resistance against almost all the β -lactams drugs and hence the exposure to lactam type of antibiotics was a huge significant risk factor for hospital-produced *P. aeruginosa* metallo- β -lactamases infections. Long term use of β -lactams group of antibiotics is the major risk factor for transforming normal strain into multidrug resistant strain whereas patient contact with other patient having resistant strain is another big risk factor. The amount and dose of antibiotic taken is also playing an important role in the antibiotic resistant organism development including *P. aeruginosa*-producing metallo β -lactamases.

Conclusion

The current report shows that the one of the important risk factors for the infection with MDR *P. aeruginosa* is long-term hospitalization, excessive use of antibiotics (specially beta lactam class of antibiotics), use of catheter, and intake of anticancer agents, use of immunosuppressive agents like corticosteroids and some others. This infection mainly affects the immunocompromised patients and require grievous care and a better management with the combinations of antibiotics. The infections of MBL producing MDR *P. aeruginosa* linked with theadverse clinical outcome.

We also found that there are very rare antimicrobial agents left for the treatment of infection, caused by MDR *P. aeruginosa* producing metallo β -lactamase. Polymyxin B and E (colistin) are the two antibiotics which was effective during in vitro susceptibility testing, except all was ineffective against MDR strains. Till now we don't have any single effective drugs for against MDR *bla*IMP-positive *P. aeruginosa* and hence there is a requirement for the management strategy. The use of combination therapy can be one of such effective therapeutic strategies against MDR *P. aeruginosa*.

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