Prevalence and Antimicrobial Susceptibility Profile of Metallo-β-Lactamase Producing Pseudomonas aeruginosa Isolates at Kenyatta National Hospital

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Abstract

Pseudomonas aeruginosa is a major cause of nosocomial infections with high mortality rates. The organism is highly resistant to most classes of drugs used and can develop resistance during treatment. One of the resistance mechanisms of P. aeruginosasa is Metallo-β-Lactamase (MBL) production. MBL producing P. aeruginosa is a major health concern given its resistance to almost all available drugs. The prevalence of this resistant strain is unknown since there is no standardized method for testing MBL production. This was a laboratory based cross-sectional prospective study that was carried out from September 2015 to March 2016 at Kenyatta National Hospital. Ninety-nine isolates of P. aeruginosa were collected during the period and tested for antimicrobial susceptibility and isolates found to be resistant to imipenem tested for MBL production. The results indicated high resistance of P. aeruginosa to commonly used drugs. Of the isolates tested 69.7% were resistant to piperacillin, 63.6% were resistant to aztreonam, 58.6% were resistant to levofloxacin, 55.6% were resistant to cefipime, 65.7% were resistant to ceftazidime, 68.7% were resistant to ticarcillin-clavulanate, 72.2% were resistant to meropenem, 64.9% were resistance to imipenem while 86.4% of urine isolates were resistant to ofloxacin. Of the isolates resistant to imipenem 87.3% were found to be MBL producers. In conclusion, P. aeruginosasa is highly resistant to the drugs currently is used for treatment and resistance to carbapenems is largely due to MBL production.

Keywords

Pseudomonas aeruginosa, Metallo-β-Lactamase, Antimicrobial Resistance, Kenyatta National Hospital
1. Introduction

*P. aeruginosa* is a gram-negative bacteria which is widely distributed in nature. It’s a non-fastidious organism that has been isolated from sewage, distilled water, swimming pools, disinfectants, water baths, hot tubs, intravenous tubes and medical devices [1]. *P. aeruginosa* an opportunistic pathogen causes nosocomial infections and outbreaks with high mortality rates [2] [3] [4]. It rarely causes infection in healthy subjects but causes infection in the immunocompromised, burns patients, and organ transplant recipients and where there is disruption of physical barriers such as in the use of invasive devices [1] [5]. Most pathogens that cause nosocomial infections exhibit resistance to antimicrobial agents. Unfortunately, selection of the right drug is complicated by the organism’s ability to develop resistance to available classes of drugs even during treatment. Choice of treatment for *Pseudomonas* infection is limited. Drugs used for treatment include: beta lactams, aminoglycosides, fluoroquinolones with ciprofloxacin being the most active, and polymyxins (polymyxin B and colistin whose use is restricted to multi drug resistant *P. aeruginosa* (MDRPA) due to toxicity) [6]. *P. aeruginosa* is a multidrug resistant (MDR) organism and has several resistance mechanisms which include; chromosomal AmpC cephalosporinase depression, loss of permeability of the outer membrane (loss of OprD proteins), over expression of active efflux pumps, amino glycoside modifying enzymes synthesis, structural alterations of topoisomerase II and IV and plasmid or integron mediated beta-lactamases [7] [8] [9]. Carbapenems (meropenem, doripinem, imipenem) are the last line drugs for treatment of MDRPA but resistance to these drugs has been detected in some strains [10]. These strains produce carbapenem hydrolyzing enzymes (carbapenemases) which mediate resistance to carbapenems. Carbapenemases are mostly MBLs and include: Imipenemase (IMP), Australian imipenemase (AIM), Sao Paolo MBL (SPM), Verona integron encoded MBL (VIM), Seoul imipenemase (SIM) German imipenemase (GIM) and most recently New Delhi MBL (NDM) [11]. MBLs have a worldwide distribution and have been identified virtually in all continents and their spread is continuing [12]. This study was carried out to determine the resistance of *P. aeruginosa* against commonly used antibiotics and to determine MBL production.

2. Materials and Methods

2.1. Study Site

This was a laboratory based cross-sectional prospective study that was carried out from September 2015 to March 2016 at Kenyatta National Hospital which is the largest referral hospital in Kenya.

2.2. Antimicrobial Susceptibility Testing

A total of 99 isolates were collected from samples of patients at Kenyatta National Hospital (KNH) and antimicrobial susceptibility carried out using agar plate method according to Clinical and Laboratory Standards Institutes (CLSI)
guidelines. The zone of clearance was measured using a ruler and recorded in millimeters (mm) and was interpreted sensitive, intermediate or resistant according CLSI guidelines [13] Antibiotics tested included piperacillin 100 µg, aztreonam 30 µg, cefepime 30 µg, levofloxacin 5 µg, ceftazidime 30 µg, ticarcillin-clavulanate 75/10 µg, ofloxacin 5 µg (urine isolates) imipenem 10 µg and meropenem 10 µg.

2.3. Mbl Detection

A 0.5 M Ethylene diaminetetraacetic acid (EDTA) solution was prepared by dissolving 186.1 g of disodium EDTA. 2H₂O in 1000 ml of distilled water and the pH was adjusted to 8 using NaOH. The mixture was then sterilized by autoclaving. A4 (microliters) pipette the EDTA solution was poured on imipenem disks (the EDTA works by blocking MBL production). The EDTA impregnated antibiotic disks were dried immediately in an incubator. A broth culture of test strain (opacity adjusted to 0.5 McFarland opacity) was inoculated on a plate of Mueller Hinton Agar (BD Biosciences, Germany). One 10 µg imipenem disk was placed on the agar plate. Each of EDTA impregnated disk was placed on the same agar plate. The plate was incubated at 37˚C for 16 - 18 h the zone of clearance was measured using a ruler and comparison was made between the imipenem discs and the EDTA impregnated discs. A zone of clearance of at least 7 mm around the imipenem-EDTA disk as compared to imipenem disk without EDTA was recorded as an MBL producing strain [14].

3. Results

The antimicrobial susceptibility of P. saeruginosa is given in Table 1 and Table 2.

More than half of the isolates (52.5%) were males and 44.4% were females.

More than three quarters (77.8%) of the isolates were from the intensive care unit (ICU), 15.2% were from other wards while 2% were obtained from outpatient department. The specimens taken were mainly tracheal aspirates (67.7%) while 23.2% were urine samples and 8.1% pus.

3.1. Antibiotic Susceptibility of P. aeruginosa

P. aeruginosa had high level of resistance to the antibiotics tested.

As shown in Table 3, in all the instances, isolates from the ICU had a higher level of resistance to the listed drugs than isolates from other wards. ICU isolates were 68.8% resistant to ceftazidime, 74% to piperacillin, 74% to ticarcillin-clavulanate, 66.2% to aztreonam and 100% to ofloxacin.

3.2. MBL Production

MBL production was evaluated in the imipenem resistance isolates (63) and 87.3% (55) of the isolates were MBL producers while 12.7% (8) were non MBL producers.
Table 1. Antibiotic susceptibility of *P. aeruginosa*.

| Drug                  | Sensitive n (%) | Intermediate n (%) | Resistant n (%) |
|-----------------------|-----------------|--------------------|-----------------|
| Cefepime              | 37 (37.4)       | 7 (7.1)            | 55 (55.6)       |
| Ceftazidime           | 29 (29.3)       | 5 (5.1)            | 65 (65.7)       |
| Piperacillin          | 27 (27.3)       | 3 (3.0)            | 69 (69.7)       |
| Ticarcillin-clavulanate | 23 (23.2)     | 8 (8.1)            | 68 (68.7)       |
| Aztreonam             | 26 (26.3)       | 10 (10.1)          | 63 (63.6)       |
| Levofloxacin          | 38 (38.4)       | 3 (3.0)            | 58 (58.6)       |
| Ofloxacin (n = 22)    | 3 (13.6)        | 0                  | 19 (86.4)       |
| Meropenem (n = 97)    | 24 (24.7)       | 3 (3.1)            | 70 (72.2)       |
| Imipenem (n = 97)     | 34 (35.1)       | 0                  | 63 (64.9)       |

Table 2. Antibiotic susceptibility by type of specimen.

| Drug                  | Tracheal aspirates | Urine | Pus |
|-----------------------|--------------------|-------|-----|
| Cefepime              |                    |       |     |
| Sensitive             | 26 (38.8)          | 4 (17.4) | 6 (75.0) |
| Intermediate          | 6 (9.0)            | 1 (4.3)  | 0   |
| Resistant             | 35 (52.2)          | 18 (78.3) | 2 (25.0) |
| Ceftazidime           |                    |       |     |
| Sensitive             | 20 (29.9)          | 4 (17.4) | 5 (62.5) |
| Intermediate          | 5 (7.5)            | 0      | 0   |
| Resistant             | 42 (62.7)          | 19 (82.6) | 3 (37.5) |
| Piperacillin          |                    |       |     |
| Sensitive             | 18 (26.9)          | 4 (17.4) | 5 (62.5) |
| Intermediate          | 3 (4.5)            | 0      | 0   |
| Resistant             | 46 (68.7)          | 19 (82.6) | 3 (37.5) |
| Ticarcillin-clavulanate |                 |       |     |
| Sensitive             | 17 (25.4)          | 3 (13.0) | 3 (37.5) |
| Intermediate          | 5 (7.5)            | 1 (4.3)  | 1 (12.5) |
| Resistant             | 45 (67.2)          | 19 (82.6) | 4 (50.0) |
| Aztreonam             |                    |       |     |
| Sensitive             | 19 (28.4)          | 4 (17.4) | 3 (37.5) |
| Intermediate          | 9 (13.4)           | 0      | 1 (12.5) |
| Resistant             | 39 (58.2)          | 19 (82.6) | 4 (50.0) |
| Levofloxacin          |                    |       |     |
| Sensitive             | 29 (43.3)          | 4 (17.4) | 4 (50.0) |
| Intermediate          | 2 (3.0)            | 0      | 1 (12.5) |
| Resistant             | 36 (53.7)          | 19 (82.6) | 3 (37.5) |
| Meropenem             |                    |       |     |
| Sensitive             | 16 (24.6)          | 4 (17.4) | 4 (50.0) |
| Intermediate          | 3 (4.6)            | 0      | 0   |
| Resistant             | 46 (70.8)          | 19 (82.6) | 4 (50.0) |
| Imipenem              |                    |       |     |
| Sensitive             | 27 (40.9)          | 4 (18.2) | 3 (37.5) |
| Resistant             | 39 (59.1)          | 18 (81.8) | 5 (62.5) |

*Sputum specimen excluded due to small numbers (n = 1).*
Table 3. Antibiotic susceptibility by source of isolates.

| Antibiotic            | Patients’ source* | ICU     | Other wards |
|-----------------------|-------------------|---------|-------------|
|                       |                   | ICU     | Other wards |
| Cefepime              | Sensitive         | 26 (33.8) | 9 (60.0)   |
|                       | Intermediate      | 7 (9.1)  | 0           |
|                       | Resistant         | 44 (57.1) | 6 (40.0)   |
| Ceftazidime           | Sensitive         | 19 (24.7) | 9 (60.0)   |
|                       | Intermediate      | 5 (6.5)  | 0           |
|                       | Resistant         | 53 (68.8) | 6 (40.0)   |
| Piperacillin           | Sensitive         | 17 (22.1) | 9 (60.0)   |
|                       | Intermediate      | 3 (3.9)  | 0           |
|                       | Resistant         | 57 (74.0) | 6 (40.0)   |
| Ticarcillin-clavulanate | Sensitive     | 15 (19.5) | 7 (46.7)   |
|                       | Intermediate      | 5 (6.5)  | 2 (13.3)   |
|                       | Resistant         | 57 (74.0) | 6 (40.0)   |
| Aztreonam             | Sensitive         | 17 (22.1) | 8 (53.3)   |
|                       | Intermediate      | 9 (11.7) | 1 (6.7)    |
|                       | Resistant         | 51 (66.2) | 6 (40.0)   |
| Levofloxacin          | Sensitive         | 27 (35.1) | 9 (60.0)   |
|                       | Intermediate      | 3 (3.9)  | 0           |
|                       | Resistant         | 47 (61.0) | 6 (40.0)   |
| Ofloxacin             | Sensitive         | 0        | 3 (42.9)   |
|                       | Resistant         | 11 (100.0) | 4 (57.1)  |
| Meropenem             | Sensitive         | 15 (20.0) | 8 (53.3)   |
|                       | Intermediate      | 2 (2.7)  | 0           |
|                       | Resistant         | 58 (77.3) | 7 (46.7)   |
| Imipenem              | Sensitive         | 25 (33.3) | 8 (53.3)   |
|                       | Resistant         | 50 (66.7) | 7 (46.7)   |

*Outpatients were excluded due to small numbers (n = 2).
4. Discussion

*P. aeruginosa* is a serious threat in health care settings. In this study, most isolates were from the ICU (77.8%) and the highest resistance was in ICU. This is because the ICU has one of the highest occurrence rates of nosocomial infections 20% - 30% [15]. Of the isolates tested 69.7% were resistant to piperacillin, 63.6% were resistant to aztreonam, 58.6% were resistant to levofloxacin, 55.6% were resistant to cefepime, 65.7% were resistant to ceftazidime, 68.7% were resistant to ticarcillin-clavulanate, 86.4% of urine isolates were resistant to ofloxacin, 72.2% were resistant to meropenem while 64.9% were resistance to imipenem. Of the isolates resistant to imipenem 87.3% were found to be MBL producers. A previous study carried out in Kenya in a private hospital showed that 53% of the isolates of *P. aeruginosa* were resistant to piperacillin and aztreonam, whereas 100% were resistant to ceftazidime, cefepime, tobramycin, gentamicin, amikacin and ciprofloxacin, in this study the results differ slightly [16]. Also, majority of our isolates were from the (ICU 77.8%), 15.2% were from other wards while 2% were from outpatients. Majority of our isolates were tracheal aspirates 67.7%, 23.2% were urine samples while 8.15 % were from pus, a similar study carried out Kenyain the Aga Khan University Hospital, of the isolates tested three (3%) were isolated from urine, four (7%) from blood, 17 (30%) from wounds (purulent), 30 (53%) from respiratory tract specimens, and the remaining three (5%) from various other specimens [16].

The results obtained are comparable to a study carried out in Iran in 2015 [17] whereby the rate of resistance to imipenem was 72% while MBL production identified on isolates resistant to imipenem was 88.9% this was slightly higher compared to our study. A study carried out in a private hospital in Kenya in 2008 during an outbreak of *P. aeruginosa* infection to characterize the betalactamases content of carbapenem resistant *P. aeruginosa* found that all carbapenem resistant isolates were MBL producers and the gene isolated was VIM-2 [16]. These findings were slightly higher than the results we obtained. A study carried out in Tunisia showed that all the strains tested were resistant to all anti-pseudomonal drugs that is betalactams, aminoglycosides and fluoroquinolones, only 67% of the strains tested were MBL producers [18]. Variation in results obtained differ due to multiple factors which include geographical location, the drugs that are prescribed to treat *P. aeruginosa* infections ,the dosing regimen and local hospital practices in dealing with patients with resistant pathogens [19] [20].

It’s important to note that resistance to carbapenems is not only due to MBL production but could also be due to many other mechanisms. These mechanisms include secondary changes in regulatory system of MBL gene expression, outer membrane permeability, active efflux systems in bacterial membrane and/or multiplication of structure gene. The most common mechanism of resistance to carbapenems besides MBL production is loss or alteration of the outer membrane porin protein OprD [11] [21]. The porin protein OprD is the major portal
of entry for carbapenems [22] while impermeability due to loss of the OprD porin or upregulation of the active efflux pump system in the cytoplasmic membrane of the *P. aeruginosa* causes non-MBL resistance. In our study (12.7%) of the isolates were non-MBL producers [23]. This type of resistance requires the presence of AmpC (inducible or stably derepressed) [24]. From this study it is quite evident that MBL poses a serious risk in health setups considering that MBL resistant isolates can be resistant to all betalactams posing a serious problem in the treatment of *P. aeruginosa* infections.

5. Conclusion

*P. aeruginosa* highly resistant to the drugs currently used for treatment and also resistance to carbapenems is largely due to MBL production. It’s necessary to have a routine surveillance of MBL production in order to guide the physicians on the most effective treatment regimen and also to prevent further spread of the enzymes to other bacterial groups like the enterobacteriaceae family. It’s also important to test various combinations of drugs for treatment of infections that are resistant to carbapenems and use of polycationic antimicrobials (colistin and polymyxin B) should be considered for the carbapenem resistance isolates and also such antimicrobial susceptibility should regularly be tested.

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Author’s Contribution

JN developed the concept and drafted the proposal and the manuscript. Miss. SO, Dr. AM and Dr. MM guided in the drafting the proposal and the manuscript and clean-up of the same.

Conflicts of Interest

None.

Ethical Approval

This study was approved by KNH-UoN Ethics and research committee which is a committee that regulates research and ensures adherence to ethical principles to help safeguard the dignity rights safety and well-being of all actual or potential research participants by vetting proposals and overseeing conduct of research.

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