

Molecular Characterization of Beta-lactams Resistance in *Pseudomonas aeruginosa* Isolated from Clinical Sources at the Nairobi Hospital

Armstrong Ndiokubwayo^{1*} Caroline W. Ngugi¹ Paul Makau² Andrew K. Nyerere¹

1. Department of Medical Microbiology, School of Biomedical Sciences, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, P.O BOX 62000-00200, Nairobi, Kenya

2. Department of Pathology, The Nairobi Hospital, P.O BOX 30026-00100, Nairobi, Kenya

Abstract

The increase of Beta-lactamases producing organisms can cause major therapeutic failure and poses a significant clinical challenge in healthcare settings. A total of 185 clinical isolates of *Pseudomonas aeruginosa* strains were collected from in-and out-patients at The Nairobi Hospital, 74.1 % were inpatients and 25.9% were outpatients with the high prevalence of this bacterium among the male gender (61.1%) than female(38.9%); and preponderantly comprising the patients above 45 years old (64.3%). The highest numbers of *P. aeruginosa* were isolated from pus swab (39.5%), respiratory secretions (25.9%), and urine (18.9%). The resistance rate of *P. aeruginosa* against carbapenem was 31.5% among the isolates. The prevalence of MBL producing *P. aeruginosa* was 22.7% as compared to non-MBL isolates (77.3%). The MBL isolates were resistant to the examined antibiotics. There were two predominant genes VIM-2 (28.57%) and NDM-1 (66.67%) types among MBL *P. aeruginosa*, and more prevalent genes were isolated from Critical care nursing ward; Intensive Care Unit (45.2%) and High Dependency Unite (28.6%) at The Nairobi Hospital. These findings suggest that the early detection of Metallo-Beta-Lactamases-producing isolates and the cooperation between medical professionals and infection control team may help in appropriate antimicrobial therapy and avoid further spread of these multidrug resistance strains.

Keywords: *Pseudomonas aeruginosa*, Metallo-Beta-Lactamases, Resistance, Beta-lactams.

1. Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is responsible for 10–15% of the nosocomial infections worldwide. Often these infections are hard to treat due to the natural resistance of the species, as well as to its remarkable ability of acquiring further mechanisms of resistance to multiple groups of antimicrobial agents (Strateva and Jordanov, 2009). The global emergence of *P. aeruginosa* resistance to multiple antibiotics including Beta-lactams represents an extraordinary threat to public health (Toval et al., 2015). *P. aeruginosa* develops resistance by various mechanisms like multi-drug resistance efflux pumps, outer-membrane permeability, biofilm formation, production of β -lactamases and aminoglycoside modifying enzymes (Mahmoud et al., 2013). In this context, infections by *P. aeruginosa* harboring acquired mechanisms of resistance, such as production of MBLs and ESBLs have high clinical impact. These “newer Beta-lactamases” are capable of hydrolyzing a wide range of Beta-lactam antibiotics, notably the extended-spectrum penicillins and the third and fourth generation cephalosporins, which include the carbapenems (Oberoi et al., 2013; Wadekar et al., 2013). Carbapenems are the drug of choice for treatment of serious infections due to ESBL-producing organisms including *P. aeruginosa* but yet, carbapenem-resistant isolates have been reported (Ejikeugwu et al., 2012). MBL-producing *P. aeruginosa* isolates are responsible for several nosocomial outbreaks from different parts of the World, illustrating the need for proper infection control practices (Pitout et al., 2008). Those strains are particularly problematic leading to increased mortality, longer hospital stays and higher hospital costs over and above the values associated with susceptible strains of this pathogen (Hong et al., 2015). Its current impact on antibacterial therapy failure encouraged us to find out the Beta-lactamases mediated resistance rate and current antibiotic options for effective treatment of this opportunistic organism (Ansari et al., 2016). This study aimed to address the molecular characterization of Beta-lactams resistance in *P. aeruginosa* isolated from clinical sources in different wards at The Nairobi hospital in Kenya.

2. Materials and Methods

2.1 Collection of strains

P. aeruginosa used in this study were clinical isolates obtained from in-and out-patients at The Nairobi Hospital, Nairobi-Kenya. A total of 185 non-duplicate consecutive isolates were collected from the Pathology Department of the hospital between May 2017 and April 2018 and were identified from clinical Samples as *Pseudomonas aeruginosa* by use of Vitek 2 Compact System and stored at -20°C in a Brain Heart Infusion (BHI) Medium (Himedia, India) with glycerol (30%) according to standard techniques.

2.2 Antimicrobial Susceptibility patterns (David H., BioMérieux, USA)

Isolates on MacConkey (Oxoid, UK) were subcultured on Nutrient Agar plate (Oxoid, UK) and incubated at 35°C overnight. Inoculums were prepared from a pure culture, according to good laboratory practices. The 0.5McFarland bacterial suspension was diluted with 1.5×10^7 /ml in 0.45% saline and measured with DensiCheck. Aseptically, 3.0 ml of sterile saline (0.45% to 0.5% NaCl, pH 4.5 to 7.0) were transferred into a clear plastic (polystyrene) test tube (12 mm x 75 mm). A sterile swab was used to transfer a sufficient number of morphologically similar colonies to the saline tube. In a second tube containing 3.0 ml of saline, 145 µl of the suspension was transferred for AST-GN cards. (BioMérieux, USA) Then this tube was placed in the cassette with a susceptibility card. All the cards were automatically filled, sealed, and loaded into the Vitek 2 compact for instrument and reading. The MICs of each antimicrobial agent (piperacillin/tazobactam PTZ 110µg, cefotaxime CTX 30 µg, ceftazidime CAZ 30 µg, cefepime FEP 30 µg, meropenem MER 10 µg, aztreonam AZM 30 µg, gentamycin CN 10 µg, amikacin 30 µg and ciprofloxacin 5 µg) tested against *Pseudomonas aeruginosa* were generated by the Vitek 2 Compact according to MIC interpretation Standard in line with CLSI recommendations. In the Same time, the resistance to meropenem was interpreted by the expert system as a mechanism of Carbapenemase production for *P. aeruginosa*. The results were finally generated by the Expert Advanced System (EAS) of the Vitek 2 Compact for MBL phenotype. Alternative test methods such as targeted PCR amplification were required to confirm MBL-producing the isolates.

2.3 Extraction of total DNA

The total DNA content from $\leq 5 \times 10^6$ bacterial cells was extracted using Quick-DNA Mini-Prep plus Kit (Zymo Research, USA) via mechanical method according to kit's instructions. The eluted DNA was stored at $\leq -20^\circ\text{C}$ for future use.

2.4 PCR assays for the detection of Metallo-Beta-Lactamases (MBL) encoding genes

Four pairs of specific primers introduced by Shibata et al. (2003) with one pair primer experienced by Mushi et al. (2014) (See Table 1) were used in this study, which specifically detected vim1, vim2, imp1, imp2, and ndm1 genes. The mixture for amplification of these genes consisted of Taq 2x Master Mix (BioLabs, UK) 12.5 µl followed by Forward-primer (10µmol/µl) 0.5 µl and Reverse-primer (10µmol/µl) 0.5 µl then Nuclease free water 9µl and lastly the DNA template 2.5 µl, in final volume of 25 µl. The PCR conditions were 5 minutes at 94°C and 35 cycles of 1 min at 94°C for the denaturation of the template, 45 seconds at an annealing temperature (A.T) of 51°C and 1 min 30 seconds at 72°C extension before a final extension at 72°C (Sepehriseresht et al., 2012).

2.5 Agarose gel electrophoresis

Horizontal electrophoresis system containing 2% agarose (AMRESCO, USA) gel in the TAE Buffer was used. Voltage was set at 110V for 35 minutes. E-Z vision, in gel solution staining (AMRESCO, USA) was performed for 20 minutes with 100 bp DNA Ladder (BioLabs, UK) to determine molecular weight. Additionally, the PCR products were visualised by gel imaging instrument.

2.6 Statistical analysis

The results were expressed using descriptive analyses (mean, standard deviation, frequency, and proportion). The data was analysed with the Statistical Software SPSS version 21.0 for windows. Chi-square or Fisher's exact test was used where applicable. The data presentation by tables, graphs or figures was done using Excel Software version 2013 (Microsoft Corporation, USA). This investigation was approved by Bioethics and Research Committee of The Nairobi Hospital.

Table 1. Primers sequences used for detection of MBL genes (Shibata et al., 2003; Mushi et al., 2014)

Genes	Oligonucleotides sequences	A.T (°C)	Product size	Reference
vim1	F: AGT GGT GAG TAT CCG ACA G R: ATG AAA GTG CGT GGA GAC	55	261	Shibata et al.(2003)
vim2	F: ATG TTC AAA CTT TTG AGT AAG R: CTA CTC AAC GAC TGA GCG	51	801	Shibata et al.(2003)
imp1	F: ACC GCA GCA GAG TCT TTG CC R: ACA ACC AGT TTT GCC TTA CC	51	587	Shibata et al. (2003)
imp2	F: GTT TTA TGT GTA TGC TTC C R: AGC CTG TTC CCA TGT AC	51	678	Shibata et al. (2003)
ndm1	F:GGT TTG GCG ATC TGG TTT TC R: CGG AAT GGC TCA TCA CGA TC	52	621	Mushi et al. (2014)

3. Results

Out of 185 clinical isolates, the high prevalence of the bacterium isolates was seen among the male gender than female (61.1% and 38.9%, respectively), and the high rate of isolates were found among the age groups above 45 years (64.3%) with the mean age of patients of 56.1 ± 21.6 years old, ranging from 4 to 100 years (Table 2). This table 2 demonstrates also that out of 185 subjects who showed the growth of *P. aeruginosa* isolates, 137 (74.1 %) were inpatients and 48 (25.9%) were outpatients.

Table 2. Distribution of demographic characteristics for patients with *P. aeruginosa* infections

Age/Year	Number of isolates	%
Age group <15	4	2.2
Age group 15-30	23	12.4
Age group 31-45	39	21.1
Age group 46-60	37	20.0
Age group 61-75	39	21.1
Age group 76-90	38	20.5
Age group >90	5	2.7
Sex	Frequency (N=185)	%
Female	72	38.9
Male	113	61.1
Type of patient	Frequency (N=185)	%
Inpatient	137	74.1
Outpatient	48	25.9

Table 3. Distribution of *P. aeruginosa* isolates by type of clinical samples

Type of specimen	Number of isolates	Proportion (%)
Pus and wound swab	73	39.5
Sputum	48	25.9
Body fluid	9	4.9
Urine	35	18.9
Blood	3	1.6
Catheter tip	11	5.9
Tissue or Biopsy	6	3.2
Total	185	100.0

In the present study, the specimens showing the highest isolation of *P. aeruginosa* were from pus and wound swab (39.5%), sputum and respiratory aspirates (25.9%), and urine (18.9%), followed by the lowest isolation of *P. aeruginosa* such as catheters tips (5.9%), body fluid (4.9%), biopsy (3.2%) and blood (1.6%) (Table 3).

Table 4. Antimicrobial susceptibility profile for *Pseudomonas aeruginosa* isolates

Antibiotics tested	Sensitive (%)	Intermediate (%)	Resistant (%)
Piperacillin/tazobactam (PTZ)	102 (55.1)	14 (7.6)	69 (37.3)
Cefepime (FEP)	125 (67.6)	8 (4.3)	52 (28.1)
Ceftazidime (CAZ)	126 (68.1)	7 (7.6)	52 (28.1)
Cefotaxime (CTX)	0 (0)	2 (3.8)	183 (98.9)
Meropenem (MER)	122 (65.9)	5 (2.7)	58 (31.4)
Gentamycin (CN)	125 (67.6)	1 (0.5)	59 (31.9)
Amikacin (AK)	126 (68.1)	0 (0)	59 (31.9)
Ciprofloxacin (CIP)	126 (68.1)	1 (0.5)	58 (31.4)
Aztreonam (AZM)	70 (37.8)	3 (1.6)	40 (21.6)

The resistant rates for β -lactam antibiotics including cefepime, ceftazidime, meropenem, piperacillin/tazobactam, and aztreonam were respectively 28.1%, 28.1%, 31.4%, 37.3%, 21.6%. The resistant rates for non- β -lactam antibiotics including amikacin, gentamycin, and ciprofloxacin were 31.9%, 31.9% and 31.4% (Table 4).

Of the One Hundred and Eighty Five (185) *P. aeruginosa* isolates, forty two (22.7%) positively expressed MBL Carbapenemases enzymes. The antimicrobial susceptibility patterns of MBL producing *P. aeruginosa* showed that all the MBL isolates were resistant to the antibiotics tested. Chi-square (χ^2) or two-tailed Fisher's exact test was used where applicable. The difference in resistance levels between MBL and non-MBL producing isolates for all the antimicrobial drugs (fluoroquinolones, aminoglycosides, and Beta-lactams) tested were statistically significant, ($p < 0.05$, $P < 0.001$) (Table 5).

In this study, the highest numbers of cases of MBL-producing *P. aeruginosa* were isolated from Intensive Care Unit (ICU) (45.2%) and High Dependency Unit (HDU) (28.6%) with predominant distribution in pus swab (38.1%) and sputum (33.3%). The isolates with resistant pattern to meropenem and MBL production were subjected to PCR for MBL genes BlaVIM-1, BlaVIM-2, BlaIMP-1, BlaIMP-2 and BlaNDM-1.

Table 5. Antibiotic Resistance for MBL and Non-MBL *P. aeruginosa* isolates

Variable	MBL(=42)	Non-MBL(=143)	Significance level
Antibiotics tested	Resistant (%)	Resistant (%)	P-Value
Piperacillin/tazobactam (PTZ)	42 (100)	27 (18.9)	P<0.001
Cefepime (FEP)	42 (100)	10 (7.0)	P<0.001
Ceftazidime (CAZ)	42 (100)	10 (7.0)	P<0.001
Meropenem (MER)	42 (100)	16 (11.2)	P<0.001
Gentamycin (CN)	42 (100)	17 (11.9)	P<0.001
Amikacin (AK)	42 (100)	17 (11.9)	P<0.001
Ciprofloxacin (CIP)	42 (100)	16 (11.2)	P<0.001
Aztreonam (AZM)	42 (100)	19 (20.7)	P<0.001

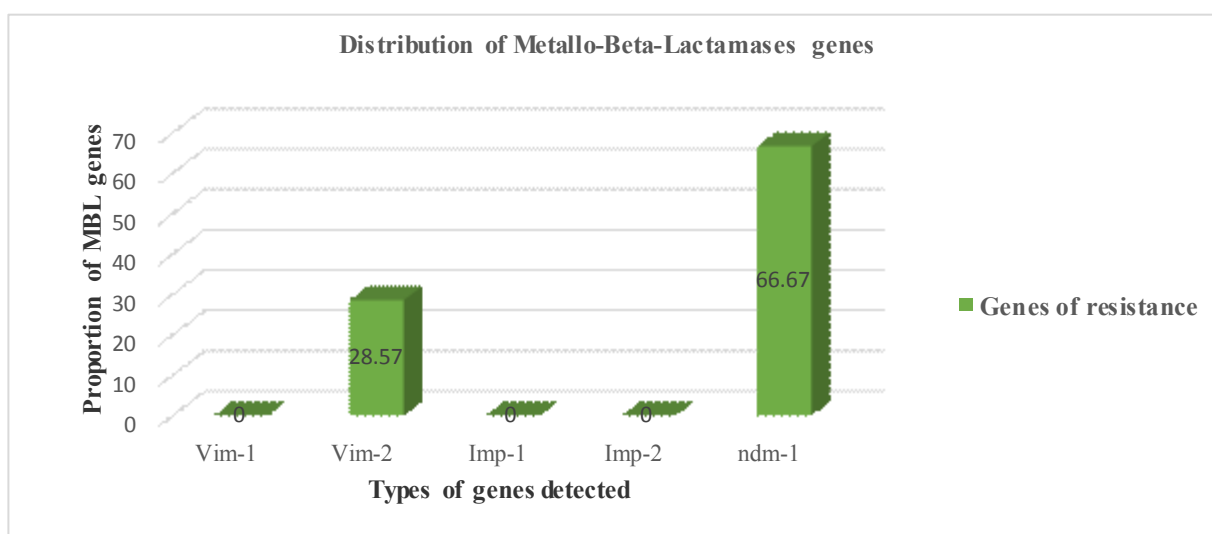


Figure 3. Distribution of Metallo-Beta-Lactamases genes among the *P. aeruginosa* isolates

The PCR assays performed for metallo-beta-lactamases were positive in twelve MBL isolates (28.57%) for BlaVIM-2 and twenty eight MBL isolates (66.67%) for BlaNDM-1. None of the MBL *P. aeruginosa* isolates carried BlaVIM-1, Bla IMP-1 and BlaIMP-2. From the 42 MBL isolates, three (7.14%) isolates had both BlaNDM-1 and BlaVIM-2 genes (Figure 1, 2, 3).

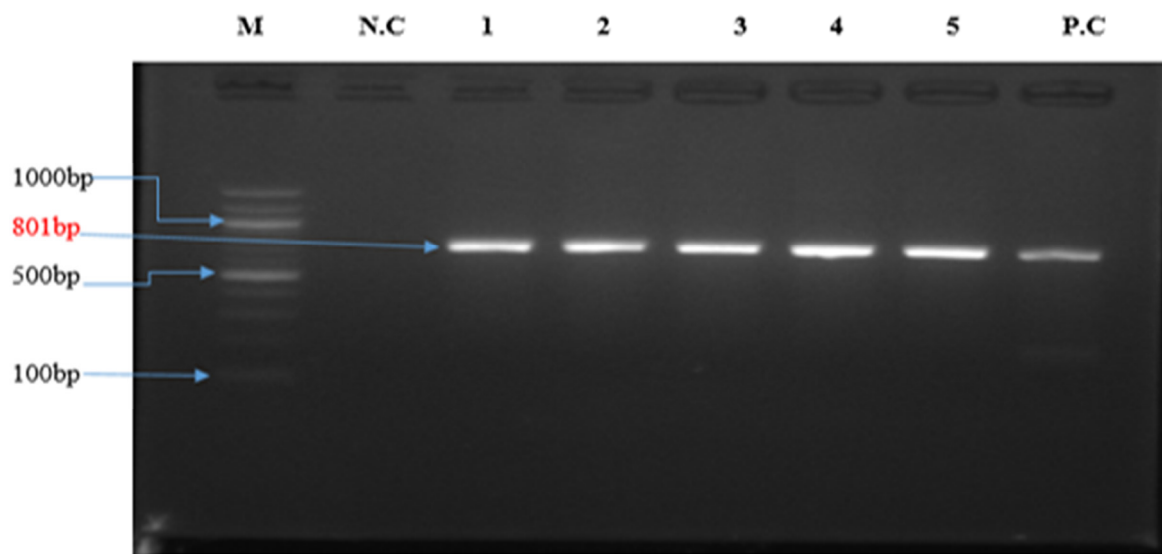


Figure 1. Agarose gel electrophoresis of BlaVIM-2 PCR products
Lanes 1, 2, 3, 4 and 5 show isolates positive for BlaVIM-2. M shows 100 bp DNA Ladder. Lane P.C shows the positive control. Lane N.C is the Negative Control.

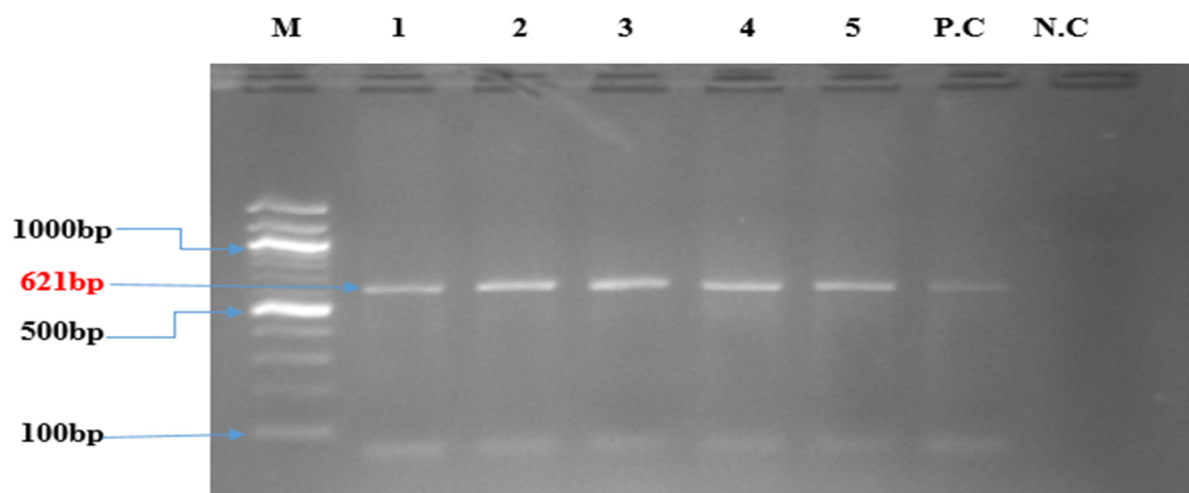


Figure 2. Agarose gel electrophoresis of BlaNDM-1 PCR products.
Lanes 1, 2, 3, 4 and 5 show isolates positive for BlaNDM -1. M shows 100 bp DNA Ladder. Lane P.C shows the positive control. Lane N.C is the Negative Control.

4. Discussion

This is one of the few studies that has evaluated the magnitude of Metallo-Beta-Lactamases producing isolates and characterized the antimicrobial resistance among *Pseudomonas aeruginosa* isolates obtained from clinical sources in Kenya. In the present study, a total of 185 isolates from clinical samples were identified as *Pseudomonas aeruginosa* from May 2017 to April 2018. According to gender and age group distribution, our findings agree with the study of Mahmoud *et al.* (2013) and Kiana *et al.* (2016) where *P. aeruginosa* infections were common in males than female cases and their mean age were 44.6 ± 25 and 49.39 ± 16 years old, respectively (Mahmoud *et al.*, 2013; Shirani *et al.*, 2016). These results differ with the study of Zafer *et al.* in which the highest number of cases were found in males with a low mean age of 24.5 years with the highest number of cases in younger patients (54%)

Same observation has been made by Al-Zaida (2016) in which results of the studies showed higher occurrence of the bacterium in male (55.6%) and younger patients (38.9%) (Zafer *et al.*, 2014; Al-Zaida *et al.*, 2016). The preponderance of males can be explained by greater number of cases from Critical Care Nursing ward (Intensive Care Unit) which should have more admissions of male patients in this hospital. This particular age group should be explained by the immunocompromised condition due to lifestyle disease among the old age

groups (>45 years old). In our study *P. aeruginosa* isolates are more prevalent in inpatients (74.1 %) as compared to the outpatients (25.9%). A similar finding was observed by others authors (Sandhya and Swathi, 2014). These results are importantly expected because *Pseudomonas aeruginosa* is most frequently associated with hospital-acquired infection compared to the community-acquired infection of this organism reported in different studies (Zafer *et al.*, 2014; Sandya and Swathi, 2014; Farajzadeh *et al.*, 2014; Jayarani *et al.*, 2014).

The specimens showing the highest isolation of *P. aeruginosa* were from pus and wound swab (39.5%), followed by the sputum (25.9%), and urine (18.9%). This is also comparable with studies of many researchers in which the highest number of *P. aeruginosa* strains were isolated from pus and wound swab followed by urine and sputum (Mahmoud *et al.*, 2013; Zafer *et al.*, 2014; Mohanam and Thangam, 2017; Wassef *et al.*, 2015). These findings should be explained by the long hospital stay of the patient in ICU in which is found the application of medical devices, which prompt the selection of nosocomial pathogens. *P. aeruginosa*, as nosocomial strain, is well known to be the leading cause of cystic fibrosis among lower respiratory tract infections, and surgical wounds infections. *P. aeruginosa* from patients demonstrated resistance to piperacillin/tazobactam, gentamycin, amikacin, ciprofloxacin, meropenem, ceftazidime and aztreonam in the following order respectively: 37.1%, 31.9%, 31.9%, 31.4%, 31.4%, 28.1%, 28.1%, and 21.6%. Similar results have been found by Bangera *et al.* (2016) where 38.0% of the isolates showed resistance to gentamicin followed by ceftazidime (31.69%) and meropenem (33.03%) (Bangera *et al.*, 2016). This is in contrast with the study of Radan *et al.* (2016) in which the resistance rate to ciprofloxacin, imipenem, meropenem, amikacin, ceftazidime, and ceftazidime was higher than 90%, while the resistance rates to piperacillin/tazobactam and aztreonam were 70.7% and 86%, respectively (Radan *et al.*, 2016). This difference can be attributed to the production of different factors through the resistance genes and mutational processes. Resistance of *P. aeruginosa* to antibiotics is the result of the production of enzymes that inactivate and degrade antibiotics, reducing the membrane permeability and the multidrug resistance efflux system (Mahmoud *et al.*, 2013; Wassef *et al.*, 2016). Our study revealed good activity of ceftazidime (67.6%), and Ceftazidime (68.1%). In contrast, cephalosporins tested in a study conducted in Egypt, showed that 90% of the isolates were resistant to ceftazidime (91%) and Ceftazidime (98%) (Mahmoud *et al.*, 2013). This difference may be due to the absence of Extended Spectrum β -Lactamases (ESBLs) production in our *P. aeruginosa* isolates used β -lactamase mediated resistance against cephalosporins.

Out of 185 strains of *P. aeruginosa* 31.5 % were meropenem resistant. This prevalence of carbapenem resistance is comparable with the study of Ghasemian *et al.* (2018) in which the prevalence of Carbapenem-Resistant *P. aeruginosa* (CRPA) was 37.7% among the isolates (Ghasemian *et al.*, 2018). In most countries, the reported Carbapenem-resistance of *P. aeruginosa* (CRPA) ratio ranged from 10 to 50%. Many CRPA strains have been identified and their prevalence is increasing gradually (Hong *et al.*, 2015; Meradji *et al.*, 2016; Dogonchi *et al.*, 2018). This discrepancy can be explained by both carbapenem use and the application of medical devices, which prompt the selection of resistant organisms. Both factors are related to patient characteristics, clinical practice policies and antibiotic prescription patterns of health care facilities in each hospital.

The rate of MBL producing *P. aeruginosa* (22.7 %) was not alarming and still awareness compared to the study of others researchers in which MBL producing *P. aeruginosa* were 14% in a tertiary hospital in Kenya and 17 % in Egypt (Pitout *et al.*, 2008, Abaza *et al.*, 2017). Different studies have reported higher rates of MBL production among *P. aeruginosa* isolates (E.g.: 84.5% in Brazil and 68.6% in Nepal) (Acharya *et al.*, 2017; Kalluf *et al.*, 2017). This difference can be attributed to the continuous development of Multi-drug resistant strains in different parts of the world, also a considerable geographic difference in the prevalence of MBL in different countries.

Our study showed also that all MBL producing-*Pseudomonas aeruginosa* were resistant to the antibiotics tested. These results were similar with the results of others researchers in which MBL-producing *P. aeruginosa* isolates were resistant to the examined antibiotics (Sandhya and Swathi, 2014; Liew *et al.*, 2018). This high level of resistance (100%) to all classes of antibacterial agents tested can be probably explained by the co-production of MBL and others non-investigated mechanisms of the resistant strains in our study. Despite these facts, the difference in resistance levels between MBL and non-MBL producing isolates for all the antimicrobial drugs (Beta-lactams, fluoroquinolones and aminoglycosides) tested were statistically significant, ($p < 0.05$, $P < 0.001$). Our findings showed that the highest rate of isolation of MBL producers was found from critical Care Nursing ward, ICU (45.2%) and HDU (28.6%) with predominant distribution in pus swab and sputum. Similar results have been observed by Subramaniyan *et al.* (Subramaniyan *et al.*, 2018). This finding should be explained by the increase of incidence of infection associated with the use of invasive devices for the diagnostic purpose or medical applications as well as inadequate sterilization and immunocompromised condition due to lifestyle disease.

Among carbapenemases, Metallo-Beta-Lactamases (MBL) and mostly Verona integron-mediated (VIM) and New Delhi MBL (NDM) have played the crucial role in carbapenem-resistance *Pseudomonas aeruginosa* emergence. Our study showed that twenty eight (66.67%), and twelve (28.57%) of the isolates had blaNDM-1

and blaVIM-2 genes in their PCR results, respectively. The PCR assays performed for metallo-beta-lactamases were negative in all MBL isolates for BlaVIM-1, BlaIMP-1 and BlaIMP-2. Different findings have been observed by the study of Pitout *et al.* (2008) in which fifty seven *Pseudomonas aeruginosa* were screened for MBL producers for VIM-type and IMP-type genes through the PCR method; and showed that all the MBL isolates (100%) carried blaVIM-2 genes from a tertiary care Centre in Kenya (Pitout *et al.*, 2008). Moyo *et al.* demonstrated that all carbapenem-resistant isolates from a tertiary care hospital in Dar-es-Salaam (Tanzania) were phenotypically positive for MBL production. PCR followed by sequencing showed that the isolates harbored blaVIM-2 (Moyo *et al.*, 2015). To our knowledge, this is the first study done on molecular characterization of blaNDM-1-producing *P. aeruginosa* in Kenya. Few others studies and cases reports have shown the presence of VIM-2 and NDM-1 from the African continent including South Africa, Ghana, Nigeria, Tunisia, Algeria and Egypt (Manenzhe *et al.*, 2015, Sekyere *et al.*, 2016). VIM-2 and NDM-1 are increasingly reported worldwide. Similar studies have recently reported the emergence of the *Pseudomonas aeruginosa* carrying the blaNDM-1 which exhibited resistance to carbapenems for the first time in Iraq, blaVIM-2 in Spain, and blaNDM-1 and VIM-2 genes in Malaysia (Bellés *et al.*, 2018; Ismail *et al.*, 2018; Liew *et al.*, 2018). The negative PCR results for VIM-1- and IMP-types can be explained by a considerable geographic difference in the distribution of Metallo-beta-Lactamases genes in different countries.

The authors did not investigate the clonality of the isolates and the sequence of the genes and also did not use primers to target all known MBL genes. Despite these limitations, the study has provided the distribution of the common MBLs genes and the magnitude of the problem in The Nairobi Hospital.

5. Conclusion and Recommendation

The high level of resistance to antibiotics of MBL-producing *P. aeruginosa* is present in this hospital with a prevalence of 22.7 % that suggests the spread of resistance among the population of nosocomial strains. To overcome the problem of emergence and the spread of multidrug resistant *P. aeruginosa* a combined interaction and cooperation between the microbiologists, clinicians and the infection control team is required in order to develop infection control strategies that can limit the dissemination of resistant strains. We also recommend further studies on epidemiology of MBL-producing *P. aeruginosa*, others mechanisms of antimicrobial resistance in *P. aeruginosa* isolates, proper antibiotic stewardship, and rapid diagnostic methods for proper detection of Beta-Lactamases-producing isolates.

6. Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

7. Acknowledgements

The authors appreciate and thank the Government of Burundi through East African Public Health Laboratory Network Project (EAPHLNP) for their financial support for this research and Molecular Biology Laboratory of Pan African University Institute for Basic Sciences, Technology and Innovation (PAUSTI) for their technical support. More thanks go also to The Nairobi Hospital for their permission to undertake this study and their guidance and assistance in providing necessary facilities to carry out the research work.

8. References

- Abaza F. A., Soraya A. E.S., Heba S. A. S., and Gehan A. A. (2017) "Metallo-Beta-Lactamases - producing *Pseudomonas aeruginosa* in a Healthcare Setting in Alexandria, Egypt", *Polish Journal of Microbiology*, 66, 3, 297-308.
- Acharya M., Prabhu R. J., Kamal T., Rajan A., Trishna K., Supriya S. (2017) "Detection of MBL-encoding genes among clinical isolates of *P. aeruginosa* in a Tertiary Care Hospital, Kathmandu, Nepal", *BMC research Notes*. 10 (1), 718.
- Al-Zaida, J.R. (2016) "Antibiotic susceptibility patterns of *Pseudomonas aeruginosa* isolated from clinical and hospital environmental samples in Nasiriyah, Iraq", *African Journal of Microbiology Research*, 10, 844-849.
- Ansari S., Rabindra D., Shrestha S., Thapa S., Puri R., Chaudhary N., Khatiwada S., and Rajendra G. (2016) "Growing menace of antibacterial resistance in clinical isolates of *Pseudomonas aeruginosa* in Nepal: An insight of...", *Biomed Research International*, 2-9.
- Bangera D., Shenoy S.M., Saldanha D.R. (2016) "Clinico-microbiological study of *Pseudomonas aeruginosa* in wound infections and the detection of MBL production", *International Wound Journal*, 13 (6), 1299-1302.
- Bellés A., Bueno J., Rojo-Bezares B., Torres C., Javier C.F., Saenz Y., and Seral C. (2018) "Characterization of VIM-2 producing *Pseudomonas aeruginosa* isolates from lower tract respiratory infections in a Spanish hospital", *European Journal of Clinical Microbiology and Infectious Diseases*, 1-10.
- David H. Pincus, Biomérieux, inc Hazelwood, Mo, USA; "Microbial identification using the Biomérieux Vitek - 2 System", *Encyclopedia of rapid microbiological methods*.

- Dogonchi A. A., Ghaemi E. A., Ardebili A., Yazdansetad S., Pournajaf A. (2018) "MBL-mediated resistance among clinical carbapenem-resistant *Pseudomonas aeruginosa* isolates in Northern Iran: A potential threat to clinical therapeutics", *TZu-Chi Medical Journal*, 30 (2), 90-92.
- Ejikeugwu P.C., Ugwu, Araka C.O., Gugu T.H., Iroha I.R., Adikwu M.U., and Esimone C.O. (2012) "Imipenem and meropenem resistance among ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates", *International Research Journal of Microbiology*, 3(10), 339-344.
- Farajzadeh S.A., Rostami S., Abbas J., Tabatabaiefar A. M., Khorvash F., Saki A., Shoja S., and Sheikhi R. (2014) "Detection of Metallo-Beta Lactamases Among Carbapenem-Resistant *Pseudomonas aeruginosa*", *Jundishapur Journal of Microbiology*, 7 (11): e12289.
- Ghasemian A., Kobra S. R., Hassan R. V., and Farshad N.(2018) "Prevalence of clinically isolated of MBL-producing *Pseudomonas aeruginosa*, coding genes, and possible risk factors in Iran", *Pathological winter*, 13 (1),1-9.
- Hong D. J., Kwon B., Jang I., Jeong S.H., Kang H., and Kyungwon L. (2016) "Epidemiology and characteristics of Metallo-Beta-Lactamases-producing *Pseudomonas aeruginosa*", *Journal of Infection and chemotherapy*, 47(2), 81-97.
- Ismail, S.J., and Mahmoud, S.S. (2018) "First detection of New Delhi metallo-Beta-Lactamases variants (NDM-1, NDM-2) among *Pseudomonas aeruginosa* isolated from Iraqi hospitals", *Iranian journal of Microbiology*, 10 (2), 98-103.
- Jayarani K., Sandhyarani T., Kumar C.N., Swathi S, Jayaranjani K. (2014) "Detection of MBL producing *Pseudomonas aeruginosa* in tertiary care hospital, Pondicherry" *International journal of recent scientific research*, 5 (8), pp.1460-1463.
- Kalluf K.O., Arend L.N., Wuicik T.E., Pilonetto M., Tuon F.F. (2017) "Molecular Epidemiology of SPM-1-producing *Pseudomonas aeruginosa* by rep-PCR in hospitals in Parana", *Brazil. Infectious and Genetic Evolution*; 49, 130-133.
- Liew M. S., Ganeswari R., Savithri D. P., and Kek H. C. (2018) "Detection of VIM-2, IMP-1 and NDM-1 producing multidrug-resistant *Pseudomonas aeruginosa* in Malaysia", *Journal of Global Antimicrobial Resistance*, 13, 271-271.
- Mahmoud A. B., Zahran W. A., Hindawi G. R., Labib A. Z. and Galal R. (2013) "Prevalence of Multidrug-Resistant *Pseudomonas aeruginosa* in Patients with Nosocomial Infections at a University Hospital in Egypt, with Special Reference to Typing Methods", *Journal of Virology and Microbiology*, 1-13.
- Manenzhe R.I., Zar H.J., Mark P. Nicoll M.P., and Kaba M. (2015) "The spread of carbapenemase-producing bacteria in Africa: a systematic review", *Journal of Antimicrobial Chemotherapy*, 70 (1), 23–40
- Meradji S., Barguigua A., Bentakouk M.C., Nayme K., Zerouali K., Mazouz D., Chettibi H., Timinouni. (2016) "Epidemiology and virulence of VIM-4 MBL-*Pseudomonas aeruginosa* isolated from burn patients in eastern Algeria", *Burns*, 42 (4), 906-918.
- Mohanam L., and Thangam, M. (2017) "Coexistence of MBL-Encoding Genes in *Pseudomonas aeruginosa*", *Indian Journal of Medical Research*, 146 (Suppl 1), S42-S56.
- Moosavian, M., and Mohammad, R. (2015) "Molecular detection of metallo-β-lactamase genes, blaIMP-1, blaVIM-2 and blaSPM-1 in imipenem resistant *Pseudomonas aeruginosa* isolated from clinical specimens in teaching hospitals of Ahvaz, Iran", *Iranian Journal of Microbiology*, 7 (1), 2-6.
- Moyo S., Haldorsen B., Aboud S., Blomberg B., Maselle S.Y., Sundsfjord A., Langeland N. (2015) "Identification of VIM-2-producing *Pseudomonas aeruginosa* from Tanzania is associated with Sequence Types 244 and 640 and the location of blaVIM-2 in a TniC integron", *Antimicrobial Agents and Chemotherapy*, 59, 682–685.
- Mushi M.F., Stephen E. M., Imirzalioglu C., and Freddie B. (2014) "Carbapenemase Genes among Multidrug Resistant Gram Negative Clinical Isolates from a Tertiary Hospital in Mwanza, Tanzania", *BioMedical Research International*, 1-7.
- Oberoi, L., Singh N., Sharma P., and Aggarwal A. (2013) "ESBL, MBL and AmpC Beta-lactamases producing Superbugs-Havoc in the intensive care Units of Punjab India", *J. of Clinical and Diagnostic Research*, 7(1), 70-73.
- Pitout J., Revathi G. Chow B.L., Kabera B., Kariuki S., Nordmann P. and L. Poire. (2008) "Metallo-Beta-lactamase-producing *Pseudomonas aeruginosa* isolated from a large tertiary Centre in Kenya", *Clinical Microbiology and Infection*, 14(8), 755-759.
- Radan M., Moniri R., Khorshidi A., Gilasi H., Norouzi Z., and Goli D. Y. (2016) "Emerging Carbapenem-Resistant *P. aeruginosa* isolates carrying blaIMP among Burns Patients in Isfahan", *Iran Arch Trauma Research*, 5 (3), e33664.
- Sandhya R. T., and Swathi, S. (2014) "Phenotypic detection of MBLs producing *Pseudomonas aeruginosa* in wounds", *International Journal of Pharma and Bio Sciences*, 6 (1), 1255 - 1259.
- Sekyere J.O., Govinden U., and Essack S. (2016) "The Molecular Epidemiology and genetic environment of

- carbapenemases detected in Africa", *Microbial Drug Resistance*, 22 (1), 59-68.
- Sepehrisresht, S., Boroumand A.M., Pourgholi L., Anvari M.S., Habibi E., and Tabrizi M.S.(2012) "Detection of vim- and ipm-type Metallo-Beta-Lactamases in *Pseudomonas aeruginosa* clinical isolates", *Archives of Iranian Medicine*, 15(11), 670-673.
- Shibata N., Doi Y., Yamane K., Yagi T., Kurokawa H., Shibayama K., Kato H., and Arakawa Y. (2003) "PCR typing of genetic determinants for MBLs and integrases carried by gram-negative bacteria isolated in Japan, with focus on the class 3 integron", *Journal of Clinical Microbiology*, 41 (12), 5407-5413.
- Shirani K., Behrooz A., and Fardad R. (2016) "Antibiotic resistance pattern and evaluation of MBL genes (VIM and IMP) in *Pseudomonas aeruginosa* strains Producing MBL enzyme, isolated from patients with secondary immunodeficiency", *Advanced Biomedical Research*, 5, 124.
- Strateva, T., and Yordanov, D. (2009) "*Pseudomonas aeruginosa* - a phenomenon of bacterial resistance", *Journal of Medical Microbiology*, 1133-1148.
- Subramaniyan J. S., and Jeya, M. S. (2018) "Occurrence of bla genes encoding Carbapenem-Resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from Intensive Care Unit in a tertiary care hospital", *Journal of Laboratory Physicians*, 10 (2), 208-2013.
- Toval F., Guzman-Marthe A., Madriz V., Somogyi T., Rodriguez C. and Garcia F. (2015) "Predominance of carbapenem-resistant isolates carrying bla-IMP and bla-VIM metallo-Beta-lactamases in a major hospital in Costa Rica", *Journal of Medical Microbiology*, 64(1), 37-43.
- Wadekar, M. D., Anuradha, K., and Venkatesha D. (2013) "Phenotypic detection of ESBL and MBL in clinical isolates of Enterobacteriaceae", *International of Current Research and Academic Review*, 1(3), 89-95.
- Wassef M., Mahallawy H.E., Zafer M.M., Ghaith D., Hamid R.A. (2015) "Laboratory Based Surveillance of Multidrug Resistant *Pseudomonas aeruginosa* in Cairo", *Journal of Microbiology and Experimentation*, 2(1), 00039.
- Zafer M.M., Hadir A.E., Ashou S.M., and Amin A.M. (2014) "Characterization of MBL Producing *Pseudomonas* in Egypt", *Egyptian Journal of Medical Microbiology*, 23 (1), 69-75.