Antimicrobial Resistance Profile and Genetic Profiling of *Pseudomonas aeruginosa* Strains Obtained from Different Inpatient Wards at Kenyatta National Hospital

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Abstract: *This study sought to determine the antimicrobial susceptibility profiles of Pseudomonas aeruginosa isolates from inpatients populations at the Kenyatta National Hospital. A total of 188 P. aeruginosa strains were obtained from different inpatient wards from August 2015 to January 2016. Minimum inhibitory concentrations (MICs) were conducted on the Vitek 2-Compact (Biomereux company-France). P. aeruginosa ATCC 27853 and E. coli ATCC 25922 were used as reference strains for drug resistance testing and interpretation done based on the CLSI 2017 guideline.*

High resistance was recorded towards Tetracycline (92%) with an MIC of ≥ 128μg/ml followed by Cefotaxime (88.8%) and Ceftriaxone (86.2%) with MICs of ≥ 64μg/ml. Lowest resistance was recorded towards Piperacillin (25%) and Amikacin (46.3%). Pseudomonas aeruginosa isolates recovered from the Critical care unit (CCU) recorded the highest resistance of 83% to all the antimicrobial tested while least resistance was observed in strains from the Newborn unit (NBU) ward (38%). On the other hand, isolates obtained from urine (92%) sample were the most resistant while lowest resistance was recorded from blood samples (29%). PCR screening revealed 68 Metallo β-lactamase (MBL) positive strains amongst 127 isolates that were Meropenem resistance. Resistance to Aztreonam amongst the 68 MβL positive producers was 89.7%. Resistance to CAZ, CIP, CN, and AMK was 82.4%, 80.9%, 88.2% and 78% respectively. At least 52(76.5%) of these MβL positive isolates were recovered from patients in the Critical Care Unit. Among the total 188 recovered P. aeruginosa, 48 (25.5%) carried class-1 integron with a single strain among them also harbouring a class-3 integron. Carriage of integron among the 64 bla$_{VEB}$ positive isolates was 70.3%. Among the 68 isolates that were positive for bla$_{NDM}$, 47 (69.1%) carried class-1 integron. Overall, 45 (23.9%) among the 188 P. aeruginosa isolates were positive for a co-carriage of bla$_{NDM}$, bla$_{VEB}$ and class I integron. Plasmid screening revealed 3 types of incompatibility groups. One P. aeruginosa isolate had both incW and incFIB, while another isolate had an incN. Phylogenic cluster analysis using the Gelcompar2® revealed four major clusters based on age, specimen type and wards. The four clusters had a significant genetic similarity of >80% amongst P. aeruginosa strains obtained from different wards which is indicative of cross-infection.

Keywords: *New Delhi Metallo-beta-lactamase; MβL – Metallo βeta lactamase; VEB-type beta-lactamases; Pseudomonas aeruginosa, Kenya*

I. Introduction

Pseudomonas aeruginosa is a common flora of the skin, gut and also ambiguous in the environment. This organism however has a significant clinical importance as one of the major cause Health-care associated infections (HAI) and has been implicated in severe opportunistic infections in immunocompromised individuals. High antimicrobial resistance has globally been reported in clinical *P. aeruginosa* strains raising alarm due to associated high mortality rates ranging between 18% and 61% due to treatment failure [1]. Infections caused by this organism range from bacteremia, respiratory, urinary tract, skin and burn wound infections with a positive culture from blood, urine and tracheal aspirate specimens of infected patients [2]. Nosocomial infections caused by *P. aeruginosa* is approximated to be 8% in United States alone, however, prevalence in developing countries like Kenya remain largely underestimated. In the 2014 WHO report, Africa was identified as one of the regions that lack an established antimicrobial surveillance system. This underestimation is partially attributed to the complex HAI diagnosis and inadequate surveillance due to limited resources.

Antimicrobial resistance *in Pseudomonas aeruginosa* has partially been attributed to over-use and miss-use of antimicrobial agents resulting in the emergence of multiple drug resistance (MDR) strains. Another

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mechanism of resistance involves production of β-lactamases and acquisition of plasmid-borne integron through horizontal gene transfer (HGT). Carriage of multiple integron containing long arrays of resistance cassettes consequently withstand high antibiotic selection pressure and are therefore likely to spread faster in hospitals, and in particular, in intensive care units. Class 1 integron implicated in resistance to important anti-Pseudomonal drugs such as third generation Cephalosporin, fluoroquinolones and advanced classes of Aminoglycosides such as Amikacin have been reported in *P. aeruginosa* [3-5]. Plasmid-borne integron contains a pool of gene cassettes therefore are more common among multiple drug resistance (MDR)-strains of *P. aeruginosa.*

Previous studies has shown that MDR strains of *P. aeruginosa* are predominant in the Intensive Care Units possibly due to the immune-compromised status of hospitalized patients and associated high volumes of antimicrobials used in such settings[6]. Other risk factor for colonization in such settings includes concurrent infections, prolonged hospitalization and use of invasive procedures such as catheters and mechanical ventilation [7]. The spread of plasmid borne integron has partially been attributed to usage of medical devices such as indwelling urinary catheters as well as person-to-person contamination. The emergence of Carbapenemase capable of hydrolyzing carbapems has aggravated the antimicrobial resistance problem in this burgs. This is because such drugs are considered the last resort for treatment of serious Gram-negative infections. Of particular importance are the Metallo-beta-lactamase (MβL) such as the bla_{NDM} that confer high resistance to Carbapenem and are harbored in plasmid bearing integron. These MβL borne in integron are therefore have a higher potential to spread to other susceptible bacteria. Among the MβL variants, bla_{VIM} and bla_{IMP} are the most prevalent in *P. aeruginosa* and have been widely implicated in numerous nosocomial outbreaks. Other MBL of clinical and epidemiological importance includes the bla_{SPM}, bla_{NDM} and bla_{VEB}.

Despite of the enormous threat associated with *P. aeruginosa* burgs, data on prevalence of Carbapenemase producers in the Africa continent remains scarce. In Kenya, only bla_{VIM-2} has been reported in *Pseudomonas aeruginosa* isolates from a tertiary hospital [4]. To the best of our knowledge, there is no data on other Carbapenemases and mobile genetic elements in multidrug resistance isolates of *Pseudomonas aeruginosa* in the country. We therefore set this cross-sectional study to determine the antimicrobial resistance profile and carriage of Carbapenemases, plasmids and integron in *P. aeruginosa* recoverable from different in-patient ward at Kenyatta National hospital. In order to access possible cross-infection within the hospital, we also sought to determine the genetic relatedness of recovered burgs using low resolution fingerprinting (ERIC-PCR).

II. Methods

Recruitment of patients and sample collection

In this hospital-based cross-sectional study, recruitment of the hospitalized participants and sample collection was done between August 2015 and January 2016. Upon participant consenting, specimens (pus swab, blood, urine, aspirates) were collected by clinicians in their respective wards/units using previously published method [8]. Clinical isolates were obtained from patients admitted in the Critical Care Unit (103), Renal Unit Ward (1), Burns Unit (2), Newborn Unit (4) and Medical Wards (77) (admission wards, maternity wards, oncology wards, theatres, accident and emergency, cardiology unit, Infectious Respiratory Disease and Orthopedic Units). Approximately 1ml of blood was collected into EDTA-coated vacationers while midstream urine was collected in sterile containers for analysis. Wound specimen were obtained from aspirated pus from ruptured or incised abscesses and transferred into a leak-proof sterile container.

Culture and susceptibility testing

Standard blood culture was done as previously published [8]. Aspirates were first homogenized by vortexing for 1 minute before culture on MacConkey agar and Blood agar [8]. Samples from urine were cultured on CLED and blood agar [8]. Standard colony and biochemical tests were used for identification of *Pseudomonas aeruginosa* [8]. Since *Pseudomonas aeruginosa* infections are rarely multi-clonal, a single colony from each successful culture was analyzed. Antimicrobial susceptibility testing disc diffusion method on Mueller-Hinton was performed for 12 antimicrobial agents; Ceftriaxone (CRO, 30μg), Ceftazidime (CAZ, 30μg), Cefotaxime (CTX, 30μg), Carbenicillin (CAR, 100μg), Piperacillin (PRL, 100μg), Aztreonam, (ATM, 30μg), Levofloxacin (LEV, 5μg), Ciprofloxacin (CIP, 5μg), Gentamicin (CN, 10μg), Amikacin (AK, 30μg), Tetracycline (TET, 30μg), Piperacillin/tazobactam (100/10μg) and Meropenem (MEM,10μg). Minimum inhibitory concentrations (MICs) were conducted on the Vitek 2-Compact (Biomereux Company-France). *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used as reference strains. Interpretation of the zones was done using the CLSI guidelines.

2.2. PCR Amplification

Bacterial DNA extraction was done using the Chelex method as previously described [9]. PCR amplification of the DNA was done using selected consensus primers for detection of Carbapenemases frequently reported in *P. aeruginosa* that includes *bla*PER, *bla*GES, *bla*NDM, *bla*GIM*, *bla*SPM and *bla*VEB (table 1). The PCR amplification program consisted of an initial denaturation (94 °C, 5 minutes) followed by 35

cycles of denaturation (94 °C, 60 seconds), annealing temperature depending on the primer and a single final extension for 5 minutes at 72 °C. Replicon typing of plasmid was done using 5 multiplex and 3 simplex PCR assays as previously described by Carattoli [10] (table 2). Separation of the amplified DNA was done suing 1.5% agarose gel and visualization of the bands done on UV Gelmax imager.

Table1. Amplification primers for Metallo βeta-lactamases and integron

Primer Name	5' -3' Sequence	Base pairs	Annealing temperature (°C)	Reference
VEB-F	CGACTTCCATTTCCCGATGC		56	[8]
VEB-R	TGTTGGGGGTTGCCCAATTTT	371		
NDM-F	ACTTGGCCTTGCTGTCCTT	621		[9]
NDM-R	CATTAGCCGCTGCATTGAT		56	
SPM-F	AAAATCTGGGTACGCAAACG	271	52	[11]
SPM-R	ACATTATCCGCTGGAACAGG			
PER-F	ATGAATGTCATTATAAAAGC	933	50	[12]
PER-R	AATTTGGGCTTAGGGCAGAA			
GES-F	ATGCGCTTCATTCACGCAC	863	56	[9]
GES-R	CTATTTGTCCGTGCTCAGGA			
GIM-F	TCGACACACCTTGGTCTGAA	477	52	[11]
GIM-R	AACTTCCAACTT TGCCATGC			
5_CS	GGCATACAAGCAGCAAGC	Variable	52	[12]
3_CS	AAGCAGACTTGACCTGAT			
IntM1_U	ACGAGCGCAAGGTTTCGGT	441	60	[13]
IntM1_D	GAAAGGTCTGGTCATACATG			
INT_1U	GTTCGGTCAAGGTTCTG	923	60	[13]
INT_1D	GCCAACTTTCAGCACATG			
INT2-L	CACGGATATGCGACAAAAAGGT	789	50	[13]
INT2-R	GTAGCAAACGAGTGACGAAATG			
Int3-F	AAATGACAAACCTGACTG	922	60	[15]
Int3-R	CGAATGCCCCAACAACTC			
ERICR	ATGTAAGCTCCTGGGGATC	variable	50	[14]
ERIC2	AAGTAAGTGACTGGGGTGAGCG			

Table 2: Amplification primers incompatibility group plasmids

	Sequence (5'-3')	Primers	Annealing temperature (°C)	Product
Multiplex -1	GGAGCGATGGATTACTTCAGTAC	HI1 – F	60	471 bp
	TGCCGTTTCACCTCGTGAGTA	HI1 – R		
	TTTCTCCTGAGTCACCTGTTAACAC	HI2- F	60	644 bp
	GGCTCACTACCGTTGTCATCCT	HI2 – R		
	CGAAAGCCGGACGGCAGAA	I1 – F	60	139 bp
	CGTCGTTCCGCCAAGTTCGT	I1 – R		
Multiplex -2	AACCTTAGAGGCTATTTAAGTTGCTGAT	X – F	60	376 bp
	GAGAGTCAATTTTTATCTCATGTTTTAGC	X –R		
	GGATGAAAACTATCAGCATCTGAAG	L/M – F	60	785 bp
	CTGCAGGGGCGATTCTTTAGG	L/M – R		
	GTCTAACGAGCTTACCGAAG	N – F	60	559 bp
	GTTTCAACTCTGCCAAGTTC	N – R		
Multiplex -3	CCATGCTGGTTCTAGAGAAGGTG	FIA – F	60	462 bp
	GTATATCCTTACTGGCTTCCGCAG	FIA – R		
	GGAGTTCTGACACACGATTTTCTG	FIB – F	60	702 bp
	CTCCCGTCGCTTCAGGGCATT	FIB – R		
	CCTAAGAACAACAAAGCCCCCG	W – F	60	242 bp
	GGTGCGCGGCATAGAACCGT	W – R		
Multiplex -4	AATTCAAACAACACTGTGCAGCCTG	Y – F	60	765 bp
	GCGAGAATGGACGATTACAAAACTTT	Y – R		
	CTATGGCCCTGCAAACGCGCCAGAAA	P – F	60	534 bp
	TCACGCGCCAGGGCGCAGCC	P- R		
	GTGAACTGGCAGATGAGGAAGG	FIC – F	60	262 bp
	TTCTCCTCGTCGCCAAACTAGAT	FIC – R		
Multiplex -5	GAGAACCAAAGACAAAGACCTGGA	A/C – F	60	465 bp
	ACGACAAACCTGAATTGCCTCCTT	A/C – R		
	TTGGCCTGTTTGTGCCTAAACCAT	T – F	60	750 bp

CGTTGATTACACTTAGCTTTGGAC	T – R		
CTGTCGTAAGCTGATGGC	FII – F	60	270 bp
CTCTGCCACAAACTTCAGC	FII – R		
TGATCGTTTAAGGAATTTTG	FrepB - F	60	270 bp
GAAGATCAGTCACACCATCC	FrepB - R		
GCGGTCCGGAAAGCCAGAAAAC	K/B – F	60	160 bp
TCTTTCACGAGCCCGCCAAA	K – R		
GCGGTCCGGAAAGCCAGAAAAC	B/O – F	60	159 bp
TCTGCGTTCCGCCAAGTTCGA	B/O – R		

DNA fingerprinting of recovered bacterial isolates

Enteric repetitive intergenic consensus (ERIC-PCR) using published primers (table 1) was used to determine the genetic relatedness of bla_{NDM} and bla_{VEB} Positive *Pseudomonas aeruginosa* isolates recovered from different wards. The PCR products were separated by running on 1% agarose with ethidium bromide gel for 1 hour. Banding patterns were visualized under ultraviolet light using a Gelmax® imager. Cluster analysis was done using Gelcompar®2 software version 6.6. Cluster analysis was done using the dice method based on banding pattern with arithmetic mean UPGMA. Isolates that had a correlation of $\geq$ 80% were considered genetically related [15].

Ethical consideration

All specimens were collected and processed in accordance with ethical Clearance approved by The National Ethics Committee number: SERU 3048 and Institutional Ethical Committee of Kenyatta National Hospital, reference number: UP44/02/2010.

III. Results

Bacterial Isolates

One hundred and eighty-eight non-duplicate clinical *P. aeruginosa* strains were isolated during a six-month period (August 2015 - January 2016) from CCU (103), Renal Unit Ward (1), Burns Unit (4), Newborn Unit (2), and Medical Wards (77). A total of 153 isolates were obtained from patients aged 50 years and below and 62% of 188 isolates were obtained from males while the rest (38%), were from females. A total of 103 tracheal aspirates, 55 pus swabs, 26 urine samples and 4 blood samples yielded clinical isolates of MDR *P. aeruginosa* respectively.

Antimicrobial susceptibility test based on disc diffusion method

All the 188 *Pseudomonas aeruginosa* isolates recovered in this study were multiple drug resistance strains ($\geq$3 antimicrobial class). High resistance was recorded towards Piperacillin-tazobactam (96%) while Ciprofloxacin (34%) was the least resisted antimicrobial. All the isolates were resistance to one or more of extended cephalosporin (CAZ, CTX and CRO) with a percentage resistance of 63.1%, 82% and 79.7% respectively (figure 1). A high resistance to Carbapenemases (Meropenem, 54%) and Aztreonam (54%) was also revealed in this study.

Figure 1: Resistance profiles of Pseudomonas aeruginosa based on disc diffusion method
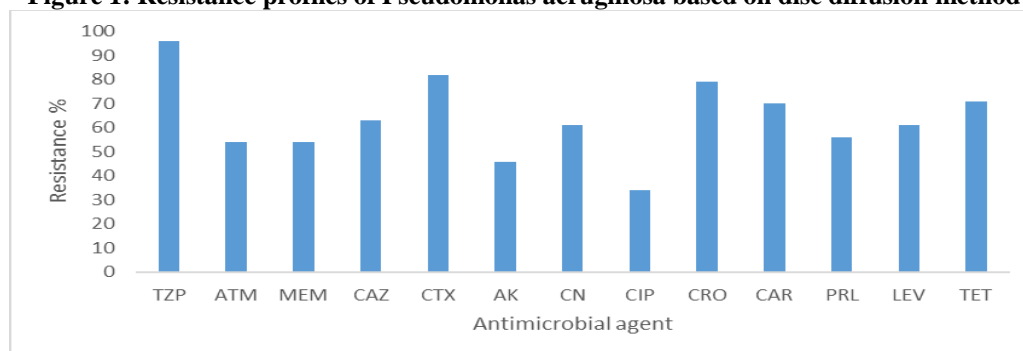
MEM: Meropenem, ATM: Aztreonam, CAZ: Ceftazidime, CTX: Cefotaxime, AK: Amikacin, CN: Gentamycin, CIP: Ciprofloxacin, CRO: Ceftriaxone, CAR: Carbenicillin, PRL: Piperacillin, LEV: Levofloxacin, TET: Tetracycline, TZP: Piperacillin/tazobactam

Antimicrobial susceptibility testing based on the Minimum inhibitory concentration

More than 70% of *P. aeruginosa* were resistant to β-lactams tested including Ceftazidime, Cefotaxime, and ceftriaxone with an MIC value of $\geq$ 32 µg/ml. High resistance to Aztreonam (67.4%) was recorded in this study. Resistance to Meropenem was 67.6% with an MIC value of $\geq$ 8µg/ml (table 3). However, contradictory to

disc diffusion results, most resistance towards Tetracycline was recorded (92%) with MIC value of ≥ 8 µg/ml. The MIC test also revealed relatively high resistance to Ciprofloxacin (52.7) compared to the disc diffusion method where resistance was 34.3%. Piperacillin was the most effective antimicrobial, where only 47 (25.1%) out of the 188 *P. aeruginosa* isolates recovered been resistance. *P. aeruginosa* isolates obtained from the critical care unit (CCU) were the most resistant to overall antimicrobial (83%) used which includes. Isolates recovered burn unit also revealed a high resistance of (58%) followed by medical wards (43%) while those obtained from newborn unit (38%) were least resistance (figure 2). *Pseudomonas aeruginosa* isolates recovered from urine samples were the most resistant to tested antimicrobial with an overall resistance of (72.2%) been recorded. High resistance in *P. aeruginosa* isolates from Tracheal aspirates (76%) was also recorded while blood isolates were the least resistance (figure 3).

Table 3: MIC of *P. aeruginosa* isolates obtained from various sample types from different wards.

| Drug	Resistance breakpoint* (µg/ml)	% resistance	Mode MIC	MIC$_{50}$ (µg/ml)	MIC$_{90}$ (µg/ml)
MEM	≥ 8	67.6	128	16	128
ATM	≥ 32	67.4	128	64	128
CAZ	≥ 32	70	64	64	64
CTX	≥ 32	88.8	64	64	64
AK	≥ 64	46.3	128	32	128
CN	≥ 16	67.9	128	64	128
CIP	≥ 4	52.7	128	32	128
CRO	≥ 32	86.2	64	64	64
CAR	≥ 64	57.4	64	64	64
PRL	≥ 128	25.	64	64	128
LEV	≥ 8	73.9	128	64	128
TET	≥ 8	92	128	64	128
TZP	≥ 128	50.5	128	64	128

MIC: Minimum inhibitory concentration, MEM: Meropenem, ATM: Aztreonam, CAZ: Ceftazidime, CTX: Cefotaxime, AK: Amikacin, CN: Gentamycin, CIP: Ciprofloxacin, CRO: Ceftriaxone, CAR: Carbenicillin, PRL: Piperacillin, LEV: Levofloxacin, TET: Tetracycline, TZP: Piperacillin/tazobactam.

Figure 2: MIC antimicrobial resistance profiles of isolates recovered from different wards
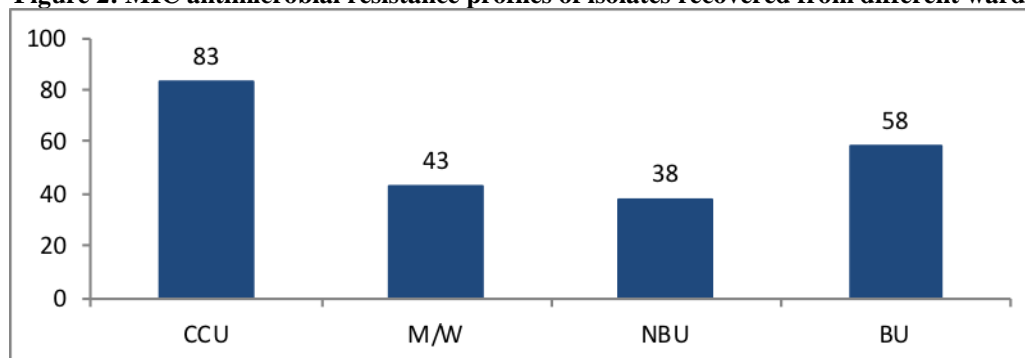
CCU: Critical care unit, B/U: Burn unit, NBU: New born unit, R/U: Renal unit

Figure 3: MIC antimicrobial resistance profiles of isolates based on sample type
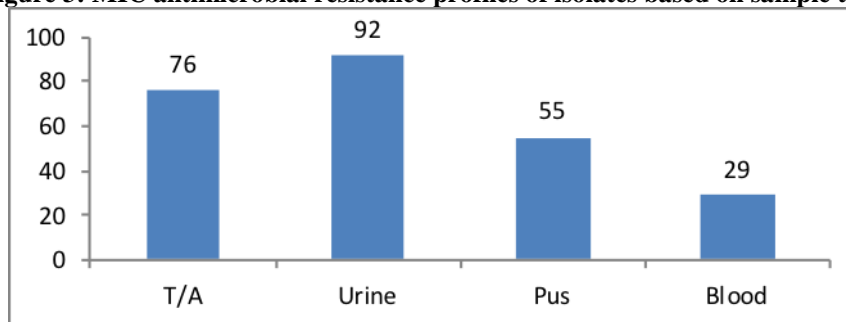
T/A: Tracheal aspirate

PCR analysis of Metallo-β-lactamase, Integron and Plasmid typing carriage in *P. aeruginosa* isolates

Amplified PCR products were obtained respectively for bla_{VEB} and bla_{NDM} using consensus primers PCR experiments with primers specific for bla_{TEM} bla_{SHV}, bla_{GES}, bla_{PER}, bla_{KPC}, bla_{GIM} bla_{SPM} were negative. Integron class I, II and III were screened in all *P. aeruginosa* isolates positive for bla_{NDM} and/or bla_{VEB}. The prevalence of bla_{NDM} carriage was the highest at 51.9% while carriage of bla_{VEB} was 49.6%. Fifty-two (76.5%) of these isolates were obtained from patients in Critical Care Unit among the 188 *P. aeruginosa*, 48 (25.5%) of these isolates carried class-1 integron with a single strain among them also harbouring class-3 integron. Carriage of class 1integron among the 64 bla_{VEB} positive isolates was 70.3%. Amongst the 68 isolates that were positive for bla_{NDM}, 47 (69.1%) carried class-1 integron. Overall, 45 (23.9%) among the 188 *P. aeruginosa* isolates were positive for a co-carriage of bla_{NDM}, bla_{VEB} and class I integron. Chi-square analysis found significant association in antimicrobial resistance to the drugs tested except for CTX and TET with carriage of integron (table 4). Plasmid screening revealed 3 types of incompatibility groups. One *P. aeruginosa* isolate had both W-Plasmid and a FIB-Plasmid, while another isolate had an N-Plasmid. The nucleotide sequences of the bla_{NDM} and the bla_{VEB} reported in this paper have been submitted to the EMβL/GenBank nucleotide sequence database under accession numbers KX857136 (https://www.ncbi.nlm.nih.gov/ nuccore/KX857136) and KX857137 (https://www.ncbi.nlm.nih.gov/nuccore/KX857137), respectively.

Table 4: Integron carriage association to antimicrobial resistance

Antimicrobial	Integron positive (N=48) No.(%)			Integron negative (N=140) No.(%)			Chi-square P-value	Test significance
	R	I	S	R	I	S		
MEM	44 (91.6)	2 (4.2)	2 (4.2)	83 (59.2)	0 (0)	57 (40.7)	0.00001	S*
ATM	44(91.6)	2 (4.2)	2 (4.2)	82 (58.6)	0 (0)	58 (41.4)	0.0001	S*
CAZ	40 (83.3)	2 (4.2)	6 (12.5)	91 (65)	0 (0)	49 (35%)	0.004955	S*
CTX	47 (97.9)	0 (0)	1 (2)	119 (85)	10 (7.1)	11 (7.9)	0.141657	NS*
AK	40 (83.3)	0 (0)	8 (16.6)	41 (23.9)	5 (3.6)	94 (67.1)	0.00001	S*
CN	42 (87.5)	0 (0)	6 (12.5)	85 (60.7)	2 (1.4)	51 (36.4)	0.006661	S*
CIP	42 (87.5)	2 (4.2)	4 (8.3)	57 (40.7)	1 (0.7)	82 (58.6)	0.00001	S*
CRO	46 (95.8)	2 (4.2)	0 (0)	116(82.9)	1 (0.7)	23 (16.4)	0.028865	S*
CAR	44 (91.6)	2 4.2)	2 4.2)	63 (45)	45 (32.1)	32 (22.9)	0.00001	S*
PRL	38 (79.1)	8 (16.6)	2 4.2)	9 (6.4)	58 (41.4)	73 (52.1)	0.00001	S*
LEV	42 (87.5)	2 4.2)	4 (8.3)	96 (68.6)	1 (0.7)	43 (30.7)	.003063	S*
TET	48 (100)	0 (0)	0 (0)	124(88.6)	0 (0)	16 (11.4)	0 .137591	NS*
TZP	44 (91.6)	4 (8.3)	0 (0)	50 (35.7)	36 (25.7)	54 (38.6)	0.00001	S*

R=Resistant, I=Intermediate, S=Susceptible, S*=significant, NS*=Non-significant. The test was considered significant at P < 0.05.

Enterobacterial repetitive intragenic consensus polymerase chain reaction (ERIC-PCR)

Cluster analysis revealed four major clusters based on banding patterns with >80% similarity (figure 3). Isolates in the first cluster (C-1) harbored bla_{NDM} and bla_{VEB} in exception of a single strain that harbored only bla_{NDM}. Five out seven isolates were recovered from critical care unit (CCU) while only 2 were from medical ward. This cluster also revealed a sub-cluster (a) with 2 isolates from male CCU ward that harbored bla_{NDM} and bla_{VEB} with a 94% similarity. Another sub-cluster (b) showed 4 *P. aeruginosa* isolates obtained from patients in medical and critical care unit ward which had a significant genetic similarity of 96%. The second cluster revealed 8 isolates with a similarity matrix of 96%. All the isolates in the second cluster (C-2) were obtained from CCU patients in urine and tracheal aspirate samples. Cluster 3 (C-3) uncovered a sub-cluster (d) with isolates obtained in CCU and medical ward that had a 96% similarity. With exception of a single strain that carried bla_{NDM}, all other isolates in this sub-cluster harbored class 1 integron, bla_{NDM} and bla_{VEB}. Another sub-cluster (e) had 5 *P. aeruginosa* isolates obtained from CCU ward with a homogeneous resistance pattern and a significant similarity of 96%. A single isolate in this sub-cluster harbored *inc*W and *inc*FIB in addition to the other resistance genetic elements. Cluster 4 (C-4) on the other hand uncovered 3 homogenous isolates all of which were recovered from the critical care unit. The 3 isolates had a similarity matrix of >90%, all carrying class 1 integron, bla_{NDM} and bla_{VEB}.

Figure 3: Showing cluster analysis
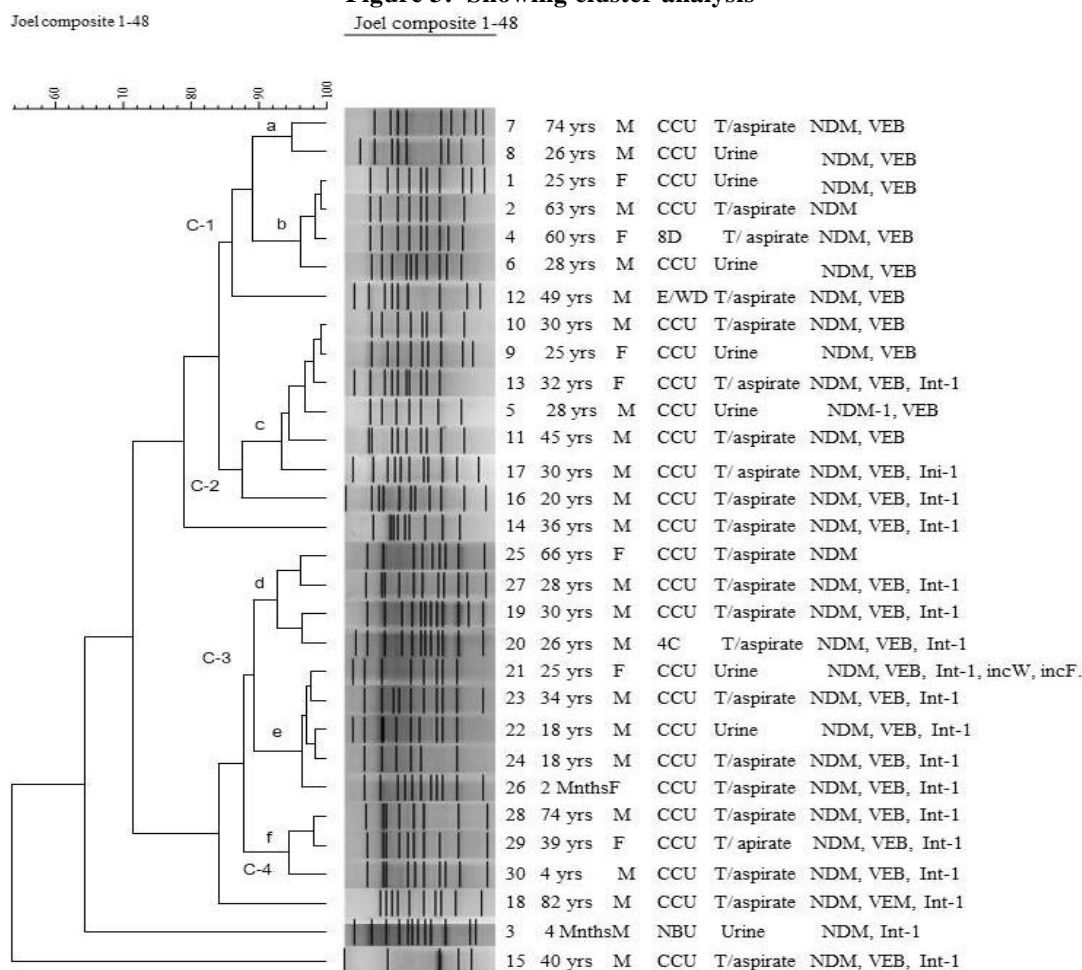
C- cluster, F- female, M- male, CCU- critical care unit, NBU- new-born unit, 8D,4C & E/WD- medical wards, T/aspirate- tracheal aspirate, NDM- New Delhi Metallo-βeta lactamase, VEB- Verona extended βeta lactamase, Int-1- class 1 integron, *inc*- incompatibility group.

IV. Discussion

Pseudomonas aeruginosa is an opportunistic pathogen which plays a major role in nosocomial infections amongst immunocompromised patients. In the wake of widespread antibiotic resistance strains in both environmental and hospital settings, control of this pathogen has been futile. Of major concern are *P. aeruginosa* strains with inherent resistance to multiple antimicrobial agents that are heavily used in inpatient wards. Although clinical isolates of *P. aeruginosa* are generally sensitive to Ceftazidime, Amikacin and Ciprofloxacin, our findings were contradictory. We recorded high levels of resistance to Cephalosporin's, Aminoglycosides, and fluoroquinolones. Ciprofloxacin has been widely used in Kenya in combined therapy with Ceftazidime among ICU patients, which potentially increases the risk of resistance build-up due to selective pressure. However, resistance to Ciprofloxacin (53.2%) was found to be relatively lower compared to that of Levofloxacin (73.7%).

Carbapenemase *P.aeruginosa* producers are normally susceptible to Monobactam such as Aztreonam[16], however, this study revealed high resistance to this antimicrobial agent (67.4%). Although we only screened for MBLs in our isolates, previous studies have associated Aztreonam resistance in Carbapenemase producers to carriage of Extended Spectrum β-lactamases (ESBL) and AmpCs such as CMY-1 and CMY-2[17]. The high level of resistance observed in Aminoglycosides, B-lactams and fluoroquinolones suggest addition resistance mechanisms in addition to the *bla*NDM and *bla*VEB detected in our isolates. Resistance to β-lactam antibiotics has also been associated with the production of Extended spectrum β-lactamase enzymes (ESBLS) that are mostly plasmid-encoded[18]. Therefore, such genes can easily spread to susceptible strains and in turn impact negatively on our ability to combat serious life-threatening infections in multiple wards. The combined resistance to β-lactams, Fluoroquinolones and Aminoglycosides which are widely used as anti-Pseudomonal agents makes

treatment problematic especially in developing countries where Carbapenem are not ready available or affordable. Resistance to Carbapenem further narrows the treatment of MDR-*P. aeruginosa* resultant infections.

Although 127 out of 188 *P. aeruginosa* isolates were resistant to Meropenem, only 62 (48.8%) were positive for bla_{NDM} and/or bla_{VEB}. This findings are contradictory with other studies conducted in Kenya that reported bla_{VIM-2} from all imipenem resistance *P.aeruginosa* isolates obtained from urine and blood samples[22]. However, the current study revealed higher resistance to Carbapenem compared to later study that reported a prevalence of 53%. Our findings also differ from the Agha Khan study where resistance to Ceftazidime, Ciprofloxacin, Gentamicin and Amikacin was 100%. In both studies however, most of the multidrug resistance strains of *P. aeruginosa* were obtained from the critical care unit.

To date, only a handful of studies in Africa have reported bla_{VEB} positive *P. aeruginosa*, and this includes a study in Egypt and South Africa[9;23]. The prevalence of VEB among the Carbapenem resistance isolates in the current study was however lower compared to the later studies[24-26]. In the East Africa region, *bla*NDM-1 has been reported in Carbapenem-resistant *Acinetobacter baumannii* clinical isolates [19]. In other parts of the world, NDM-1 positive *P. aeruginosa* has been reported in Serbia, France, India, Italy and Singapore [26;28-30]. In another study, bla_{VEB-1} -like genes were present as a gene cassette on class 1 integron in *P. aeruginosa* from Thailand [25]. This, therefore, means that these resistance mechanisms may be spreading across continents. This is most likely been fuelled by international travels.

To the best of our knowledge, this is the first report of co-carriage of bla_{NDM-1} and bla_{VEB-1} genes in clinical isolate of *P. aeruginosa* in Kenya. Isolates that harbored these 2 resistance determinants were also resistant to the third-generation cephalosporin, Aminoglycosides, Fluoroquinolones and also Aztreonam. Although we did not confirm the content of the integron detected, high MDR phenotype and carriage of MβL genes was associated with carriage of an integron. Previous studies have implicated resistance antimicrobial resistance to carriage of bla_{VEB} in Pseudomonas aeruginosa isolates. The Co-carriage of bla_{NDM} and bla_{VEB} in *P aeruginosa* therefore means only a few anti-Pseudomonal agents are effective to such strains. The high resistance recorded in both studies particularly in Gentamicin and Amikacin in the ICU pose a serious health-care problem in the country. This is injectable antimicrobials are amongst the few available treatment options for patients in coma where oral treatment is not feasible.

Notably, recovery of multiple-drug resistance *P. aeruginosa* strains was predominant in the critical care unit. This observation is in line with previous studies that have shown that Critical Care Units (CCU) are a hotspots of MDR strains[20]. Multiple-drug resistance *Pseudomonas aeruginosa* organisms are highly adaptable to antimicrobial agents' selective pressure [3]. This selective pressure is even higher in hospital settings such as the ICU due to stronger antimicrobial therapy including injectable drugs such Amikacin and ceftriaxone. Other risk factors of colonization by MDR *P aeruginosa* in this settings included concurrent disease, previous exposure anti-*Pseudomonas* antibiotics and use of mechanical ventilation. The current study found high levels of resistance to Meropenem and Amikacin in this ward, both of which are widely used in such settings. Our results are consistent with findings of a study conducted in a tertiary hospital in Nepal[20] where most of MβL-producing *P. aeruginosa* were from ICU.

In the current study, 36 multidrug-resistant *P. aeruginosa* isolates were found to carry of class 1 integron. We also identified a single strain that harbored both class 1 and 3 integron. The high detection of class-1 integron opposed to class 2 and 3 is consistent with findings of previous studies conducted in south Nigeria which reported 57.4% incidence rate[9.] Integron class 1 has also been reported in *P. aeruginosa* encoding bla_{GES-2} extended β-lactamases in a study conducted in South Africa [21]. We also recorded significant statistical association of class-1 integron with high resistance recorded in β-lactams, aminoglycosides, and fluoroquinolones. Our findings are in line with a study conducted by Odumosu *et al* 2013 where resistance to Carbenicillin (80.6%), Ceftriaxone (87.1%) and Tetracycline (100%) was significantly associated with carriage of class-1 integron in *P. aeruginosa* isolates. Other studies have also implicated resistance to these antimicrobial agents to genes carried by integron [31]. Resistance to extended spectrum β-lactams and Carbapenem is mostly plasmid-borne bearing integron [22]. Genetic elements carried by these integron are very stable and can easily spread to other strains and bacterial species leading more resistance[15].

Plasmid have also been reported to transfer resistance agents to other bacterial strains or species via horizontal gene transfer. Plasmid-mediated horizontal gene transfer has been implicated in *P. aeruginosa* resistance to β-lactams, Carbapenem and Aminoglycosides[16]. We detected 2 multidrug *P. aeruginosa* that harbored both *inc*FIB and *inc*W and a single isolate that carried *inc*N. It was however not clear the kind of resistance conferred by these incompatibility plasmids groups. To the best of our knowledge, this is the first report of these types of plasmids in *Pseudomonas aeruginosa* isolates in Africa. These plasmids have been implicated with resistance to Ampicillin, Streptomycin, Gentamicin, Amikacin, Trimethoprim, Nalidixic Acid and Chloramphenicol in *Escherichia coli*. Previous studies have demonstrated transferability of such resistance genes harbored in plasmids to *P. aeruginosa* isolates [23].

The genotype cluster created from *P. aeruginosa* isolates collected in 5-months period revealed a significant similarity of >80%. Significant genetic similarity was observed in isolates obtained from diverse inpatient population from different wards in Kenyatta National Hospital. The distinct temporal association observed is therefore a strong evidence of persistent spread of MDR clones among patients in different wards. This finds may also reflect a lack or dysfunctional Antimicrobial resistance monitoring, prevention and control unit in this health facility.

In conclusion, reliable surveillance and control initiative programs should be initiated to prevent the spread of antimicrobial resistance in our medical facilities. Consequently, this will impact on early detection of strains with unique resistance and possible spread control initiatives enactment. Early detection also means appropriate treatment regime which is important in the prevention of antimicrobial resistance build up.

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