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Research Article

Prevalence of Multidrug-Resistant *Pseudomonas aeruginosa* at Kenyatta National Hospital

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Abstract: This cross-sectional study was designed to determine the prevalence of multidrug resistance *P. aeruginosa* in Kenyatta National Hospital. Recruitment of patients and bacterial isolation was done in the period between August 2015 to January 2016. Aspirates, blood, urine and pus swab samples were obtained from patients in the critical care unit, new-born unit, renal unit and medical wards. A total of 188 non-duplicate *P. Aeruginosa* isolates were recovered. Antimicrobial susceptibility testing on 13 drugs was done using Kirby technique. *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used for quality control of all susceptibility testing. Our findings revealed that all the 188 isolates were multidrug resistant. Piperacillin-tazobactam (96%) was the most resisted antimicrobial while Ciprofloxacin (65.7%) was the most susceptible. High resistance to Carbapenem (Meropenem 54%) and *β*-lactams (CAZ 63.1%, CTX 82%, CRO 79.7%, CAR 70.1% and ATM 54%) was uncovered. Notably, *P. aeruginosa* isolates recovered from Critical Care Unit (73.4%) were the most resistant.

Keywords: America Type Culturecollection (ATCC), Burns Unit (BU), Critical Care Unit (CCU), Kenyatta national hospital (KNH), Multiple-Drug Resistance (MDR), New-born Unit (NBU), *Pseudomonas aeruginosa* (*P. aeruginosa*), Renal Unit Ward (RU)

INTRODUCTION

Kenyatta National Hospital (KNH) is the largest hospital in Kenya with a bed capacity of 1800. This hospital gives priority to serious medical conditions (chest infection, severe head injury, sepsis, diabetes complications, cardiac complications, burns, autoimmune-related diseases, kidney complications among others). Previous independent observations have noted that more than 60% bacterial isolates from clinical specimen analyzed in Microbiology laboratory (KNH) from critical care areas are resistant to at least three antimicrobials. Multiple drug resistance has also been noted among the outpatients with over 35% representation (Microbiology Laboratory Kenyatta National Hospital). Although the actual drive to this observation has not yet been established, extended hospitalization has been suggested as a risk factor for acquisition of multidrug resistance in the hospital. Although it is illegal to buy drugs over the counter without the Doctor's prescription in Kenya, most of the correspondents admitted to previous use of un-prescribed medications. The high prevalence of *P. aeruginosa* MDR strains from urine revealed in the current study may reflect a corresponding heavy use of antimicrobials among these patients (Kiiru *et al*., 2012). The heavy use of antimicrobials has also been attributed as a major cause of resistance brought about by selective pressure to these agents (Gales *et al*., 2003).

The global threat of nosocomial multidrug-resistant *P. aeruginosa* is a growing concern among hospitalized patients. Infections caused by *P. aeruginosa* are severe and often associated with high mortality and morbidity rates. *P. aeruginosa* frequently develops resistance during therapy hence becoming challenging to treat (El Solh and Alhajhusain, 2009). *P. aeruginosa* has been reported to be resistant to structurally unrelated antibiotics attributed to a vast array of chromosomal and plasmid-mediated antibiotic resistance mechanisms (El Solh and Alhajhusain, 2009). Antimicrobial resistance in these strains has also been due to the acquisition of newer resistant genes from other organisms such as *Acinetobacter baumannii, Klebsiella pneumoniae* and *Salmonella* spp (Bonomo and Szabo, 2006).

Previous studies have attributed antimicrobial resistance in *P. aeruginosa* to the presence of one or more of these genetic elements (Su *et al*., 2010). Genetic elements such as plasmids, transposons and integron are means through which resistance genes are

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acquired leading to rising multidrug resistance *P. Aeruginosa* (Szabo *et al*., 2006a). These MDR strains have been implicated with high mortality and morbidity rates resulting from severe nosocomial infections ranging from the bloodstream, wound, urinary tract and respiratory tract infections especially in patients in ICU (Rossolini and Mantengoli, 2005; Varaiya *et al*., 2008).

This cross-sectional study sought to unravel the prevalence of Multidrug-Resistant *P. aeruginosa* at Kenyatta National Hospital.

MATERIALS AND METHODOLOGY

Kenyatta national hospital: The current study was conducted at Kenyatta National Hospital (KNH). This is the biggest referral hospital in East Africa and the sub-Sahara region located in the capital of Kenya, Nairobi. The hospital was founded in 1901 as a Native Civil hospital with a bed capacity of 40 which has since grown to 1800.

Recruitment of patients and sample collection: A cross-sectional study design was used to obtain aspirates, blood, pus swabs and urine samples from In-patients and Out-patients seeking medical attention at Kenyatta National Hospital between August 2015 and January 2016. These samples were obtained from consenting patients (relatives' approval sort for unconscious patients) in Critical Care Unit (CCU), Renal Unit Ward (RU), Burns Unit (BU), New-born Unit (NBU) and Medical Ward.

Clinical samples were obtained from patients using previously published methods (Monicah, 1999). In brief, a 1 mL blood sample was collected into EDTA-coated vacutainers while midstream urine was collected in a urine tube. Wound specimen and aspirates from incised abscesses were transferred into a leak-proof sterile container.

Bacterial isolation and Bio-typing: Blood cultures were done using the previously published methods (Monicah, 1999). Aspirate samples were first homogenized by vortexing before culture on Blood agar and MacConkey agar (Monicah, 1999). Fresh urine samples were cultured on CLED and blood agar (Monicah, 1999). Presumed *P. aeruginosa* on MacConkey and Blood agar were verified by biotyping using published methods.

Antimicrobial susceptibility testing: Kirby-Bauer disc diffusion method was used to perform antimicrobial susceptibility testing for *P. aeruginosa* isolates. Susceptibility testing was done using oxoid Mueller-Hinton agar on Tazobactam and Piperacillin (TZP, 110 µg), Amikacin (AK, 30 µg), Aztreonam, (ATM, 30 µg), Carbenicillin (CAR, 100 µg), Cefotaxime (CTX, 30 µg), Ceftazidime (CAZ, 30 µg), Ceftriaxone (CRO, 30 µg), Ciprofloxacin (CIP, 5 µg), Gentamicin (CN, 10 µg), Levofloxacin (LEV, 5 µg), Meropenem (MEM, 10 µg), Piperacillin (PRL, 100 µg) and Tetracycline (TET, 30 µg).*P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922 were used for quality control susceptibility testing. The antimicrobial discs' zones were interpreted using CLSI guidelines (27th edition).

Ethical consideration: Ethical Clearance approved by Scientific Ethical Review Unit (SERU), KEMRI reference number 3048 and the Institutional Ethical Committee of Kenyatta National Hospital/University of Nairobi, reference number: UP44/02/2010.

RESULTS

Bacterial isolates: A total of 188 non-duplicate *P. aeruginosa* isolates were obtained from MacConkey and Blood agar cultures. Out of the 188 *P. aeruginosa* isolates,103 were from patients in CCU, 4 in Burns Unit, 77 in Medical Wards, 2 in New-born Unit and 1in the Renal Unit Ward. One hundred and seventeen (117) of these isolates were obtained from male participants while (71) were obtained from females.

Table 1: Antimicrobial resistance patterns of *P. aeruginosa* isolates recovered from various samples types

Antimicrobial agent	Resistance (%)	CCU (103) 73.4% R	M/W (77) 46.8% R	R/U (1) 46% R	NBU (2) 23.1% R	B/U (4) 44.2% R	T/A (103) 69% R	Urine (26) 77.2% R	P/S (55) 41.1% R	Blood (4) 13.5% R
TZP	96	72	22	0	0	2	67	19	9	1
ATM	54	74	25	0	0	2	71	18	12	0
MEM	54	75	24	1	0	1	71	17	13	0
CAZ	63.1	75	39	1	0	3	72	23	23	0
CTX	82.9	89	60	1	2	3	85	25	44	1
AK	46.5	64	23	0	0	0	59	17	11	0
CN	61	73	37	1	0	3	68	23	23	0
CIP	34.3	67	14	0	0	0	61	17	3	0
CRO	79.7	89	56	1	1	3	86	25	38	0
CAR	70.1	71	56	1	1	2	66	16	45	4
PRL	56.7	73	31	0	0	2	66	19	21	0
LEV	61	74	36	0	0	0	73	21	20	0
TET	71.1	84	45	0	2	2	70	21	32	1

TZP = Tazobactam/Piperacillin; MEM = Meropenem; ATM = Aztreonam; CAZ = Ceftazidime; CTX = Cefotaxime; AK = Amikacin; CN = Gentamycin; CIP = Ciprofloxacin; CRO = Ceftriaxone; CAR = Carbenicillin; PRL = Piperacillin; LEV = Levofloxacin; TET = Tetracycline; CCU = Critical Care Unit; B/U = Burn Unit; NBU = New born unit; R/U = Renal Unit; T/A = Tracheal Aspirate; P/S = Pus Swab; M/W = Medical Ward; R = Resistance

Antimicrobial susceptibility test: All of the 188 *P. aeruginosa* isolates were resistant to ≥ 1 drug from ≥3 class of antimicrobial and therefore multidrug resistant. Piperacillin-tazobactam (96%) was the most resisted antimicrobial while Ciprofloxacin was the most effective drug for *P. aeruginosa* isolates (Table 1). High resistance to Meropenem (54%) and β-lactams (CAZ 63.1%, CTX 82%, CRO 79.7%, CAR 70.1% and ATM 54%)) was revealed in this study. *P. aeruginosa* isolates obtained from patients in Critical Care Unit (CCU) were the most resistant as compared to other wards. Isolates obtained from urine samples were also revealed to be the most resistant to the tested antimicrobial.

DISCUSSION

P. aeruginosa has been implicated in severe infections among immune-compromised patients. This organism develops resistance to antimicrobial agents during treatment (El Solh and Alhajhusain, 2009) or through resistance genes acquisition via horizontal transfer from resistant strains or other species (Bonomo and Szabo, 2006). Infections caused by multidrug resistant *P. aeruginosa* have proven problematic to treat and have also been implicated with high mortality rate in hospitalized patients.

In the current study, all the 188 isolates of *P. aeruginosa* were multidrug resistant. High level of resistance to Carbapenem (Meropenem 54%) was revealed in the present study. Our findings are higher compared to results of a survey conducted in Nigeria (Odumosu *et al*., 2013) where Imipenem resistance of 9.6% among *P. aeruginosa* isolates was recorded. Resistance to Amikacin (25.5%), Gentamicin (51.6%), Ceftazidime (22.5%) and Cefotaxime (77.4%) was comparatively low compared to the current study. However, level of resistance to Tetracycline (100%), Ceftriaxone (87.1%) and Carbenicillin (80.6%) was higher compared to the current study which was at 71. %, 79.7% and 70.1%, respectively. In both studies, however, all the *P. aeruginosa* isolates were multidrug resistant. These findings therefore suggest that emergence and spread of MDR strains of *P. aeruginosa* isolates are on the rise. Resistance in these strains has been attributed to chromosomal and plasmid-mediated antimicrobial resistance determinants (El Solh and Alhajhusain, 2009). Integron that carries resistance gene cassettes has also been implicated in multidrug resistance *P. Aeruginosa* (Jeong *et al*., 2009). These integrons have been reported to carry genes that mediate resistance to *β*-lactams, aminoglycosides and other antimicrobial agents (Elbourne and Hall, 2006; Jeong *et al*., 2009)

In the current study, we revealed a high level of Resistance in *P. aeruginosa* isolates recorded in Critical Care Unit (73.4%). Our findings are supported by results of a previous study conducted in the ICU unit in Iran (Vaez *et al*., 2015). In the current study, however, resistance frequency was lower compared to the survey conducted in Iran; Meropenem (100%), Aztreonam (90%), Ceftazidime (90%), Cefotaxime (90%) and Ciprofloxacin (90%). The findings of the current study support findings of previous studies which have reported ICU to be a hotbed for MDR strains (Vaez *et al*., 2015). These resistances are associated with certain medical procedures like the use of catheters and mechanical ventilators (Rodrigues *et al*., 2011). Other risk factors for MDR-*P. aeruginosa* colonization in the ICU includes extended hospitalization and concurrent diseases.

The high level of resistance in Meropenem revealed in this study may suggest inefficacy of the drug in the affected patients. The high Carbapenem resistance poses a serious threat in treatments of serious infections caused by multidrug resistance *P. aeruginosa*. This is because these drugs are regarded the last resort drugs for the treatment of severe infections caused by gram-negative bacteria. Our study, however, revealed a low level of resistance to Ciprofloxacin (34.3%). This drug has been used to treat serious infections caused by *P. aeruginosa* and therefore proves to be still active.

CONCLUSION

The high prevalence of multidrug resistance *P. aeruginosa* uncovered by this study is an indication of continued emergence and spread of resistant strains. This situation therefore requires an urgent need to formulate Multidrug surveillance and control initiatives in hospitals to curb this menace. Clinical studies geared towards identifying risk factors for MDR development and establishing most efficacious antimicrobial regimes should also be encouraged. The hospital should also make it a mandatory undertaking, to conduct environmental surveillance through swabbing surfaces in wards such as sinks, to obtain samples for laboratory analysis. Regular fumigation in precincts and their environs should be embraced. The staff should also be screened on regular basis to avoid chances of clinicians becoming carriers, hence, source of transmission.

CONFLICT OF INTEREST

The author declares no conflict of interest in this study.

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