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Human Body Burden of *Pseudomonas aeruginosa*, Antibiotics Susceptibility Pattern and Presence of Extended Spectrum β-lactamase and Carbapenemase encoding Genes in Lagos State, Nigeria

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ABSTRACT: *Pseudomonas aeruginosa*, frequently associated with a wide range of community and nosocomial infections, is notorious for being resistant to several classes of antibiotics with only a handful of antibiotics still effective. This study determined the human body burden of *P. aeruginosa* as well as antibiotics susceptibility pattern and presence of extended spectrum beta-lactamase (ESBL) and carbapenemase encoding genes in Lagos state, Nigeria using standard methods. Out of 103 bacterial cultures collected, 31 *P. aeruginosa* isolates were obtained, mostly originating from wound and urine samples. High rates of antibiotics resistance were observed to fluoroquinolone and cephalosporins with 24 (77.4%) resistant to ciprofloxacin, 19 (61.3) to cefotaxime, and 18 (58.1%) to ceftriaxone as well as amoxicillin clavulanic acid. However, resistance to ceftazidime and meropenem were low with only 6 (19.4%) and 5 (16.1%) resistant isolates respectively. ESBL production was detected in 10 (32.3%) of the isolates with ESBL genes detected in 6 (60%) of the 10 isolates. Ceftazidime and meropenem are viable therapeutic options for *P. aeruginosa* infections. Selection and dissemination of ESBL producing P. aeruginosa must be curtailed to prevent the loss of efficacy in currently available viable therapeutic options.

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Pseudomonas aeruginosa is a Gram-negative facultatively anaerobic straight or slightly curved rod 1 to 3µm long and 0.5 to 1.0µm wide that produces one or more water soluble pigments including pyoverdine a yellow-green fluorescent pigment, pyorubin a red-brown pigment, pyocyanin a blue-green pigment, and pyomelanin a dark pigment (Ezeador *et al*., 2020). It is an opportunistic pathogen and member of the ESKAPE group of pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species) notorious for being highly virulent and resistant to antibiotics. Although *P. aeruginosa* can degrade recalcitrant materials as well as produce secondary metabolites used industrially and in medicine, it is frequently responsible for infections in patients with cystic fibrosis, bacteremia, ventilator-associated pneumonia, urinary tract infections, skin and soft-tissue infections, surgical site infections, as well as sepsis in intensive care unit patients (Anayo *et al*., 2019). It is the most pathogenic Gram-negative rod, second most common cause of

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pneumonia, third most common Gram-negative cause of blood stream infection, and fourth most commonly isolated nosocomial pathogen accounting for 10% of all hospital-acquired infections (Pachori *et al*., 2019). *P. aeruginosa* is intrinsically resistant to a variety of antibiotics and antiseptics, has minimum nutrition requirements, the ability to form biofilm, as well as acquire and discard genomic segments in response to environmental stresses, making it able to survive in adverse environments such as dry surfaces of hospitals, medical equipment like catheters and ventilators, distilled water, among others, and disseminate in intensive care units with high selection pressure due to the increased use of antibiotics (Pachori *et al*., 2019). Acquisition of several mechanisms of antibiotics resistance including the extended spectrum beta lactamases (ESBLs) by strains of *P. aeruginosa* has made their infections more difficult to treat as these organisms often multidrug resistant, expressing resistance to third generation cephalosporins, monobactams, aminoglycosides and fluoroquinolones. Carbapenem has been the drug of choice for treatment of infections caused by ESBL producing organisms but carbapenem resistant bacteria have been increasingly reported worldwide (Ponce-de-Leon *et al*., 2018).

Carbapenem resistant *P. aeruginosa* (CRPA) have been reported worldwide and are a major threat to public health. In fact, the World Health Organization (WHO) has classified CRPA as a critical priority pathogen for which novel antibiotics are urgently needed (WHO, 2017). This study researched the prevalence of *P. aeruginosa* strains encoding extended spectrum beta lactamases and carbapenemases in Ikeja, the heart of Lagos state, Nigeria. Therefore, the objective of this paper is to evaluate the human body burden of *Pseudomonas aeruginosa*, antibiotic susceptibility pattern of isolated *P. aeruginosa*, and prevalence of extended spectrum β-lactamase and carbapenemase encoding genes in Lagos state, Nigeria.

MATERIALS AND METHOD

Study Design: Bacterial samples were collected over a period of two months from different hospitals and clinical laboratories in Ikeja, Lagos state, Nigeria, and analyzed at the Department of Medical Microbiology and Parasitology, Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos state, Nigeria.

Bacterial Collection and Identification: One hundred and three bacterial samples were collected in Brain Heart Infusion broth (Oxoid, Basingstoke, Hampshire, United Kingdom), obtained from patients' urine (41), stool (25), wound (23) and high vaginal swab (14). Samples collected were cultured on MacConkey agar (Oxoid, Basingstoke, Hampshire, United Kingdom) and identified using conventional methods.

Antibiotics Susceptibility Testing: Antibiotics susceptibility testing was performed using Kirby-Bauer disc diffusion method according to standards by the Clinical and Laboratory Standards Institute (CLSI, 2020). Commercial antibiotics discs used include ceftazidime (30µg), cefotaxime (30µg), gentamycin (10µg), ciprofloxacin (5µg), amoxicillin clavulanic acid (30µg), ceftriaxone (30µg) and meropenem (10µg) (Oxoid, Basingstoke, Hampshire, United Kingdom). *P. aeruginosa* ATCC 27853 was used for quality control.

Detection of ESBL Production: Phenotypic determination of ESBL production was performed according to standards set by the Clinical Laboratory Standards Institute (CLSI, 2020). Double disc synergy test was performed using cefotaxime and ceftazidime in combination with amoxicillin-clavulanic acid.

Detection of Carbapenemase Production: All isolates non-susceptible to carbapenem (imipenem and/or meropenem) were tested for the production of carbapenemase using the modified carbapenem inactivation method according to CLSI 2020 standards.

Table 1. Primers used in Detection of Genes Encoding ESBL and Carbapenemase

Target gene	Primer name	Primer Sequence	Amplicon size (bp)	Annealing Temperature
PER-1	PER-1-F PER-1-R	ATG AAT GTC ATT ATA AAA G TTG GGC TTA GGG CAG	920	49ºC
IMP	IMP-F IMP-R	GAGTGGCTTAATTCTCRATC CCAAACYACTASGTTATCT	183	56ºC
OXA	OXA-F OXA-R	AACGGGCGAACCAAGCATTTT TGAGCACTTCTTTTGTGATGGCT	585	58ºC
TEM	TEM-R TEM-F	AGCGATCTGTCTAT AAACGCTGGTGAAAGTA	822	56ºC
SHV	SHV-R SHV-F	TGCTTTGTTATTCGGGCCAA ATGCGTTATATTCGCCTGT	753	56ºC
CTX-M	CTX-M-R CTX-M-F	CGATATCGTTGGTGGTGCCATA TTTGCGATGTGCAGTACCAGTAA	590	56ºC

Genotypic Detection of ESBLs and Carbapenemases: DNA was extracted from all isolates that tested positive for ESBLs and/or carbapenemases production by boiling method. Multiplex polymerase chain reaction (PCR) was performed in a DNA thermal cycle using the kit Maxima Hot Start PCR Master Mix (2x), Cat. No. K1051 according to manufacturer's instructions and primers for genes encoding ESBL and carbapenemase production (Table 1). The PCR amplification products were separated on 1.5% agarose gel and electrophoresis carried out at 80V for 1 hour 30 minutes using Gene Ruler 1Kb plus DNA ladder as DNA molecular weight marker. After electrophoresis, DNA bands were visualized by ethidium bromide staining.

Data Analysis: Descriptive analysis of the results was performed using SPSS 20.

RESULTS AND DISCUSSION

Over the two-month period, a total of 31 isolates of *Pseudomonas aeruginosa* were obtained from the samples collected, with the majority (19; 61.3%) originating from wounds (Table 2). No *P. aeruginosa* was isolated from stool. The isolated *P. aeruginosa* displayed high levels of resistance to antibiotics, particularly to ciprofloxacin with 24 (77.4%) resistant isolates (Fig 1).

Table 2. Prevalence of *P. aeruginosa* Isolated

Sample Source	Total Samples Collected	Total Isolates Obtained, n (%)
Urine	41	10 (24.4%)
Wound	23	19 (82.6%)
Stool	25	0
High Vagina Swab	14	2 (14.3%)
Total	103	31 (30.1%)

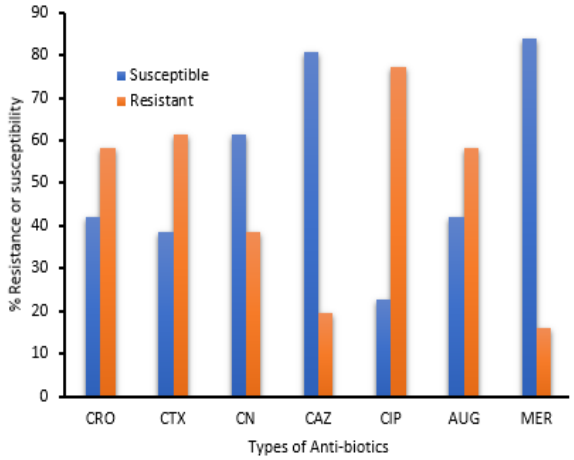

Fig 1. Antibiotics Sensitivity of Isolated Bacteria

CRO – Ceftriaxone, CTX – Cefotaxime, CN – Gentamicin, CAZ – Ceftazidime, CIP – Ciprofloxacin, AUG – Amoxicillin-clavulanic acid, MER - Meropenem

However, the isolates showed high susceptibility to ceftazidime and meropenem. ESBL production was phenotypically detected in 10 (32.3%) of the *P. aeruginosa* isolates (Fig 2). The highest prevalence (6; 60%) of ESBL producers was obtained from wound, followed by urine with 3 (30%) and high vagina swab with only 1 (10%) ESBL producer. Genes encoding ESBL, including bla_{SHV} and $bla_{CTX\text{-}M}$, were observed in 6 (60%) of the ESBL producers (Fig 3). There were no bla_{TEM} or $bla_{PER\text{-}1}$ genes detected. All isolates with ESBL genes were observed to carry more than one gene except an isolate with only $bla_{CTX\text{-}M}$ (Fig 4). Only 1 (10%) isolate was observed to carry bla_{OXA}.

Pseudomonas aeruginosa is one of the most frequently isolated Gram-negative bacteria in the hospital environment due to its high level of antimicrobial resistance and ability to persist in harsh environments. It is responsible for a myriad of infections, particularly in immunocompromised patients, and is the leading aetiology of nosocomial infections such as urinary tract infections and surgical site infections. In this study, *P. aeruginosa* was majorly isolated from wound, with 82.6% of the wound samples collected found to harbour this bacterium. This correlates with the studies by Adejobi et al. (2021) in southwest Nigeria, and Hosu et al. (2021) in South Africa who obtained the majority (44% and 80.4% respectively) of their *P. aeruginosa* isolates from wound and pus. *P. aeruginosa* is known to be a common pathogen in wound, often present as an antibiotic resistant biofilm, and causes prolonged morbidity, delayed healing and severe wound outcomes (Raizman *et al*., 2021). *P. aeruginosa* is not a regular member of the human microflora and is not often an aetiology of gastrointestinal infection as evidenced by the lack of isolates from stool in this study. It has however been known to colonize the gastrointestinal tracts of hospitalized patients, particularly immunocompromised patients already on antimicrobial therapy (Ettu *et al*. 2021).

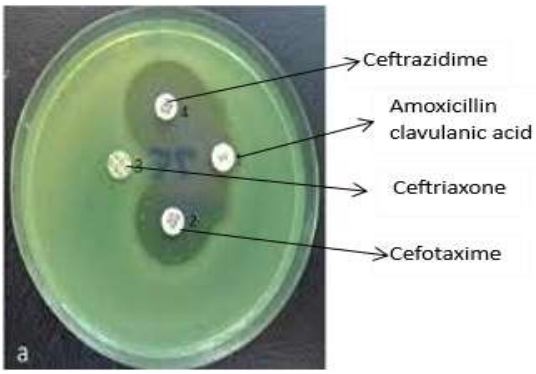

Fig 2. Double disc synergy test positive for ESBL production
Ceftrazidime and cefotaxime display synergy with amoxicillin clavulanic acid.

Antibiotics resistance in *P. aeruginosa* remains a major concern urgently requiring the development of new antimicrobials to improve treatment options and outcomes. This study showed a high level of antibiotics resistance in fluoroquinolone (ciprofloxacin 77.4%), third generation cephalosporins (cefotaxime 61.3% and ceftriaxone 58.1%), and penicillin + β-lactamase inhibitor (amoxicillin clavulanic acid 58.1%).

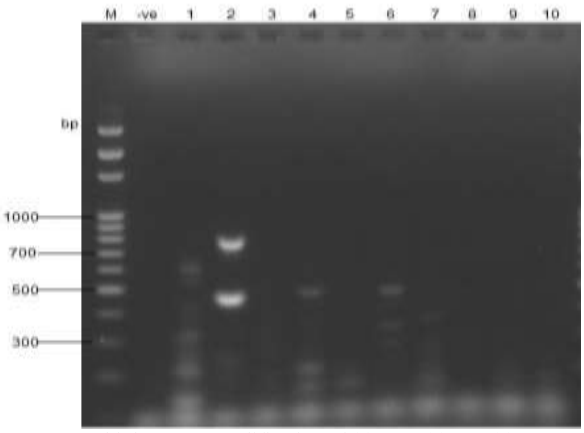

Fig 3. Multiplex PCR amplification of *bla*$_{PER-1}$, *bla*$_{OXA}$, *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$

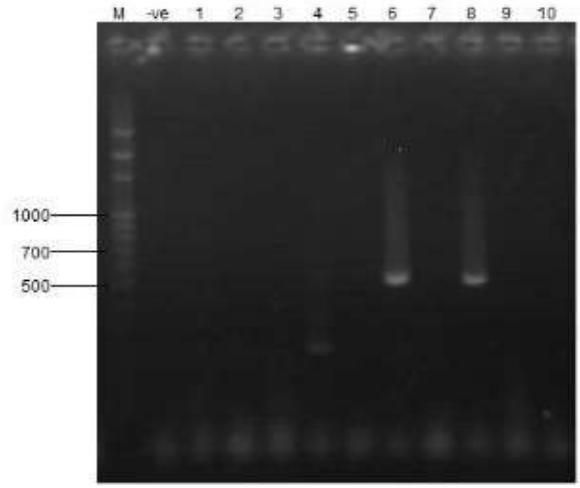

Fig 4. PCR amplification of *bla*$_{CTX-M}$

The aminoglycoside gentamicin, third generation cephalosporin ceftazidime, and carbapenem meropenem were however found to be effective against *P. aeruginosa* with 61.3%, 80.6%, and 83.9% susceptibility respectively. Although gentamicin seems effective in this study, it had a resistance rate of 38.7%, higher than the 35.4% resistance reported in the study by Adejobi et al. (2021) in which gentamicin had the highest resistance rate. A lower susceptibility to meropenem was also observed in this study, with Adejobi et al., (2021) reporting 89% susceptibility. Antibiotics surveillance is expedient to monitor the effectiveness of commonly used antibiotics in order to mitigate selection of antibiotics resistant strains. Antibiotics prescription, particularly empiric therapy should be well guided, and prolonged antibiotics treatment of infections caused by antibiotic resistant bacteria discouraged to prevent further selection of resistant strains (Tamma *et al*., 2021).

The expression of ESBL by *P. aeruginosa* further aggravates the level of antibiotics resistance displayed by the isolates. ESBL production was observed in 32.3% of the *P. aeruginosa* obtained of which the genes responsible were detected in 60% of the isolates. *Bla*$_{OXA}$ which encodes carbapenem resistance was also detected. *Bla*$_{CTX-M}$ was the most prevalent gene encoding ESBL detected, concurring with reports that *bla*$_{CTX-M}$ is now the most widely distributed ESBL encoding gene worldwide (Rahman *et al*., 2018). CTX-M is commonly associated with co-resistance as it is usually expressed along with other resistance genes (Sawa *et al*., 2020). The presence of ESBL encoding genes, particularly *bla*$_{CTX-M}$, as well as *bla*$_{OXA}$ in this study is alarming as they expand the antimicrobial resistance threshold of *P. aeruginosa*, a bacterium already with a fully stocked arsenal of resistance mechanisms, further complicating treatment of infections, particularly in immunocompromised patients.

Conclusion: This study detected a high human body burden of *Pseudomonas aeruginosa* with a number being highly resistant to antibiotics including carbapenem, and possessing genes encoding extended spectrum β-lactamase as well as carbapenemase. Ceftazidime and meropenem remain viable options for reducing the burden of antibiotics resistant *P. aeruginosa*. Antibiotics surveillance systems are expedient to conserving the efficacy of currently available therapeutic options.

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