Short Research Article

Prevalence and Antibiotic resistance Profile of *Pseudomonas aeruginosa* from hospital sinks in South Western Nigeria

RUNNING TILE: Prevalence of *pseudomona aeruginosa* in Hospital sinks

ABSTRACT

Background: *Pseudomonas aeruginosa* (*P. aeruginosa*) has been identified as a major pathogen in man, causing both opportunistic and nosocomial infections. Pseudomonas is a ubiquitous organism often isolated from various surfaces, which have the ability to form biofilms, making it a unique organism of medical importance.

Objective: The aim of this study is to determine the prevalence of *P. aeruginosa* isolated from hospital sinks and their antibiotic resistance profile.

Methods: Swab samples were collected from hospital sinks in five health care institutions and inoculated unto Nutrient agar and sub cultured on cetrimide agar. Isolated *P. aeruginosa* were subjected to antibiotic susceptibility testing using CSLI guidelines.

Results: Prevalence of *Pseudomonas* species isolated from the hospitals' sinks was 56%. High level resistance was recorded against amoxicillin/clavunalate, ampicillin and ceftriaxone. Resistance profile of the isolates clustered into two main clades clade A and clade B, with clade A isolates recording a higher MARI score.

Conclusion: Isolation of multi-resistant *P. aeruginosa* from hospital sinks calls for improved hospital infection control practices. We advocate for inclusion of environmental surveillance, particularly of opportunistic pathogens in our hospitals.

Keywords: Prevalence, *Pseudomonas aeruginosa*, Antibiotic resistance, Hospital sinks, MARI, Nigeria.

INTRODUCTION

Pseudomonas spp is a gram negative rod-like bacteria belonging to the family Pseudomonacea, responsible for a host of infectious diseases in both man and animals such as Otitis externa, folliculitis, septicemia, and ventilator associated pneumonia[1]. Pseudomonas possesses certain properties such as quorum sensing and biofilm formation which allow it to spread and survive under adverse conditions [1]. Previous reports have also identified *Pseudomonas spp* to be responsible for responsible for outbreaks of dermatitis, conjunctions and otitis externa from recreational waters in the US [2]. Due to the ubiquitous nature of *Pseudomonas spp* it has been identified in various water sources [3, 4, 5], and as such water contributes to its cross contamination in clinical environments making it a primary agent of nosocomial infections [5,6].

Among organisms within *Pseudomonas spp*, *Pseudomonas aeruginosa* is the most medically important, it is responsible for chronic lung infections among cystic fibrosis patients [7]. *Pseudomonas aeruginosa* has also been identified as the singular most incriminated pathogen in nosocomial infection [8]. *Psuedomonas aeruginosa* is also unique for ability to acquire both plasmid borne and chromosomal antibiotic resistance genes [9]. Mutidrug resistance observed at high levels among P. aeruginosa, involves several mechanisms, including the overexpression of active efflux systems, production of beta-lactamase modifying enzymes, a decrease in outer membrane (OM) permeability as well as structural alterations of topoisomerases II and IV, involved in quinolone resistance (10, 11). In majority of MDR *P. aureginosa*, some of these antibiotic-resistance genes are harbored within plasmids, clustered in a cassette carried by a class 1 integron (11). Figure 1 represents an example of a gene map of an MDR plasmid showing the location of various antibiotic resistance genes. The *P. aeruginosa* genome, about 6.3 million bp encodes more than 8 virulence genes, making *P aeruginosa* one of the most pathogenic bacteria with high mortality rates resulting from infections [12]. In Nigeria there have several reports on *P. aeruginosa* infections in clinical settings in Nigeria [8]. However there is a paucity of data on isolation of *P. spp* from fomites or contaminated surfaces such as sinks in hospital settings in Nigeria, despite its importance as a principal nosocomial pathogen able to survive on various surfaces and form biofilms. This study is aimed at determining the prevalence and antibiotic resistance profile of *P. aeruginosa* and other species of psuedomonas in hospital sinks in some Nigerian hospitals.

MATERIALS AND METHODS

Study design and sample collection

This study is a cross sectional survey of pseudomonas species isolated from sinks in 5 tertiary health institutions in South western Nigeria, namely Lagos University Teaching Hospital (LUTH), University College Hospital, Ibadan (UCH), Nigeria Navy Reference Hospital, Lagos (NNRH), Pentecost Medical Centre, Lagos (PMC) and Bowen University Hospital, Iwo (BUH). Ethical approval was sought from sought the relevant ethics committee of the various facilities.

Sinks used for hand washing were randomly selected from the nursing department of each of the study facility, and swabbed with the aid of a sterile swab stick around the edges and around the tap knobs. Swab sticks were then transported aseptically to the Microbiology laboratory for sample processing.

Isolation of *Pseudomonas spp*

Samples were swabbed unto Nutrient/Mackonkay agar and incubated at $36^{O}C$ for 48hrs. Colonies were then subcultured on Cetrimide agar. Isolated pure cultures were then gram stained and tested using standard biochemical tests [12].

Antibiotic susceptibility testing

Antibiotic susceptibility testing was done on identified *Pseudomonas* isolates using commercially available single disks from Oxiod (U.K). Single colony of each isolate was inoculated into peptone water and 1.5 = 10 cfu/ml Macfarland suspension was inoculated unto Muller Hinton Agar. Single disks of Gentamicin (GEN), 10µg; Ciprofloxacin (CPR), 5µg; Ofloxacin (OFL), 5µg; Amoxicillin/clavulanate (AUG), 30µg; Nitrofurantoin (NIT), 300µg; Ampicillin (AMP), 10µg; Ceftazidime (CAZ), 30µg and Cefuroxime (CRX), 30µg. Agar plate were then incubated at $36^{O}C$ for 24 hrs. Zone of inhibition were measured and interpreted following Clinical Laboratory Standards Institute guidelines [13].

Antibiotic resistance relatedness

DendroUPGMA ulility software was used to build a dendogram, and the distance matrix used to calculate a similarity matrix and transform coefficients into distances using the Unweighted pair group method with arithmetic mean (UPGMA) algorithm [14].

Statistical analysis

All the data generated was grouped into tables, charts were drawn with the aid of Microsoft excel. Analysis of data was done using SPSS Vs 20.0.

RESULTS

The prevalence of *Pseudomonas* species isolated from the hospitals' sinks are shown in Table 1 From Lagos University Teaching Hospital, University College Hospital, Ibadan, Bowen University Hospital, Nigerian Navy Reference Hospital, Pentecost Medical Centre, the occurrence of *Pseudomonas* species were 85.71%, 57.14%, 57.14%, 50%, 20% respectively. The antibiotic sensitivity test is shown in Figure 2. Most strains were sensitive to gentamicin, ciprofloxacin and ofloxacin, while they showed relative resistance to other antibiotics including amoxicillin clavulanate, nitrofurantoin, ampicillin, ceftazidime and cefuroxime.

The antibiotic resistance profile of the isolates is shown in a dendogram Figure 3, indicating their clustering pattern and antibiotic multiresistant index (MARI). *Pseudomonas aeruginosa* recorded a MARI of between 0.25 to 0.625, while *Pseudomonas spp* recorded MARI of 0.025

to 1.0. The isolates also clustered into two main clades clade A and clade B, with clade A isolates recording a higher MARI score.

DISCUSSION

Pseudomonas remains one of the most important pathogens known to man, with its ubiquitous nature and ability to form biofilms [1], makes it an effective nosocomial pathogen. This study surveyed the prevalence and resistance profile of *Pseudomonas* isolated from hospital sinks in 5 different hospitals. The total prevalence of *Pseudomonas ssp* isolated was 56%, while UCH recorded the highest number of isolations, while PMC recorded the lowest. This observation could be attributed to the size and number of consultations, UCH for instance is one the largest teaching hospitals in Nigeria. Regardless of the level of infection control, some nosocomial pathogens such as pseudomonas can be introduced into the hospital environment accidentally by attending care givers. In our study, the highest level of antibiotic resistance was recorded in Cefuroxime, and Amoxicillin, with 88% and 82% respectively. This is a clear indication of high level beta lactamase resistance among our pseudomonas isolates. Previous studies have recorded a high level of resistance to 2rd generation chephalosporins [16]. Although there have been other reports of low resistance rates of 2nd generation chephalosporins to pseudomnas, such as Yetkin et al [17]. In our study there was also high level resistance observed against augumentin (amoxicillin/clavunalate) with 65% resistance. This high level resistance is indicative of the presence of extended spectrum beta lactamase (ESBL) among our isolates, even though ESBL testing was not conducted on the isolates; a resistance to both cephalosporin and augumentin shows the presence of ESBL enzyme [18-20]. *Pseudomonas aeruginosa*, resistance to b-lactam antibiotics and aminoglyco-sides has been reported to be associated with the production of enzymes, such as, carbenicillin hydrolyzing b-lactamases, extended-spectrum b-lactamases (ESBL), oxacillinases, metallo-b-lactamases(MBLs) ae well as aminoglycoside-modifying enzymes [10, 11].A limitation of this study was our inability to determine the type of β-lactamase gene responsible for the observed β-lactamase and probable ESBL properties observed among our isolates. Another interesting finding in our study is the low level resistance observed to the quinolones among our pseudomonal isolates, this is not in concordance with several reports that have reported high level quinolone resistance in Nigeria[21]. There was also very little resistance observed against the aminoglycoside Gentamycin, a study by Akingbade et al [8] reported 66% and 26% resistance to Streptomycin, and Gentamycin respectively, most other studies report a moderate to low level resistance to aminoglycosides [22]. This might be attributed to lack of abuse of this class of antibiotics as they are injectable drugs and are often administered in proper health care facilities. The antibiotic resistance relatedness among the isolates showed a distinct clustering pattern with the isolates falling into 2 main clades, A and B. Clade A isolates had a MARI of 0.5 to 0.625 indicating a uniform antibiotic resistance pattern. Most of the isolates in this clade was pseudomonas species, showing multi-drug resistance with high a MARI score of 0.625. Clade B isolates however showed a much lower MARI score ranging from 0.00 to 0.375. Studies have reported that a MARI index score of >

0.2 is indicative of multi-drug resistance occurring from indiscriminate use of antibiotics [23].

CONCLUSION

The current observation of high MARI is evidence of the wide spread dissemination of *pseudomonas spp* harboring transmissible multi-resistant genes such as ESBL and Carbapenem resistance genes KPC in our environment. The detection of this pathogen in hospital sinks is worrisome, because pseudomonas is able to form biofilm on various materials including plastic tubing used in most invasive medical devices such as catheter and naso-gastric tube. Effort should be made toward incorporating practices such as hospital environmental surveillance and effective environmental disinfection in various hospital infection prevention and control units.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding this paper.

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Table 1: Prevalence of *Pseudomonas* species isolated from hospital sinks in South Western Nigeria.

Location	Total number of samples	Number of Pseudomonas spp isolated	Prevalence %
LUTH	7	6	85.71
UCH	7	4	57.14
BUH	7	4	57.14
NNRH	4	2	50
PMC	5	1	20
Total	30	17	56.67

Key: Lagos University Teaching Hospital (LUTH), University College Hospital, Ibadan (UCH), Nigeria Navy Reference Hospital, Lagos (NNRH), Pentecost Medical Centre, Lagos (PMC) and Bowen University Hospital, Iwo (BUH).

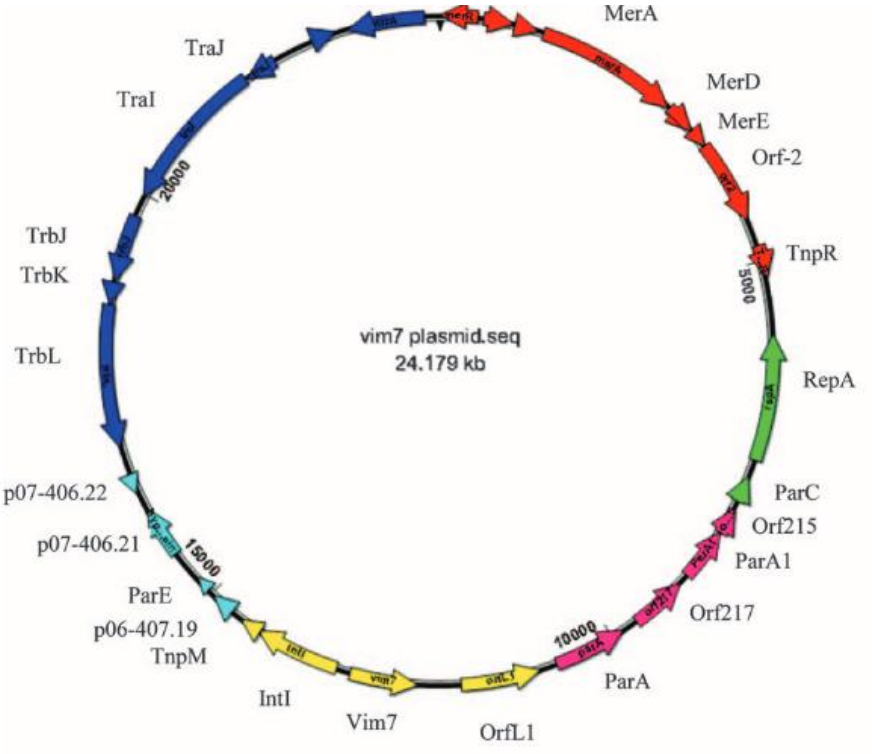

Figure 1: Genetic map of plasmid p07-406 (accession number AM778842) showing the arrangement of the major DNA segments. Blue, Tra region; red, Mer; green, Rep; pink, ParA and ParC; yellow, class 1 integron containing blaVIM-7; and aqua, ParE.

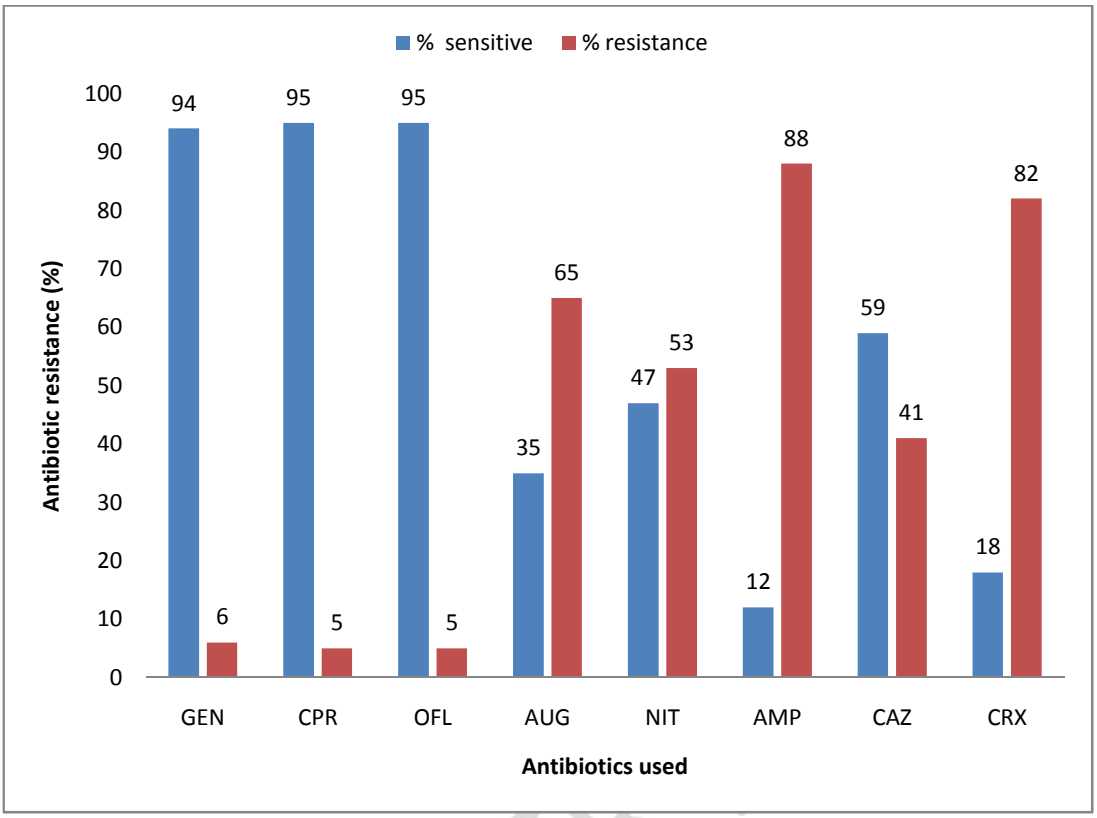

Figure 2: Overall antibiotic sensitivity pattern of the isolated *Pseudomonas* species. The y-axis represents percentage susceptibility/resistance, x-axis represents antibiotics used for testing.

Antibiotic key: GEN-Gentamycin, CPR-Ciprofloxacin, OFL-Ofloxacin, AUG-Amoxicillin/Clavunalate, NIT-Nitrofurantoin, AMP-Ampicillin, CAZ-Ceftrazone, CRX-Ceftazidime

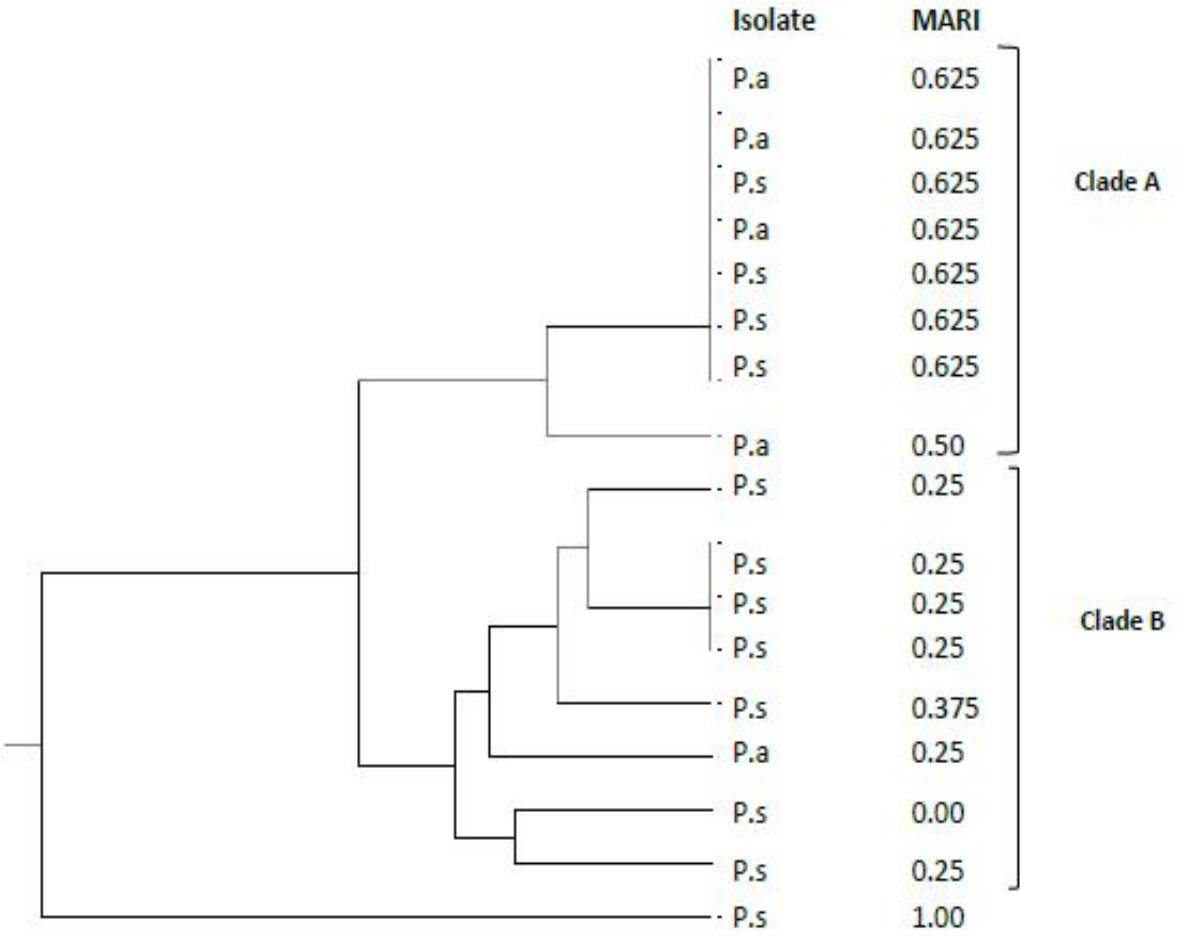

Figure 3: Antibiotic resistant relatedness of *Pseudomonas spp* isolates obtained from hospital sinks with their respective multi-antibiotic resistance index (MARI).
N.B: P.s represents *Pseudomonas species*, P.a represents *Pseudomonas aureginosa*.