Research Article

MULTIDRUG RESISTANT *PSEUDOMONAS AERUGINOSA* FROM SOUTHWEST NIGERIA HOSPITALS

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ABSTRACT

Pseudomonas aeruginosa remains one of the most dreaded resistant pathogens mostly encountered in infections worldwide. However; serious infections due to this bacterium are predominantly hospital-acquired. This present study is aimed at investigating the resistance patterns and susceptibilities of *P. aeruginosa* isolated from hospitals in Southwest Nigeria. A total of 54 unrelated clinical strains of *P. aeruginosa* isolated from 5 hospitals in Southwest Nigeria comprises of 38.9% isolated from urine, 20.4% from wounds, and 11.1% from pus while the remaining 29.6% were distributed among other clinical sites. Antimicrobial susceptibility testing for 21 antibiotics and minimum inhibitory concentration (MIC) was done by disk diffusion and E-test method respectively. Each isolate was resistant to ≥ 3 classes of antibiotics, 24 (44.44%) were resistant to 4–9 antibiotics; 17 (31.48%) were resistant to 10–13 antibiotics; 11(20.37%) were resistant to 14 – 16 antibiotics and 2 (3.70%) were resistant to 17 – 19 antibiotics. Thirty (55.55%) isolates were resistant to ≥ 10 antibiotics. High rates of resistance were recorded for ampicillin (100%), tetracycline (100%), amoxicillin/clavulanate acid (100%), ticarcillin/clavulanate acid (87.0%), kanamycin (79.6%), carbenicillin (63.0%) and cefotaxime (46.3%). Highest number of susceptible isolates (98.1%) and (92.6%) were recorded for colistin and imipenem respectively. The highest MIC recorded for cefotaxime, ciprofloxacin, ceftazidime, piperacillin, and amikacin were 30, 240, 240, 240, and 256µg/ml respectively. The resistance profiles observed in this study suggest a prevalence of *P. aeruginosa* strains harbouring multiple resistance mechanisms in Southwest Nigeria, which could limit available therapeutic options for infections caused by these multidrug resistant strains.

Keywords: Antibiotics, multidrug resistance, *Pseudomonas aeruginosa.*

INTRODUCTION

Pseudomonas aeruginosa is a ubiquitous pathogen that can be isolated almost everywhere. However, severe infections caused by this bacterium are predominantly nosocomial.[1] *P. aeruginosa* is the second most common cause of hospital-acquired pneumonia and has been implicated as the third leading cause of hospital-acquired urinary tract infections (UTIs)[2]. Infections caused by *P. aeruginosa* are clinically similar to those caused by other Gram-negative bacteria, hence if not properly treated with appropriate empiric antibiotics specific for *P. aeruginosa* from the onset, may lead to resistance during the course of treatment and in the worse cases death[3].

Broad-spectrum anti-pseudomonal drugs such as imipenem, ceftazidime, amikacin has been recommended for treatments of infections caused by multiple drug resistant (MDR) *P. aeruginosa*[4]. However, resistance to one or more of these anti-pseudomonal drugs during therapy has been widely observed. Hence, the need for combination therapy consisting of two classes of anti-pseudomonal drug[1]. The recommended combination therapy of *β*-lactam drug and another drug from the aminoglycosides or the fluoroquinolones has recently been reported to be ineffective against some strains of MDR *P. aeruginosa*[5].

Reports from Nigeria have described *P. aeruginosa* as a major Gram-negative bacteria implicated in most nosocomial infections in Nigeria[6]. With reference to few among the many documented susceptibilities studies carried out in Nigeria[7-10], the trend in resistance phenotype of *P. aeruginosa* to the commonly prescribed antibiotics in various hospitals is increasing since the last decade. Selective pressures and indiscriminate use of the first-line drugs in Nigeria have rendered such drugs clinically ineffective against *P. aeruginosa.* There are few studies on the susceptibilities of *P. aeruginosa* to broad-spectrum anti-pseudomonal drugs in Nigeria; majority of reported studies were addressing the susceptibility with minimal antibiotics most of which have always been previously reported one way or the other. Imipenem, levofloxacin, norfloxacin, amikacin, cefotaxime, cefepime, colistin, piperacillin and piperacillin/tazobactam are wide-spectrum antibiotics with good antimicrobial activities against MDR *P. aeruginosa*. However, information on the susceptibility of clinical isolates of MDR *P. aeruginosa* against these drugs in most reports in Nigeria is scanty, it is therefore imperative to establish a correlation of their antimicrobial status with MDR *P. aeruginosa* in Nigeria.

This present study is aimed at investigating the resistance patterns and susceptibilities of *P. aeruginosa* to a panel of 21 antibiotics representing 9 classes of antimicrobial agents, some of which report against *P. aeruginosa* is scarce in our region. The information obtained is intended for use in improving empiric prescription of antibiotics for *P. aeruginosa* infections and to also provide wider information on the current status of susceptibility of *P. aeruginosa* in southwest Nigeria.

MATERIALS AND METHODS

Sample collection

Fifty-four clinical strains of *P. aeruginosa* were recovered from different specimens including urine, pus, wound swab, ear swab, blood and high vagina swab of infected patients from 5 different hospitals in 3 Southwest states of Nigeria from March through September 2010.

Isolation and identification

All isolates recovered were Gram stained and subcultured on *Pseudomonas* isolation agar base, Hifluoro *Pseudomonas* Hiveg agar base and Cetrimide agar (Hi-media, India) to obtain a pure culture. *P. aeruginosa* isolates were confirmed by standard biochemical methods as described previously[11].

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of 54 isolates of *P. aeruginosa* against 21 antibiotics (Oxoid, UK) (Table 2), representing 9 classes of antimicrobial agents was performed by the Kirby Bauer disc diffusion method and interpreted according to the Clinical and Laboratory Standards Institute guidelines[12]. Twenty clinical strains of *P. aeruginosa* among those that shows resistance ≥ 3classes of antibiotics were randomly selected for the minimum inhibitory concentration (MIC) determination. MIC of antibiotics was determined by E-Test Strip from Himedia Laboratories Pvt. Ltd., Mumbai India. Inoculated plates were incubated aerobically at 37°C and estimated after 18hrs. For quality control of the experiment *P. aeruginosa* ATCC 27853 and *E.coli* ATCC 25922 were used.

The term "resistance" in this study includes organisms that show intermediate resistance and those that are resistant according to CLSI guideline.

Table 1: Distribution of *P. aeruginosa* recovered from various clinical specimens

Source/site	No of isolates	Percentage (%)
Urine	21	38.9
Wound swab	11	20.4
Pus	6	11.1
Blood	3	5.5
Ear swab	5	9.3
HVS	2	3.7
Throat swab	3	5.6
Trachea aspirate	2	3.7
Leg ulcer	1	1.8
Total	54	100.0

RESULTS

Prevalence of Pseudomonas aeruginosa in clinical specimens

The susceptibility patterns and resistance profiles of the 54 clinical isolates of *P. aeruginosa* by disk diffusion method to a panel of 21 antibiotics was investigated. Table 1 shows the distribution of *P. aeruginosa* recovered from various clinical specimens. The predominant isolates recovered were from urine (38.9%), wound (20.4%) and pus (11.1%) while few isolates were recovered from ear swab (9.3%), blood (5.5%), HVS (3.7%), throat swab (5.6%), trachea aspirate (3.7%) and from leg ulcer (1.8%).

Table 2: The susceptibility patterns of 54 *Pseudomonas aeruginosa* strains and the percentage *(%)* resistance to 21 antibiotics

Antibiotic (Disc potency, µg)	No. and (%) Sensitive	No. and (%) Intermediate	No. and (%) Resistant
Amikacin (30)	42 (77.8)	2 (3.7)	10 (18.5)
Amoxicillin/clavulanic acid (20/10)	0(0)	NA	54 (100)
Ampicillin (25)	0 (0)	NA	54 (100)
Aztreonam (30)	30 (55.6)	6 (11.1)	18 (33.3)
Carbenicillin (100)	20 (37.0)	5 (9.3)	29 (53.7)
Cefepime (30)	44 (81.4)	5 (9.3)	5 (9.3)
Cefotaxime (30)	26 (48.1)	3 (5.6)	25 (46.3)
Ceftazidime (30)	45 (83.30	1 (1.9)	8 (14.8)
Ceftriaxone (30)	25 (46.3)	14 (25.9)	15 (27.8)
Ciprofloxacin (5)	35 (64.8)	4 (7.4)	15 (27.8)
Colistin (10)	53 (98.1)	NA	1 (1.9)
Gentamicin (10)	32 (59.3)	NA	22 (40.7)
Imipenem (10)	50 (92.6)	3 (5.5)	1 (1.9)
Kanamycin (30)	11 (20.4)	NA	43 (79.6)
Levofloxacin (5)	33 (61.1)	1 (1.9)	20 (37.0)
Norfloxacin (10)	33 (61.1)	3 (5.6)	18 (33.3)
Piperacillin (100)	33 (61.1)	NA	21 (38.9)
Piperacillin-tazobactam (100 /10)	33 (61.1)	NA	21 (38.9)
Streptomycin (10)	32 (59.3)	NA	22 (40.7)
Tetracycline (30)	0 (0)	NA	54 (100)
Ticarcillin/clavulanic (75/10)	47 (87.0)	NA	7 (13.0)

Table 3: MICs of different antibiotics for clinical *P. aeruginosa* isolates by E-Test

Strain No	Concentrations are in µg/ml							
	Antibiotics							
	PIP	CAZ	CTX	CRO	CIP	LEV	AK	GEN
1	240.0	240.0	15.0	240.0	0.01	0.01	2.0	256.0
5	240.0	240.0	15.0	240.0	0.25	1.0	4.0	256.0
8	60.0	15.0	7.5	240.0	1.0	2.0	0.5	256.0
12	5.0	7.5	3.0	3.0	0.001	0.01	0.1	0.5
16	5.0	15.0	3.0	7.5	0.01	0.25	0.1	0.1
17	30.0	30.0	15.0	240.0	0.004	0.25	0.5	256.0
24	120.0	120.0	15.0	240.0	0.5	0.5	256.0	256.0
25	120.0	5.0	15.0	240.0	1.0	1.0	256.0	256.0
27	1.0	3.0	3.0	7.5	30.0	0.005	0.05	0.1
28	1.0	1.0	3.0	15.0	0.008	0.005	0.05	0.1
32	5.0	15.0	15.0	240	240.0	240.0	256.0	256.0
34	240.0	240.0	15.0	240.0	240.0	240.0	256.0	256.0
38	5.0	1.0	3.0	240.0	240.0	240.0	256.0	256.0
40	240.0	120.0	15.0	240.0	240.0	240.0	256.0	256.0.
42	240.0	15.0	30.0	240.0	0.01	0.25	0.05	0.5
45	240.0	120.0	7.5	240.0	240.0	240.0	256.0	256.0
46	1.0	7.5	1.0	15.0	0.01	0.05	2.0	4.0
48	120.0	30.0	15.0	240.0	240.0	240.0	256	256.0
49	240.0	15.0	15.0	240.0	2.0	2.0	0.1	0.1
52	1.0	7.5	3.0	7.5	0.008	0.05	0.05	0.05

PIP; piperacillin, CAZ; ceftazidime, CTX; cefotaxime, CRO; ceftriaxone, CIP; ciprofloxacin, Lev; levofloxacin, AK; amikacin, GEN; gentamicin

Antimicrobial susceptibility and determination of MICs

The rates of resistance were highest for ampicillin (100%), amoxicillin/clavulanic acid (100%), tetracycline (100%), ticarcillin/clavulanic acid (87.0%), kanamycin (79.6%) and carbenicillin (63.0%). High susceptibility of most of the isolates was recorded for colistin (98.1%) and imipenem (92.6%). Each isolate was resistant to ≥ 3 classes of antibiotics, 24 (44.44%) were resistant to 4–9 antibiotics; 17 (31.48%) were resistant to 10– 13 antibiotics; 11 (20.37%) were resistant to 14 – 16 antibiotics and 2 (3.70%) were resistant to 17 – 19 antibiotics. Thirty (55.55%) isolates were resistant to ≥ 10 antibiotics (table 2). Table 3 depicts the MIC values for the 20 randomly selected *P. aeruginosa* strains in this study. MIC range obtained in this study for cefotaxime was 1-30 µg/ml, ceftazidime 0.25-240 µg/ml, ceftriaxone 7.5-240 µg/ml, piperacillin 1-240 µg/ml, ciprofloxacin 0.001-240 µg/ml, levofloxacin 0.005-240 µg/ml and amikacin 0.1-256 µg/ml.

DISCUSSION

This present study investigated the susceptibility of 54 *P. aeruginosa* to 21 antimicrobial agents some of which are newer drugs belonging to β–lactam, fluoroquinolones and aminoglycosides classes of antibiotics that have been shown by many studies to be active against MDR bacteria including *P. aeruginosa*. The highest number of susceptibility of *P. aeruginosa* isolates was recorded for colistin (98.1%) followed by imipenem (92.6%). Our result for colistin contradicts studies from Nwankwo and Shuaibu[13] who reported 78.9% susceptibility of *P. aeruginosa* but corroborate result of Aibinu *et al*[14] and Olayinka *et al*[15] who reported 95.6% and 94.6% susceptibility to imipenem respectively. This implies that imipenem remain a potent anti-pseudomonal antimicrobial agent in Nigeria contrary to other report outside Nigeria where imipenem resistance is prevalent[16]. All the *P. aeruginosa* (100%) investigated in this study were resistant to amoxicillin/clavulanic, ampicillin and tetracycline. This observed resistance pattern has been a frequent report about *P. aeruginosa* in many susceptibility studies in Nigeria. In clinical context, we suggest a total replacement of these drugs as a therapeutic option in the management of *P. aeruginosa* infections in Nigeria. Meanwhile, we observed a decrease in fluoroquinolones susceptibilities among our isolates as compared to earlier studies in Nigeria. Fluoroquinolones (FQ) susceptibilities in this study are as follows; Ciprofloxacin (64.8%), levofloxacin (63.0%) and norfloxacin (66.7%). This is lower than 72.0-100% susceptibilities ciprofloxacin reported by many authors (Okonko *et al*.[17] Jombo *et al*.[18] Olayinka *et al*.[15] El-Mahmood *et al*.[19] and Ogbolu *et al*.[20]). High resistance to FQ as observed in our study has also been similarly reported[14] elsewhere. However, higher resistance (98%) to ciprofloxacin was obtained in India[21]. The findings from our result also agree with the previous publication on emerging fluoroquinolone resistance *P. aeruginosa* in Nigeria[22]. The steady increment in FQ resistance in this study is alarming and the implication might be as a loss of confidence in the use of FQ alone in the management of *Pseudomonas* infection considering the fact that FQ are

potent antimicrobial agent with broad-spectrum activities that has gained a wide use in management of many infections. Increasing resistance to this broad-spectrum antibiotic might be as a result of selective pressure due to its frequent use in Nigeria. We recommend they should henceforth be used with caution and mostly in combination with broad-spectrum beta-lactams/penicillins in order to prevent total loss of activities.

The result of this study reveals an increasing trend of resistance to the cephalosporins investigated. Among the third-generation cephalosporin, ceftriaxone was found to be least active as 46.3% isolates were susceptible, followed closely by cefotaxime (48.1%). Previous report in Nigeria[8, 9, 23] have also obtained low susceptibilities in their studies with ceftriaxone and cefotaxime. Ceftazidime was most active as 83.30% of the isolates were susceptible to it. Aibinu *et al*[14] also reported a close value of 79.4% of this drug against *P. aeruginosa* in their study. Although a contrary report or rather decrease in activity was observed for ceftazidime as compared to previous reports[13, 24] where over 90% of susceptibilities to ceftazidime were reported for *P. aeruginosa*. This result also agrees with other study in India[21] where less resistance were shown to this antibiotic by *P. aeruginosa*.

A high level of resistance (MIC ≥ 240µg) was observed in 15% and 70% of the resistant strains tested in this study against ceftazidime and ceftriaxone respectively. This means that these strains are speedily acquiring resistance against third-generation cephalosporins, unless it is henceforth used in combination with an aminoglycosides or fluoroquinolones that are resistant to extended-spectrum beta-lactamase, resistance to cephalosporin will be imminent. An interesting finding in this study is the emerging resistance to cefepime. Cefepime is a fourth-generation cephalosporin, one of the few remaining agents that have a reliable activity against *P. aeruginosa*[25]. However, resistance observed against cefepime (18.4%) in this study is troubling, considering the fact that its use in Nigeria is rarely reported. Prompt attention is hereby imperative to check this emergence in order to prevent the complications in the treatments of *P. aeruginosa*. Cephalosporins are beta-lactams antibiotics with reported desired effect and lesser toxicity. Increase in their resistance by *P. aeruginosa* could be attributed to the increase in extended-spectrum beta-lactamase (ESBL) production among the strains of *P. aeruginosa, which* has also been previously reported[14].

Out of the three *ß*-lactamase inhibitors considered in this study, we observed that piperacillin/tazobactam inhibitory activity was the highest (61.1%) compared to ticarcillin/clavulanate (13.0%) and amoxicillin/clavulanate (0%). This result also supports the view that tazobactam inhibitory activity against ESBL is almost 10-fold greater than clavulanic acid[26]. This suggests piperacillin/ tazobactam is preferably active against *P. aeruginosa* among the other beta-lactamase inhibitors in this study. High resistance to penicillins was also observed in this study carbenicillin (53.7%), piperacillin (38.9%). Of a note is also a high resistance to Aztreonam as 44.4% of the isolates showed resistance to the drug. Earlier studies[14] has reported 36.1% resistance to Aztreonam by *P. aeruginosa* that were also reported to be ESBL producers. Aztreonam is an Oxyimino-monobactam recommended as anti-pseudomonal drug and hasn't been widely prescribed in Nigeria. These observations clearly indicate the prevalence of ESBL among the resistant strains in this study.

Susceptibility pattern among the aminoglycosides is as follows; amikacin (77.8%), gentamicin (59.3%), streptomycin (59.3%) and kanamycin (20.4%). Our result is contrary to studies by Okesola and Oni[9] who reported 66.7% susceptibility in gentamicin and 100% resistance in streptomycin, and also contradicts Iwalokun *et al* [27] who reported susceptibilities as follows; gentamicin (66.7%), streptomycin (53.3%) and Nwankwo and Shuaibu[13] who reported gentamicin (50%), streptomycin (43.7%). However, the susceptibility of *P. aeruginosa* to amikacin in this study is consistent with Ogbolu *et al*[20]. who reported 78.3% susceptibility to amikacin, Iwaloku *et al.*[27] reported (73.3%), Olayinka *et al*[15] (2009) reported (89.1%) and also correlated with studies from Aibinu *et al.*[14] who reported 78.4% susceptibility to amikacin indicating low resistance profile status for *P. aeruginosa* to amikacin in Nigeria. The MIC values for the selected amikacin and gentamicin resistant strains in this study were significantly high as 40% and 60% of the strains had MIC value (≥256µg/ml) for amikacin and gentamicin respectively. Our result corroborate previous reports where high MIC values were obtained among *P. aeruginosa* strains against aminoglycosides.[28, 29] From the result of this study it could be suggested that the first-line aminoglycoside such as gentamicin; streptomycin and kanamycin for which resistance has become persistent to be replaced or restricted. Common uses of these antibiotics have reduced their therapeutic relevancy, hence the use of amikacin should be considered more to prevent further resistance.

CONCLUSION

In conclusion, this study has shown multiple drug resistant *P. aeruginosa* at a high prevalence among the southwest hospitals investigated in Nigeria. High resistance of *P. aeruginosa* to multiple classes of antibiotics especially to recent generation of cephalosporin (cefepime) and oxyimino-monobactam (Aztreonam) is a disturbing clinical problem especially in our country where the cost of affordable antibiotics is a bit high. One of the major implications of persistent resistance of *P. aeruginosa* is the increase in the cost of its treatment due to high cost of purchasing anti-pseudomonal drugs such as imipenem, which appears to be very active in this study. However, the findings of this study underscore the need for the establishment of a functional antimicrobial surveillance system for *P.*

aeruginosa to oversee the trend of resistance to prescribed antibiotics in our environment. This will also guide clinicians in the appropriate therapy and management of *Pseudomonas* infections. We believe this move will also reduce the propensity of development of resistance in the course of treatment; and possibly curb the high rates of multiple drug resistance. The government may do well to enact laws to prohibit indiscriminate use and prescription of antibiotics and improve on public enlightenment programs to stop drug abuse.

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