Prevalence of multi-drug resistant *Pseudomonas aeruginosa* isolated from selected residential sewages in Dutsin-Ma, Katsina State, Nigeria

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Abstract

The global surge in Multidrug resistant (MDR) bacteria is an issue of great concern. *Pseudomonas aeruginosa* has been implicated in several nosocomial infections, where it has caused grave complications in immunocompromised patients. This is the first study to report the prevalence of MDR *P. aeruginosa* isolated from residential sewage in Dutsin-Ma, Katsina State, Nigeria. Pseudomonads count, isolation, biochemical characterization and antibiogram were carried out using standard microbiological procedures. This study examined sixty (60) samples from selected residential sewage in the study site collected at different intervals between July and September 2021. A total of 40 (66.7%) *P. aeruginosa* were isolated from the analyzed sewage samples. The highest ($2.84x10^4$) pseudomonad count was recorded from sewage samples collected from Kadangaru. *Pseudomonas aeruginosa* isolates from this sample site showed the highest (100%) resistance to cephalosporins (cefuroxime) and nitrofurantoin. Similarly, isolates from *Miami* area also demonstrated the highest (95%) resistance to a cephalosporin (ceftazidime). All (100%) isolates used in this study showed MDR resistance to tested antibiotics. The occurrence of MDR *P. aeruginosa* from a residential sewage site that may contaminate drinking water sources in the study area is of public health threat to the inhabitants. Surveillance and molecular epidemiology of antibiotics resistant bacteria are urgently needed in the study area.

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Introduction

Pseudomonas aeruginosa is a Gram-negative bacterium characterized by clear colonies on MacConkey agar and blue-green pigment colonies on cetrimide agar.[1] This opportunistic and ubiquitous environmental bacterium[2] has been recovered from animals, soil, plants, water,[3] and sewage.[4]

Pseudomonas aeruginosa is also a major human pathogen which causes severe complication in immunocompromised patients. These bacteria have been largely implicated in nosocomial infections.[5] Nosocomial infections associated with *P. aeruginosa* include but not limited to bacteremia, burn infection, cystic fibrosis, pneumonia and urinary tract infection.[6] Sadly, bacteremia caused by this bacterium is often associated with high mortality when compared with other Gram-negative bacteria.[7]

Large population of bacteria host antibiotic resistance genes that can be disseminated through horizontal gene transfer,[8] making the treatment of bacterial infections even more challenging. Over the years, anti-pseudomonal antimicrobials have been recommended for treatment of Multidrug Resistant (MDR) *P. aeruginosa*.[7] Despite its initial success, resistance to one or more of these anti-pseudomonal drugs has been reported in the literature.[9] Even more recently, researchers have reported a 43% prevalence of MDR *P. aeruginosa* from clinical and environmental samples in Ghana.[10]

Previous studies in Nigeria have been centered on analyzing the prevalence of multidrug-resistant *P. aeruginosa* from clinical environments and abattoir/industrial wastewater effluents.[9,11-15] However, to the best of our knowledge there is scanty information on prevalence of antibiotic resistant *P. aeruginosa* from residential sewage in Dutsin-Ma, Katsina State, Nigeria. Improper disposal of sewage has been an issue of great concern in developing countries including Nigeria, with Dustsin-Ma town (in Katsina state) as a focal point.[16] Sadly, several areas in this town had sewage flowing into open ground and drinking water sources. This portends devastating public health challenges, as the sewage run-offs may carry antibiotic resistant bacteria in them, as reported in previous litera-

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ture.[17] Therefore, this paper aimed at investigating the prevalence of multidrug-resistant *Pseudomonas aeruginosa* isolated from residential sewage in Dutsin-Ma, Katsina State, Nigeria.

Materials and Methods

Study area and sampling

This study was conducted in Dutsin-Ma, a Local Government Area (LGA) in Katsina State, Nigeria. It is located on latitude and longitude 12°27'18''N, 7°29'29''E respectively. The LGA has an area of 527 km^2 and population of 169,671 as of 2006 census, with Zobe Dam lying to the south of the town.[18] The inhabitants of this area are predominantly Hausa and Fulani by tribe. Their main occupation is farming and animal rearing.

A total of sixty (60) residential sewage samples were collected from Kadangaru and *Miami* area (30 from each location) in sterile universal bottles. For each study area, five (5) different sites were sampled weekly for six (6) weeks. Samples were transported on ice packs to microbiology laboratory, Federal University Dutsin-Ma for microbiological analysis.

Isolation of *Pseudomonas aeruginosa*

Samples were serially diluted, and 1ml each of 10^1, 10^3 and 10^5 dilution factors were transferred into separate sterile petri dishes. Sterile cetrimide agar was poured aseptically into the petri dishes, and the culture plates were incubated at 37˚C for 24-48 hours. To obtain distinct colonies, bacteria were further sub-cultured on cetrimide agar. Presumptively, blue-green pigment colonies were aseptically picked and stored on nutrient agar slants for further biochemical characterization.[11]

Biochemical characterization of *Pseudomonas aeruginosa*

Presumptive *P. aeruginosa* isolates were further subjected to biochemical tests such as Gram staining, catalase and oxidase tests, reactions on Triple Sugar Iron agar and motility test to further confirm the identity of the isolates.[19]

Antibiotics susceptibility testing

Isolates were screened for phenotypic resistance to ceftazidime (30µg), cefuroxime (30µg), gentamicin (10µg), ciprofloxacin (5µg), ofloxacin (5µg), augumentin (30µg), nitrofurantoin (300µg), and ampicillin (10µg) (Abtek Biologicals Ltd). The antibiotics susceptibility profiles were determined by agar diffusion technique on Mueller Hinton agar (MHA). An inoculum suspension was prepared and standardized to match the 0.5 McFarland's standard which corresponds to approximately 1.5×10^8 cfu/mL. Using sterile swab sticks, cells from the inoculum suspension were evenly spread on the MHA and allowed to dry. Plates were aseptically impregnated with antibiotic sensitivity discs and incubated at 37°C overnight. Zones of inhibition around the antibiotic discs were measured, and the lengths were categorized as resistant, intermediate and sensitive, following the Clinical Laboratory Standards Institute guidelines for each bacteria isolate.[20] Multi-drug resistant bacteria were selected based on their resistance to ≥3 classes of antibiotics.

Statistical analyses

The data obtained were analyzed using the Statistical Package for Social Science and EXCEL 2019. Analysis of Variance was used to determine significant differences between groups, where values of $P \leq 0.05$ were considered significantly different.

Results

Sixty (60) sewage samples, comprising thirty (30) each from Kadangaru and *Miami* areas, were collected for this study. Out of these samples, a total of 40 (66.7%) samples showed the presence of *P. aeruginosa*, while none was isolated from the remaining 20 (33.3%) samples. There was a significant difference ($P \leq 0.05$) between the occurrence of *P. aeruginosa* from Kadangaru and *Miami* area, where the analysis of data from both locations showed an actual P-value of 0.02 (Table 1).

Table 2 shows pseudomonad loads of samples recovered from this study. From Kadangaru area, the highest and lowest pseudomonad count were observed to be 2.84×10^4 and 4.6×10^3 cfu/ml, respectively. Conversely, the highest and lowest count for samples collected from *Miami* area were 3.14×10^3 and 3.40×10^2 cfu/ml, respectively.

Pseudomonas aeruginosa isolates from Kadangaru samples showed the highest (100%) resistance to each of cefuroxime and nitrofurantoin. This was followed by 90% resistance to each of amoxicillin/clavulanate, ampicillin and ceftazidime respectively. The isolates demonstrated the least (60%) resistance to gentamicin. Conversely, the *P. aeruginosa* isolates from *Miami* area demonstrated the highest (95%) resistance to ceftazidime. This was followed by 80% resistance to cefuroxime. Similarly, isolates from this area also showed the least (50%) resistance to gentamicin (Table 3).

All 40 (100%) *P. aeruginosa* isolated in this study exhibited MDR to tested antibiotics. A total of twenty-three (23) antibiotic resistance patterns were observed, with the most frequently observed antibiotic resistance pattern being CAZ, CRX, GEN, CPR, OFL, AUG, and NIT. This was observed from eight (8) isolates collected across both Kadangaru and *Miami* areas (Table 4).

Discussion

Pseudomonas aeruginosa is a non-fermentative Gram-negative bacterium widely distributed in various environmental habitats.[2] In this study, we reported a high (66.7%) occurrence of this

Table 1. Occurrence of *Pseudomonas aeruginosa* from sampled sewage sites in Dutsin-Ma.

Locations	Samples positive for *Pseudomonas aeruginosa* (%)	Samples negative for *Pseudomonas aeruginosa* (%)	Total (%)
Kadangaru	20 (66.7)	10 (33.3)	30 (100)
Miami	20 (66.7)	10 (33.3)	30 (100)
Total	40 (66.7)	20 (33.3)	60 (100)

(P=0.02).

pathogen in sewage sites (Table 1), comparably more elevated than that reported from environmental samples (18.3%) and sewage (25%) in studies conducted in Ghana[10] and Iran[17] respectively. More so, hospital environments are also heavily contaminated with *P. aeruginosa*. Occurrence rates as high as 86.4% and 33% have been reported from hospital environments in Nigeria[21] and Uganda[22] respectively. However, the variation in occurrence rates reported by these authors may be attributable to differences in sample size, study design, study locations, among other factors. Therefore, it is not surprising that *P. aeruginosa* has been previously reported as a common soil and water bacterium.[23] This bacterium has also been recovered from sinks and drains, among others.[4]

In the *Miami* area of Dutsin-Ma, Katsina State, Nigeria, a sewage system was constructed near the university clinic, presenting a possible source of nosocomial pathogens. Therefore, it is not surprising that our study recorded the highest (3.14x10^3 CFU/mL) pseudomonad count from this sample area. Cross-contamination from the hospital effluents is highly probable. More so, the high bacteria count from *Miami* could also result from a large amount of human and household wastes channeled into the sewage system, largely because the area is densely populated by students, farmers, and government workers. An even more worrying observation in this study area was sewage run-offs on the surface of the ground (personal observation). This portends severe economic and public health consequences.

The high resistance to cefuroxime (100%) and ceftazidime (95%) observed from Kadangaru and *Miami* respectively (Table 3), corroborates a 100% resistance to the same antibiotics reported in an earlier study carried out in Onitsha, Nigeria.[21] The resistance to cephalosporins observed in this study portends serious public health concerns, especially with the rising emergence of resistance to this group of antibiotics.[24] The unrestricted usage of these antibiotics in our study area is probable for the high resistance to cephalosporin we observed. Clinicians also routinely use cephalosporin antibiotics to treat common bacterial infections such as diarrhea, skin infection, sexually transmitted diseases, and others.[25] Therefore, it becomes pertinent to protect the aquatic ecosystem from contamination with sewage, as this may facilitate the dissemination of antimicrobial resistance via horizontal transfer of resistance plasmid.[26]

In general, our findings revealed that *P. aeruginosa* isolated from Kadangaru demonstrated higher resistance to tested antibiotics when compared to isolates from the *Miami* area (Table 3). This observation suggests a variation in attitudes towards antibiotic usage between the people in these two locations. It is important to note that Kadangaru is predominantly occupied by farmers and traders, who are largely uneducated, while students and civil workers dominate the *Miami* area. Therefore, this suggests that education and campaigns on antimicrobial resistance may help win this war against antimicrobial resistance.

Interviews with the locals in our study area revealed that ciprofloxacin was a commonly used antibiotic within that area (data not shown). Strikingly, we observed a 75% resistance to ciprofloxacin, which was comparably higher than the 22.9% and 45.5% reported by earlier studies in Pakistan[26] and South-Eastern Nigeria[21] respectively. Single therapy from health care practitioners, or selective pressure arising from frequent use, may be responsible for the emergence of resistance to this broad-spectrum antibiotic[27] Previously, we had reported a 50% resistance to nitrofurantoin by *Pseudomonas* spp., isolated from smoked fish in Dutsinma, Katsina State, Nigeria.[28] Therefore, observing *P. aeruginosa* isolates with a 100% resistance to nitrofurantoin in this present study is problematic and calls for immediate intervention.

Our findings reveal gentamicin to be the most effective antibiotic, with varying degrees of susceptibility to this antibiotic observed from *Miami* (50%) and Kadangaru (40%) isolates. This observation is in agreement with the high susceptibility (84% and 81.6%) to gentamicin reported by earlier studies conducted in Pakistan[26] and Sudan[5] respectively. This phenomenon may be predicated on the reduced administration of gentamicin in those regions.

The intrinsic and acquired resistance mechanism of *P. aerugi-*

Table 2. Pseudomonad load (CFU/ml) of sampled sewage sites in Dutsin-Ma.

Residential sewage samples	Pseudomonad load (cfu/ml)	
	Kadangaru	Miami
1	2.84x10^4	3.14x10^3
2	2.80x10^4	3.10x10^3
3	2.66x10^4	2.88x10^3
4	2.62x10^4	2.79x10^3
5	2.48x10^4	2.46x10^3
6	2.26x10^4	2.38x10^3
7	2.20x10^4	2.22x10^3
8	1.98x10^4	2.00x10^3
9	1.86x10^4	1.94x10^3
10	1.80x10^4	1.88x10^3
11	1.74x10^4	1.78x10^3
12	1.66x10^4	1.64x10^3
13	1.50x10^4	1.54x10^3
14	1.46x10^4	1.42x10^3
15	1.34x10^4	1.30x10^3
16	1.20x10^4	1.06x10^3
17	1.04x10^4	8.20x10^2
18	9.80x10^3	5.80x10^2
19	6.20x10^3	3.80x10^2
20	4.60x10^{3}10^3	3.40x10^2

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nosa limits choices for antimicrobial therapy. Previous literature has reported an increasing prevalence of infections caused by multidrug-resistant *P. aeruginosa*.[29] We report a remarkably high (100%) prevalence of multidrug-resistant *P. aeruginosa*, which corroborates the findings of other researchers in Nigeria.[21,30-32] This phenomenon may be primarily associated with the inherent antimicrobial resistance of *Pseudomonas* spp. to frequently administered antibiotics, as highlighted in previous literature.[33] However, in contrast to our findings, researchers from Egypt reported a relatively lower (30%) MDR prevalence.[34] Furthermore, it is noteworthy that while an earlier study on *P. aeruginosa* of clinical origin identified five (5) MDR patterns,[21] our study reports a total of twenty-three (23) different MDR patterns. This difference suggests that isolates from the environment may demonstrate more significant variations in drug resistance patterns, as they are constantly being exposed to conditions that may confer these abilities on them.

Conclusions

In conclusion, our findings revealed that multidrug-resistant *P. aeruginosa* can be isolated from sampled residential sewage that impacts the drinking water source in the study area. Gentamicin could be the potent drug of choice in treating MDR *P. aeruginosa* infection in the study area. Therefore, dilapidated drainages should be reconstructed for efficient removal of stagnant water and residential sewage flow to prevent negative environmental health

Table 3. Antibiotic resistance pattern of *Pseudomonas aeruginosa* isolates from sampled sewage sites in Dutsin-Ma.

Antibiotics	Kadangaru (n=20)		Miami (n=20)	
	Resistance No. (%)	Susceptible No. (%)	Resistance No. (%)	Susceptible No. (%)
Amoxicillin/Clavulanate	18 (90)	2 (10)	15 (75)	5 (25)
Ampicillin	18 (90)	2 (10)	12 (60)	8 (40)
Ceftazidime	18 (90)	2 (10)	19 (95)	1 (5)
Cefuroxime	20 (100)	0 (0)	16 (80)	4 (20)
Ciprofloxacin	17 (85)	3 (15)	15 (75)	5 (25)
Gentamicin	12 (60)	8 (40)	10 (50)	10 (50)
Nitrofurantoin	20 (100)	0 (0)	11 (55)	9 (45)
Ofloxacin	13 (65)	7 (35)	13 (65)	7 (35)

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Table 4. Multidrug resistance pattern among *Pseudomonas aeruginosa* isolates from sampled sewage sites in Dutsin-Ma.

S/No.	Location	No of antibiotics	Antibiotic resistance patterns	No of isolates
1	Kadangaru	7	CAZ, CRX, CPR, OFL, AUG, NIT, AMP	3
2		5	CRX, CPX, AUG, NIT, AMP	4
3		6	CRX, CPR, OFL, AUG, NIT, AMP	3
4		8	CAZ, CRX, GEN, CPR, OFL, AUG, NIT, AMP	4
5		7	CAZ, CRX, GEN, CPR, AUG, NIT, AMP	6
6	*Miami*	6	CAZ, CPR, OFL, AUG, NIT, AMP	2
7		8	CAZ, CRX, GEN, CPR, OFL, AUG, NIT, AMP	2
8		4	CAZ, CRX, AUG, AMP	1
9		4	CAZ, CRX, CPR, NIT	1
10		4	CRX, CPR, AUG, AMP	1
11		4	CAZ, GEN, CPR, AUG	1
12		5	CAZ, CRX, OFL, AUG, NIT	1
13		6	CAZ, CRX, CPF, OFL, AUG, NIT	1
14		7	CAZ, CRX, OFL, GEN, AUG, NIT, AMP	1
15		6	CAZ, CRX, GEN, OFL, NIT, AMP	1
16		5	CAZ, CRX, CPR, AUG, NIT	1
17		5	CAZ, CRX, GEN, OFL, AMP	1
18		5	CAZ, CRX, GEN, CPR, AMP	1
19		5	CAZ, CRX, CPR, OFL, AUG	1
20		6	CAZ, CRX, GEN, CPR, OFL, AUG	1
21		5	CAZ, CRX, GEN, CPR, AUG	1
22		6	CAZ, CPR, OFL, AUG, NIT, AMP	1
23		6	CAZ, CRX, GEN, CPR, OFL, AMP	1
Total				40

AMP, Ampicillin; AUG, Amoxycillin/Clavulanate; CAZ, Ceftazidime; CRX, Cefuroxime; CPR, Ciprofloxacin; GEN, Gentamicin; NIT, Nitrofurantoin; OFL, Ofloxacin.

impact in the study area. In addition, there is a pressing need to review antibiotic usage in the study area.

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