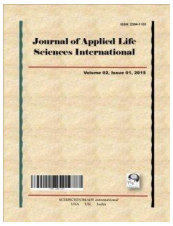

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Multi-drug Resistant *Pseudomonas* Species Isolated from the Wastewater of an Abattoir in Ibadan, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author OIF designed the study and the protocol. Authors OIF and AGR managed literature search, data acquisition and wrote the draft. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: The use of antibiotics for the promotion of animal growth and traditional therapy culminate the development of resistance in pathogenic microorganisms and their posterior transmission to humans through food. The presence of *Pseudomonas* species in aquatic environments facilitate their dissemination and further exposure to antimicrobial agents through wash down from abattoir may lead to multi-drug resistance, thereby causing serious public health problems.

Aim: This study was designed to determine the occurrence of antibiotic resistant *Pseudomonas* species in an abattoir wastewater in Ibadan, Nigeria.

Materials and Methods: Wastewater samples were collected from the slaughters slab and drainage for 6 weeks between May-June, 2015. *Pseudomonas* species were isolated using Pseudomonas Centrimide Agar. The isolates were identified using standard microbiological tests. The antibiotic susceptibility test against 10 antibiotics using disc diffusion technique was done. The antibiotics include: Ampicillin, Streptomycin, Chloramphenicol, Amoxicillin/Clavullanate, Ceftriazone, Cloxacillin, Ciprofloxacin, Ofloxacin, Tetracycline and Trimethoprim/sulfamethoxazole.

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Results: A total of 85 *Pseudomonas* species were isolated and were all (100%) resistant to ampicillin. Likewise, all the *P. fluorescens* and the other *Pseudomonas* spp. were resistant to ceftriaxone. Meanwhile, 71.8% (*P. aeruginosa),* 80.0% *(P. fluorescens)* and 78.9% (other *Pseudomonas* species) were resistant to Trimethoprim/ sulfamethoxazole.
Conclusion: The observation from this study is an indication that the wastewater of the studied abattoir could serve as important vehicle for sustenance of multi-drug resistant bacteria in aquatic ecosystem and transmission of multi-drug resistant disease-causing bacteria to humans. Hence, there is a need to ensure adequate treatment of abattoir wastewater to decimating bacteria population especially the potential pathogenic strains before they are eventually released into the environment.

Keywords: Pseudomonas; abattoir; antibiotics resistance; wastewater; environment.

1. INTRODUCTION

Pseudomonas species are Gram-negative aerobic bacilli widely distributed in the natural environment, including soil and water. These groups of bacteria are opportunistic and ubiquitous pathogens, probably due to their limited nutritional requirements and tolerance of adverse physical and chemical conditions including stream temperatures [1-3]. Some of the principal factors linked to the emergence of microbial resistance are the abusive, misuse and indiscriminate use of antimicrobial agents [4]. The emergence of strains of *Pseudomonas* species with variable and growing levels of antimicrobial resistance has generated considerable concern and various studies have sought to characterize this resistance and establish risk parameters. This phenomenon is complex and has multiple causes, some of which have already been determined whereas others still require further elucidation [5].

Antibiotics are traditionally used for animal growth promotion and therapeutic treatments [6]. One of the consequences of wide use of antimicrobial agents in animals is the development of resistance in pathogenic microorganisms and their posterior transmission to humans through food [7]. Antimicrobial agents have been easily and widely used to counter the infections caused by diverse microbial agents. However, microbial populations have developed various strategies to overcome these antimicrobial agents, a major contributing factor to the development of antimicrobial resistance world-wide [8]. Increase in the frequency of multi-drug resistant (MDR) strains of *P. aeruginosa* has severely limited availability of therapeutic options. Researches on antimicrobial resistance profiles of *P. aeruginosa* are essential to find out the susceptibilities of this pathogen against commonly prescribed antibiotics in any health care facility so as to optimize the current therapeutic treatment options [5].

The presence of *Pseudomonas* species in aquatic environments facilitates their dissemination with concurrent transmission of multi-resistance factors to humans, thus, eliciting serious public health concerns because of the implications on morbidity, mortality and healthcare costs both in hospitals and in the community [9]. The development of resistance to all available antibiotics may preclude the effectiveness of any antibiotic regimen [10,11]. Infections caused by *P. aeruginosa* are frequently life-threatening and difficult to treat as it exhibits intrinsically high resistance to many antimicrobials [12] and the development of increased, particularly multi-drug resistance in health care settings [12,13].

Pseudomonas species are intrinsically resistant to penicillin but some strains are sensitive to piperacillin, imipenem or ciprofloxacin. It has been reported that *P. aeruginosa* shows wide spectrum of resistance to different classes of antimicrobial agents, including third and fourth-generation cephalosporins (cefepime) and carbapenems (imipenem and meropenem) [2,3, 14]. Mechanisms that cause antimicrobial drug resistance and multi-drug resistance in *P. aeruginosa* are due to acquisition of resistance genes like those encoding beta-lactamase [15], aminoglycoside modifying enzymes [16] via horizontal gene transfer, biofilm formation in patients with cystic fibrosis [17].

Nonetheless, the low antibiotics susceptibility of *Pseudomonas* species-an emerging opportunistic pathogen of clinical relevance, is attributable to concerted actions of multidrug efflux pumps with chromosomally encoded antibiotic resistance genes (e.g., *mexAB-oprM*, *mexXY*) and the low permeability of the bacterial

cellular envelopes [18]. However, increasing human populations suggest corresponding increase in abattoir activities. Antimicrobial use in animals is suspected to select for resistant pathogens [19,20] with transmission possibilities to humans who come in contact with abattoir wastewater. Therefore, the discharge of untreated abattoir wastewater into water bodies comes with public health challenges. The untreated wastewater may contain multiple antibiotic resistant pathogens with human and cattle cross-infection potentials. Hence, the need to profile antibiotic susceptibility pattern of *Pseudomonas* species in water bodies especially wastewater so as to assess their potential risk within the community [21]. This study was designed to determine the antibiotics resistance profile of *Pseudomonas* species isolated from an abattoir in Ibadan, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Site

The study site was Akinyele abattoir in Akinyele Local Government Area of Oyo State, Nigeria. The abattoir was selected because it produces and releases huge amount of wastewater into the environment. The thriving cattle market within the abattoir perimeter also presupposes contact with the wastewater.

2.2 Sample Collection

Using standard method, wastewater samples were collected into sterile plastic bottles from the slaughter slab, and drainage which is about 15 metres away from the slaughter slab. The wastewater samples were analyzed within 2-3 hours of collection. The samples were collected twice weekly over a period of six weeks between May-June, 2015.

3. ISOLATION AND CHARACTERIZATION OF THE *Pseudomonas* SPECIES

Pseudomonas species were isolated using the method of Kathiravan et al. [22]. Duplicates of 1 ml aliquots of 10^0 (raw sample), 10^{-1} and 10^{-2} each of the two abattoir water samples were dispensed into labeled plates. 10 ml Pseudomonas Centrimide Agar cooled to cheek tolerant temperature was poured into the Petri dishes containing the aliquots of the samples, rocked, allowed to solidified and incubated at 35°C for 24-48 hours [22]. The isolates were characterized using standard biochemical and sugar fermentation tests [23].

4. ANTIBIOTIC SUSCEPTIBILITY TESTING

Agar diffusion technique recommended by the Clinical Laboratory Standards Institute (CLSI) [24] on Mueller-Hinton agar was used to determine the antibiotic susceptibility patterns of the isolates. Antibiotic impregnated discs that include Ampicillin (10 µg), Streptomycin (10 µg), Chloramphenicol (30 µg), Amoxicillin/clavullanate (30 µg), Ceftriazone (30 µg), Cloxacillin (5 µg), Ciprofloxacin (5 µg), Ofloxacin (5µg), Tetracycline (25 µg) and Trimethoprim/ sulfamethoxazole (25 µg) were used. The antibiotic discs were purchased from Oxoid, UK. Pure distinct colony of test organisms of about 18 to 24 hours old culture suspended in a tube containing normal saline (0.85% NaCl). The turbidity of the mixture was adjusted to 0.5McFarland standards; uniformly spread over the surface of already prepared Muller Hinton agar with a sterile swab stick. With the use of sterile forceps, the antibiotics were placed on the cultured plates. The inoculated Petri dishes were incubated in an inverted position at 37°C for 18 to 24 hours. The zones of inhibition were measured in millimeters (mm) and interpreted based on interpretive criteria of CLSI [24].

5. RESULTS

A total of 85 *Pseudomonas* spp. were isolated out of which 32 (37.6%) were *P. aeruginosa*, 15 (17.6%) were *P. fluorescens* while 38 (44.7%) represented other *Pseudomonas* spp. The occurrence percentage of *Pseudomonas* isolates from the drainage (55.3%) is higher than that of slaughter slab (44.7%). The least occurrence (7.1%) was *P. fluorescens* and was isolated from the drainage (Table 1). The result of the antibiotic susceptibility test of the isolates is as shown in Table 2. The result showed that all (100%) the isolates were resistant to ampicillin. In addition, all (100%) the *P. fluorescens* and other *Pseudomonas* spp. were also resistant to ceftriaxone while 96.9% of *P. aeruginosa* were resistant to the same antibiotic. Furthermore, the result of the study showed that 56.2% and 66.7% of *P. aeruginosa and P. fluorescens* were resistant to amoxicillin/clavullanate respectively while 90.6% of other *Pseudomonas* species were resistant to amoxicillin/clavullanate. Resistance of the isolates to trimethoprim/sulfamethoxazole was 71.8% (*P. aeruginosa)*, 80.0%

(*P. fluorescens)* and 78.9% (other *Pseudomonas* species).

The result of antibiotypes of *Pseudomonas* species is shown in Table 3. Thirty (30) different antibiotypes were obtained ranging from single antibiotic resistance (AMP) through to eight antibiotics combination resistance i.e. AMP-TET-CHL-AMC-CRO-CLX-STR-SXT and AMP-TET-CHL-CRO-OFX-CLX-STR-SXT. The result showed that 5.88%, 8.24%, 9.41% and 12.94% isolates resisted AMP-OB-SXT, AMP-C-AMC-CRO-SXT, AMC-TET-CHL-AMC-CRO-SXT and AMP-AMC-CRO-SXT respectively.

6. DISCUSSION

Isolation of *Pseudomonas* species from the wastewater samples of the present study uphold the fact that these strains of microorganisms are associated with abattoir wastewater as has been previously reported in Nigeria [25,26]. The observation from this present study that none of the *Pseudomonas* strains showed resistance to ciprofloxacin and 18.4% resistance to streptomycin is a bit lower but comparably similar to the reports of Oliveira et al. [5] from another study conducted in Brazil on samples collected from swine slaughterhouse where resistance of *Pseudomonas* species isolated was 8.8% (ciprofloxacin) and 32.4% (streptomycin). Also from a similar study conducted on abattoir wastewater in Enugu state, south eastern part of Nigeria, 28.6% of *P. aeruginosa* were reported to be resistant to ciprofloxacin [26] while it was discovered that 0% of the *P. aeruginosa* isolated from surface water from Dhaka, Bangladesh was resistant to ciprofloxacin [27]. However, a higher resistant (56%) was reported from the isolates obtained from clinical samples from Iranian Educational Hospital, Iran [28]. The disparities could be due to difference in the studied samples. Nevertheless, reports have shown increasing resistance to different anti-pseudomonas drugs; this portends a serious therapeutic challenge in the management of disease due to these organisms [8,29,30].

Strikingly, the total (100%) resistance of *P. fluorescens* and the other *Pseudomonas* species as well as 96.9% of *P. aeruginosa* to ceftriaxone in this study indicates sustained resistance to ceftriaxone. It had been previously reported that 69% of *P. aeruginosa* were resistant to the third generation cephalosporin drug-ceftriaxone [8]. This was in a study conducted on clinical isolates from hospitalized patients at a tertiary care hospital in Kathmandu, Nepal. Meanwhile, higher resistance (75%) [31], 86% [32] 90.4% [28] and 93.9% [33] to ceftriaxone had been previously reported in studies carried out in India, Bangladesh, Iran and Nepal respectively.

The result of the present study that revealed 40.6% resistance of the *P. aeruginosa* to chloramphenicol did not match the report of a study done in Kano, a city in the northern part of Nigeria with a higher resistance (97.7%) of *P. aeruginosa* isolates to chloramphenicol [25] in a study conducted on the isolates obtained from tertiary health institution. The reason for this disparity may be due to the difference in the studied samples. The isolates from the Kano study are presumably exposed to antibiotics which might be responsible for the discrepancy. In addition, the findings from this study that showed resistance of *P. aeruginosa* (40.6%) and *P. fluorescens* (40.0%) to chloramphenicol is lower compared to the report from a previous study in which a total (100%) resistance was reported for *P. aeruginosa* to chloramphenicol at the same concentration [34]. Samples sources might also be responsible for the disparity. Furthermore, the high (96.9%) resistance of *P. aeruginosa* to Ceftriaxone at the same concentration (30 µg) in this study is in agreement with the 90.4% and 85.7% reported from the study on both clinical and abattoir isolates respectively [26,28]. In addition, the same observation of total (100%) resistance of the isolates in this study to ampicillin has also been reported from previous studies on surface water samples and abattoir wastewater [26,27]. This may be a clear indication of the gross abuse and misuse of the antibiotic. However, the resistance (71.8%) of *P. aeruginosa* to Trimethoprim/sulfamethozaxole in this study though high, is lower compared to the total (100%) resistance to the drug as recently reported [26,28]. The reason for the disagreement may be as a result of the concentration (25 µg) of the drug used in this study as against the 5 µg used in the other studies.

Meanwhile, the low resistance of the *Pseudomonas* strains observed in this study to ciprofloxacin (0%), ofloxacin (9.4%) and streptomycin (18.4%), suggests the adequacy of

Table 1. Number and percentage occurrence of *Pseudomonas* from the abattoir wastewater sample in Akinyele, Ibadan

Isolates	Source		Total (%)
	Slaughter slab (%)	Drainage (%)	
Pseudomonas aeruginosa	17 (20)	15 (17.6)	32 (37.6)
Pseudomonas fluorescens	6 (7.1)	9 (10.6)	15 (17.6)
Pseudomonas spp.	15 (17.6)	23 (27.1)	38 (44.7)
Total	38 (44.7%)	47 (55.3%)	85 (100%)

Table 2. Antibiotic susceptibility pattern of *Pseudomonas* species isolated from the abattoir wastewater

	Number (%)									
Antibiotics	***P. a.***	***P. f.***	***P.* spp.**	***P. a.***	***P. f.***	***P.* spp.**	***P. a.***	***P. f.***	***P.* spp.**	**Total**
	n= 32	**n= 15**	**n= 38**							**N=85**
	Sensitive			**Intermediate**			**Resistance**			
Ampicillin (10 ug)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	32 (100)	15 (100)	38 (100)	
Amoxicillin/Clavullanate (30 ug)	14 (43.8)	5 (33.3)	9 (23.7)	0 (0)	0 (0)	0 (0)	18 (56.2)	10 (66.7)	29 (90.6)	
Ceftriaxone (30 ug)	1 (3.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	31 (96.9)	15 (100)	38 (100)	
Ciprofloxacin (5 ug)	32 (100)	15 (100)	38 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Ofloxacin (5 ug)	29 (90.6)	15 (100)	37 (97.4)	0 (0)	0 (0)	1 (2.7)	3 (9.4)	0 (0)	0 (0)	
Cloxacillin (5 ug)	24 (75)	8 (53.3)	32 (84.2)	0 (0)	0 (0)	0 (0)	8 (25)	7 (46.7)	6 (15.8)	
Chloramphenicol (30 ug)	11 (34.4)	9 (60)	20 (52.6)	8 (25)	0 (0)	8 (21.1)	13 (40.6)	6 (40)	10 (26.3)	
Tetracycline (30 ug)	12 (37.5)	6 (40)	23 (60.5)	1 (3.1)	1 (6.7)	7 (18.4)	19 (59.4)	8 (53.3)	8 (21.1)	
Trimethoprim/sulfamethozaxole (25 ug)	6 (18.8)	3 (20)	4 (10.5)	3 (9.4)	0 (0)	4 (10.5)	23 (71.8)	12 (80)	30 (78.9)	
Streptomycin (10 ug)	20 (62.5)	12 (80)	31 (81.6)	3 (9.4)	0 (0)	0 (0)	9 (28.1)	3 (20)	7 (18.4)	

P. a., Pseudomonas aeruginosa; P. f., P. fluorescens; P. spp., other Pseudomonas species

Table 3. Antibiotypes of all *Pseudomonas* species isolated from abattoir wastewater

Antibiotypes	***P. aeruginosa* n=32**		***P. fluorescens* n=15**		***P.* spp n=38**		Total
	A	**B**	**A**	**B**	**n=15**	**n=23**	**N=85**
	n=17	**n=15**	**n=6**	**n=9**	**A**	**B**	
AMP	0 (0)	1 (6.67)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.18)
AMP-OB	0 (0)	0 (0)	0 (0)	2 (22.22)	1 (6.7)	1 (4.35)	4 (4.71)
AMP-TET-CRO	2 (11.76)	1 (6.67)	0 (0)	0 (0)	0 (0)	0 (0)	3 (3.53)

Antibiotypes	*P. aeruginosa* n=32		*P. fluorescens* n=15		*P.* spp n=38		Total
	A	B	A	B	n=15	n=23	N=85
	n=17	n=15	n=6	n=9	A	B	
AMP-CRO-SXT	1 (5.88)	0 (0)	0 (0)	0 (0)	1 (6.7)	3 (13.04)	5 (5.88)
AMP-AMC-CRO	0 (0)	1 (6.67)	0 (0)	0 (0)	2 (13.33)	1 (4.35)	4 (4.71)
AMP-TET-AMC-CRO	0 (0)	0 (0)	0 (0)	0 (0)	1 (6.7)	0 (0)	1 (1.18)
AMP-TET-CRO-CLX	0 (0)	1 (6.67)	1 (16.67)	0 (0)	0 (0)	0 (0)	2 (2.35)
AMP-AMC-CRO-CLX	0 (0)	0 (0)	0 (0)	1 (11.11)	1 (6.7)	0 (0)	2 (2.35)
AMP-AMC-CRO-SXT	2 (11.76)	1 (6.67)	0 (0)	0 (0)	2 (13.33)	6 (26.10)	11 (12.94)
AMP-CHL-CRO-SXT	0 (0)	1 (6.67)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.18)
AMP-TET-CRO-SXT	0 (0)	1 (6.67)	0 (0)	1 (11.11)	0 (0)	0 (0)	2 (2.35)
AMP-CRO-STR-SXT	0 (0)	1 (6.67)	0 (0)	0 (0)	0 (0)	1 (4.35)	2 (2.35)
AMP-CHL-CRO-STR-SXT	0 (0)	0 (0)	0 (0)	0 (0)	1 (6.7)	0 (0)	1 (1.18)
AMP-AMC-CRO-CLX-SXT	0 (0)	0 (0)	0 (0)	0 (0)	3 (20.0)	0 (0)	3 (3.53)
AMP-TET-CHL-CRO-CLX	0 (0)	1 (6.67)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.18)
AMP-TET-CRO-STR-SXT	0 (0)	0 (0)	0 (0)	0 (0)	1 (6.7)	0 (0)	1 (1.18)
AMP-CHL-AMC-CRO-SXT	1 (5.88)	1 (6.67)	2 (33.33)	1 (11.11)	1 (6.7)	1 (4.35)	7 (8.24)
AMP-TET-AMC-CRO-SXT	1 (5.88)	2 (2.33)	0 (0)	0 (0)	0 (0)	0 (0)	3 (3.53)
AMP-TET-CHL-CRO-STR-SXT	1 (5.88)	0 (0)	0 (0)	0 (0)	1 (6.7)	1 (4.35)	3 (3.53)
AMP-TET-CHL-AMC-CRO-CLX	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.35)	1 (1.18)
AMP-TET-CHL-AMC-CRO-SXT	1 (5.88)	2 (2.33)	0 (0)	2 (22.22)	0 (0)	3 (13.04)	8 (9.41)
AMP-TET-AMC-CRO-CLX-SXT	0 (0)	0 (0)	1 (16.67)	1 (11.11)	0 (0)	0 (0)	2 (2.35)
AMP-CHL-AMC-CRO-CLX-SXT	2 (11.76)	0 (0)	0 (0)	0 (0)	0 (0)	2 (8.70)	4 (4.71)
AMP-TET-CHL-CRO-OFX-STR-SXT	1 (5.88)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.18)
AMP-TET-CHL-AMC-CRO-STR-SXT	2 (11.76)	0 (0)	0 (0)	0 (0)	0 (0)	2 (8.70)	4 (4.71)
AMP-TET-CHL-CRO-CLX-STR-SXT	0 (0)	0 (0)	0 (0)	1 (11.11)	0 (0)	0 (0)	1 (1.18)
AMP-TET-CHL-AMC-CRO-CLX-STR	0 (0)	1 (6.67)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.18)
AMP-CHL-AMC-CRO-CLX-STR-SXT	1 (5.88)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.35)	1 (1.18)
AMP-TET-CHL-AMC-CRO-CLX-STR-SXT	1 (5.88)	0 (0)	2 (33.33)	0 (0)	0 (0)	0 (0)	4 (4.71)
AMP-TET-CHL-CRO-OFX-CLX-STR-SXT	1 (5.88)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.18)

CIP: Ciprofloxacin; AMP: Ampicillin; TET: Tetracycline; OFX: Ofloxacin; CLX: Cloxacillin; CRO: Ceftriaxone;
CHL: Chloramphenicol; STR: Streptomycin; SXT: Trimethoprim/sulfamethoxazole; AMC: Amoxicillin/clavullanate; A: Slaughter slab; B: drainage

fluoroquinolones as drug of choice for the treatment of infections caused by *P. aeruginosa* [5]. A similar but slightly higher resistance rate of *Pseudomonas* strains to ciprofloxacin (8.8%) and streptomycin (32.4%) had been reported in another study conducted on wastewater samples collected from swine slaughterhouse in Dourados, Mato Grosso do Sul State, Brazil [5]. However, in a 10-year study at the Helios Clinic, Witten/Herdecke University, Wuppertal, Germany, a higher resistance (30.1%) of *P. aeruginosa* to ciprofloxacin was reported [35]. Nonetheless, the increasing resistance of bacterial isolates of abattoir origin to several antibiotics has been reported [36]. The result of this study showed that the isolates that were multidrug resistant were high with 80(94.1%) of the isolates that showed resistant to three or more classes of antibiotics.

7. CONCLUSION

In conclusion, the detection of multiple antibiotic resistant *Pseudomonas* strains from the studied abattoir poses potential challenges to public health particularly the inhabitants of the community where the abattoir is located. Regular monitoring of abattoir operations by saddled state establishments is recommended in order to ensure compliance with hygienic practices and hence forestall outbreak of diseases at the instance of multiple antibiotic resistant strains. Furthermore, adequate measure should be put in place for the treatment of the abattoir wastewater before being discharged into the environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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