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Isolation, identification and antibiotic resistance pattern of *Pseudomonas* species isolated from poultry feaces in owo metropolis

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Abstract
Pseudomonas species are most available pathogenic Gram negative bacteria in environmental samples. They are resistant to several drugs, opportunistic pathogen and their presence in the environment poses threat to the public health. The ability of microorganisms to survive in the presence of antimicrobial agents at a situation that can kill or enhance their growth is known as antimicrobial resistance. This has become a serious threat globally, which makes the treatment of infections in humans and animals expensive and difficult. This study aims at isolating, identifying and investigating the antibiotic resistance pattern of *Pseudomonas species* isolated from poultry feaces in Owo metropolis. Fresh feaces of 6 healthy boilers were collected separately into properly labeled and sterile capped bottles from two different privately owned poultry farms in Owo, Ondo state Nigeria. *Pseudomonas species* was isolated using Centrimide Agar, therefore, their morphological and cultural characteristics on MacConkey Agar, Nutrient Agar, and Eosin Methylene Blue Agar were equally observed, and the isolates were therefore characterized. Kirby Bauer disc (KBD) diffusion method was used for antibiogram of the isolates. The isolates presented different morphological and cultural characteristics on the saveral types of media used. At poultry farm A (43.3%) the total prevalence of the *Pseudomonas species* in the samples was low compared with (56.7%) at poultry farm B. The isolates indicated different resistant level to the antibiotics tested with resistance (3.3%) to the carbapenem (imipenem) and one of the tested cephem (cefotaxime) being the lowest and resistance (70.0%) to the nitrofuran (nitrofurantoin) being the highest; while 66.7% were multidrug resistant. This necessitates the implementation of mitigating strategies to limit the transfer of antibiotic-resistant *Pseudomonas species* from animals to humans.

Keywords: *Pseudomonas* species, pathogens microorganism, isolation, identification, morphology

Introduction

Pseudomonas species are most available pathogenic Gram negative bacteria in environmental samples. They are resistant to several drugs, opportunistic pathogen and their presence in the environment pose threat to the public health (Hesse *et al*., 2018) [1]. *Pseudomonas* species, genus *Pseudomonas* is made up of several species of Gram-negative, aerobic, rod-shaped, nonspore forming and motile organisms. Genus *Pseudomonas* are versatile metabolically with specifically ambiguous sizes of genome that ranges from 3 to 7 Mbp (Hesse *et al*., 2018) [1], which has several genetic mobile elements and mega plasmids (Cazares *et al*., 2020) [2] as well as internally and externally obtained resistance mechanisms (Lister *et al*., 2009). These properties enable them to survive in a various environments, including reservoirs such as soil and rhizosphere, pools, and others (Nadimpalli *et al*., 2020) [3].

Pseudomonas aeruginosa is the causative agent of thousands of diseases which varies from external otitis to serious pneumonia in hosts such as dogs, birds, rabbits and humans. It is a rare member of usual microbial flora in animals (Lister *et al*., 2009) [3]. Therefore *Pseudomonas aeruginosa* infections normally affect immune deficient patients. In time past, several researches shows the natural prevalence of bacteria common with birds found microorganisms that poses a threat to either human or domesticated animal (Nathan *et al*., 2011) [36]. Many of these researches gave some information on the nonepizootic prevalence of *Pseudomonas species*. Different types of antibiotics are used by veterinary experts to develop poultry products globally (Landoni and Albarellos, 2015) [5]. The abuse of such antimicrobials led to the said antibiotic resistance (Mehdi *et al*., 2018) [6], which is critical and of highly important to human health (Gonzalez Ronquillo and Angeles Hernandez, 2017) [7].

Antimicrobial resistance has become an epidemic because of their direct impact on humans health globally. Adoption of antimicrobial agents in birds is associated to the spread of the resistant bacteria (Agyare *et al*., 2018) [8]. Antimicrobial resistant pathogens in birds may result in failed treatment, causing shortage, but also can be a source of resistant bacteria/genes that poses a major risk to humans (Nhung *et al*., 2017) [9].

The resistance mechanisms
used by *Pseudomonas species* are diverse and caused by their genomic plasticity which includes multidrug efflux systems, protein loss, specific mutations, and production of enzyme (Cabassi *et al*., 2017) [10]. Currently in Owo environs, there is little information on the isolation, identification and antibiotic resistance pattern of *Pseudomonas species* from poultry feacal samples and its possible communication to humans.

Isolation, identification and antibiotic resistance pattern of *Pseudomonas species* from poultry feaces are of concern in this investigation because they are opportunistic pathogens which are a threat to both birds and humans.

The aim of this study is to isolate, identify and determine the antibiotic resistance pattern of *Pseudomonas species* isolated from poultry feaces in Owo metropolis.

Materials and methods

Description of the Sampling locations.

Site code	Description
A	Tayo farm is a private farm located in Iselu, Owo. A total of 100 birds, broilers reared in deep liter system were kept in the poultry, Antibiotics used were Augmentin, Ciprofloxacin and Amoxicillin.
B	Eric farm is a private owned poultry farm located at Opomulero, Owo. A total of 150 birds, broilers reared in folds, were kept in the poultry, Augmentin, Ciprofloxacin and Gentamicin were the antibiotics used.

Sample Collection

Between October and November 2022, Fresh feaces of 6 healthy boilers were collected separately into properlly labeled and sterile capped bottles from two different privately owned poultry farms in Owo, Ondo state Nigeria. The samples were preserved in ice packs and transported to Microbiology unit laboratory, Department of Science Laboratory Technology, RUGIPO for immediate bacteriological analyses.

Isolation of *Pseudomonas* species

1g of the poultry feacal samples was weighed into 10ml of de-ionized water to make a stock solution. Serial dilution was carried out from the stock tenfold. 1ml each of the serial diluents was poured into properlly labeled and sterile Petri dishes. Aseptically, Nutrient Agar, MacConkey Agar, Eosin Methylene Blue Agar and Centrimide Agar were respectively cooled to about 50^0C and dispensed separately into the each of samples in the three petri dishes and shacked carefully, allowed to become solid and incubated at the temperature of 37^0C for 24 hours (Egea *et al*., 2012) [11]. The generation of yellowish-green pigment on centrimide Agar is typical of Pseudomonads (Lamonth and Martins, 2003) [12]. Different colonies were sub-cultured on freshly prepared Centrimide Agar plates; repeated streaking was done to obtain pure culture of *Pseudomonas* species prior to biochemical tests. All the suspected *Pseudomonas species* isolates were identified using standard techniques (Cheesbrough, 2010) [13].

Morphological Characterization of Isolates

A 24-hour old pure culture of the isolates was characterized and the different morphologies were recorded.

Gram Staining

this was carried out according to the method described by (Becerra *et al*, 2016) [14].

Biochemical Characterization of the Isolates

The isolates were further identified through a panel of biochemical tests which were carried out following standard procedure. The tests carried out include motility, catalase, citrate, indole, Methyl Red, Voges-Proskauer and TSI (Triple Sugar Iron) test.

Antimicrobial susceptibility test of the Pseudomonas species

This was done using the standard Kirby-Bauer disk diffusion described by (Jayabarath, 2015) [15] and (Munoz-Price Silvia, 2016) [16].

Results and discussion

Results

Table 1: Morphological, Cultural and Staining Characteristics of the Isolates from the Poultry Feaces

S/N	Media Used	Colony Characteristics	Morphology (Staining Characters)
1	Centrimide Agar	Circular, raised, mucoid, smooth yellowish-green, opaque	Gram-negative, pink colour, small rod shaped appearance, arranged in single or paired short
3	Eosin Methylene Blue Agar	Circular, raised, mucoid, milky, smooth, translucent	
4	MacConkey Agar	Circular, raised, mucoid, smooth, colourless, transparent	
5	Nutrient Agar	Circular, raised, mucoid, smooth yellowish-green, translucent-opaque	

KEY: S/N = Serial number

Table 2: Biochemical Characteristics of the Isolates from the Poultry Feaces

S/N	Isolates	MOT	CAT	CIT	IND	MR	VP	TSI			Probable Organism
								Slant/Butt	Gas	H_2S	
1-30	All	+	+	+	-	-	-	P/P	-	-	*Pseudomonas* species

KEY: S/N = Serial number, + = Positive, - = Negative, MOT = Motility, CAT = Catalase, CIT = Citrate, IND = Indole, MR = Methyl red, VP = Voges-proskauer, P/P = Pink/Alkaline

Table 3: Prevalence of the *Pseudomonas* species in the Poultry Feaces

Sampling site	No. of samples collected	No. of *Pseudomonas* species isolated	Prevalence of the Isolated *Pseudomonas* species (%)
A	3	13	43.3
B	3	17	56.7
Total	6	30	100.0

Table 4: Antibiotic Susceptibility Patterns of all the *Pseudomonas* species (n=30) Isolated from Poultry Feaces

Class of antibiotics tested	Name of antibiotics tested	Antibiotics disc code	Antibiotics disc concentrations (µg)	Zone diameter breakpoint (mm)		Reaction Pattern	
				S No. (%)	R No. (%)	S No. (%)	R No. (%)
Beta-lactam combination	Augmentin	AUG	30	≥ 18	≤ 13	28 (93.3)	2 (6.7)
Cephem	Cefotaxime	CTX	30	≥ 21	≤ 14	29 (96.7)	1 (3.3)
	Ceftazidime	CAZ	30	≥ 18	≤ 14	28 (93.3)	2 (6.7)

	Cefuroxime	CRX	5	≥ 16	≤ 20	13 (43.3)	17 (56.7)
Carbapenem	Imipenem	IMP	10	≥ 19	≤ 15	29 (96.7)	1 (3.3)
Aminoglycosides	Gentamicin	GEN	5	≥ 15	≤ 12	27 (90.0)	3 (10.0)
Fluoroquinolones	Ciprofloxacin	CIP	5	≥ 21	≤ 15	21 (70.0)	9 (30.0)
	Ofloxacin	OFL	5	≥ 16	≤ 12	28 (93.3)	2 (6.7)
Monobactam	Aztreonam	AZT	30	≥ 22	≤ 15	12 (40.0)	18 (60.0)
Nitrofuran	Nitrofurantoin	NIT	300	≥ 17	≤ 14	9 (30.0)	21 (70.0)

KEY: No. = Number, % = percentage, S = Susceptible, R = Resistant

Table 5: Antibiotype of *Pseudomonas* species Isolated from Poultry Feaces

Classes of Antibiotics	Antibiotype	No. of *Pseudomonas* species isolates (%)
6	NIT-CIP-IMP-CAZ-GEN-AUG	1 (3.3)
6	AZT-CIP-IMP-GEN-CRX-AUG	1 (3.3)
6	AZT-CIP-CAZ-GEN-OFL-AUG	1 (3.3)
5	NIT-CIP-CTX-GEN-AUG	2 (6.7)
5	CIP-CRX-GEN-OFL-AUG	2 (6.7)
5	AZT-IMP-CTX-GEN-OFL	2 (6.7)
4	NIT-CRX-IMP-AUG	3 (10.0)
4	CIP-CAZ-GEN-AUG	2 (6.7)
4	CIP-CTX-OFX-AUG	3 (10.0)
4	CIP-CAZ-GEN-AUG	0 (10.0)
3	CAZ-OFX-AUG	0 (0.0)
3	CTX-GEN-AUG	2 (6.7)
2	CAZ-AUG	0 (0.0)
2	CIP-AUG	1 (6.7)
1	CIP	9 (30.3)
1	IMP	1 (3.3)
TOTAL		**30**

KEY: AUG: Augmentin; CTX: Cefotaxime; CAZ: Ceftazidime; CRX: Cefuroxime; IMP: Imipenem; GEN: Gentamicin; CIP: Ciprofloxacin; OFL: Ofloxacin; AZT: Aztreonam; NIT: Nitrofurantoin

Discussion

The colony morphology of the isolated *Pseudomonas* species in this investigation showed circular, raised, mucoid, smooth, yellow-green, opaque on centrimide agar; circular, raised, mucoid, milky, smooth, translucent on Eosin Methylene Blue agar; circular, raised, mucoid, smooth, colourless, transparent on MacConkey agar and circular, raised, mucoid, smooth, yellowish-green, translucent-opaque on Nutrient agar. These results are confirmed with the characteristics of *Pseudomonas species* as previously suggested by (Ogbukagu *et al*., 2021) [17].

All the isolates were positive to motility, catalase and citrate but negative to indole methyl red; voges Prauskaur test and gas (CO_2 and H_2S) production hence identified as *Pseudomonas species*. These findings correspond with the specific biochemical characters of *Pseudomonas species* as described by (Abedin *et al*., 2020) [18].

In this study, the prevalence of *Pseudomonas species* obtained from feacal samples of boiler collected from poultry farm B (56.7%) is higher than that obtained at poultry farm A (43.3%). This finding is in agreement with the report of previous research by (Rodrigues *et al.*, 2021) [19] that chose wild bird feaces as samples in different location. However, the prevalence observed at the two sites in this study is far higher than that of same isolates described in a research by (Vidal *et al*., 2017) [20]. According to WHO (2022) [21], poor water sources, poor hygene, and improper control of infection and control exacerbate microbial spread, which may resist antimicrobial treatments.

The spread of antimicrobial resistance among the genus *Pseudomonas* was also investigated in this study. recently, antimicrobial resistance is one of the common public health problems, as multidrug resistant bacteria associated to animals could be virulent and transmitted to humans through food and dispersed through animals (Manyi-Loh *et al*., 2018) [22]

Different level of antibiotic resistance was considered in this study. These observations are thesame with the findings of (Nhung 2017) [9] in a review where the antibiotic resistance of *E. coli* and *Salmonella* spp., etc, were seen to vary with the classes and types of antibiotic drugs used. There is an argument that the pattern of Pseudomonas spp. resistance to antibiotics may change if other regular animal drugs are examined. This is because high resistance to antibiotics such as tetracycline, cloxacillin, erythromycin, ampicillin and amoxycillin (89.4%, 100.0%, 100.0%, 91.7%, and (88.4%) respectively has been reported in poultry (Ajayi and Omoya, 2017) [25], (Kakooza *et al*., 2021) [24] and (Bushen *et al*., 2021) [23].

Based on the results of this study, the majority of *Pseudomonas* isolates had very high degrees of susceptibility to one of the cephems ceftazidime, fluoroquinolones ciprofloxacin, ofloxacin, aminoglycoside gentamycin, the beta-lactam combination augmentine (93.3%, 70.0%, 93.3%, 90.0% and 93.3%) were similar report of (Elbehiry *et al.* (2022) [26]. Nevertheless, values of susceptibility of the isolates of this study to ofloxacin, gentamicin, and augmentin are different from the report of (Bamidele *et al.* (2022) [27] who reported absence of resistance of the isolated Pseudomonads to the antibiotics in Nigeria. Also, the value of susceptibility of the isolates observed in this investigation to augmentin is more than the 50.0% reported by (Ebbo and Junaidu (2021) [28]. Also, the level of susceptibility of the isolates in this study to gentamicin is lower than the 100.0% sensitivity reported by Ebbo and Junaidu (2021) [28]. More so 70.0% susceptibility of the isolates was noticed against ciprofloxacin as reported

by (Ebbo and Junaidu (2021) [28]. This disparity may be due to different unit at which the farm workers abuse the antibiotics on the birds.

It was observed in this research that the majority of the *Pseudomonas* isolates were very sensitive (96.7%) to the carbapenem and one of the cephems (cefotaxime) respectively used. Parallel results were achieved in previous studies reported by Shenoy *et al*. (2002) [34] and by Mwinyikombo (2018) [35], In this investigation, high level of resistance was observed against different antibiotics, which includes nitrofuran [nitrofurantoin (70.0%)], monobactam [aztreonam (60.0%)] and one of the second-generation cephalosporins also known as cephems used in this work. In same vain, results regarding resistance to nitrofurantoin were gotten by (Agyare *et al*. (2018) [8].

The results from this investigation which showed 20 (66.7%) of the isolated *Pseudomonas* species exhibiting multidrug resistance to a combination of drugs of is similar to the report of (Adesoji *et* al. (2016) [32] on occurrence of antibiotic resistant bacteria in faeces from abattoir waste, processing water and products from Nigeria.

The outbreak of these MDR bacteria could be risky to consumers of these products according to (Piddock (1996) [29]. There are many mechanisms through which pseudomonads gain multidrug resistance, which includes reduction of outer membrane permeability (Lavilla Lerma *et al*., 2014) [33] and the production of beta-lactamase, and multiple drug efflux pumps with a wide spectrum (Henwood *et al*. 2001) [31]. It has been discussed that consumption of antimicrobials that can enhance transfer of genes by supporting the SOS system and equally the presence of pathogens as potential reservoirs of resistance factors, may cause increase in antibiotic resistance of pseudomonas (Lima *et al*. 2020) [30].

Conclusion

This study indicates that poultry feaces is a carrier of *Pseudomonas* species which could transmit antibiotic resistance genes to human. The use of the poultry feaces for manure is another call for concern due to the associated hazard to the general populace.

This research calls for thorough supervision of antibiotic resistance in our environs and controlled use of antibiotics in poultries.

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