# Isolation, identification and antimicrobial susceptibility profile of *Pseudomonas* species isolated from pig (*Sus scrofa)* feces in owo metropolis

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**Abstract**

*Pseudomonas* species are common pathogenic Gram negative bacteria frequently found in environmental samples. *Pseudomonas* species are responsible for different healthcare-associated infections and are inherently resistant to many commonly used antibiotics. This study was aimed at isolating; identifying and determining the antimicrobial susceptibility pattern of *Pseudomonas* species isolated from pig feaces in Owo metropolis. Freshly passed feacal samples were aseptically collected from apparently healthy pigs into appropriately labelled sterile capped universal bottles with sterile spatula from a private owned pig farms in Owo. Isolation of *Pseudomonas* species was done using Centrimide Agar, however their morphological and cultural characteristics on Nutrient Agar, MacConkey Agar, and Eosin Methylene Blue Agar were also observed; and the isolates were subsequently conventionally characterized. Antimicrobial susceptibility of the isolates was done using Kirby Bauer disc diffusion method. The isolates showed different morphological and cultural characteristics on the different types of media used in their study. The isolates showed varying level of resistance to the antibiotics tested with the lowest resistance (16.7%) to the carbapenem (imipenem) and the highest resistance (83.3%) to both the beta-lactam combination (augmentin) and one of the cephem (cefuroxime) respectively This necessitates the implementation of mitigating strategies to limit the transfer of antibiotic-resistant *Pseudomonas* species from animals to humans.

**Keywords:** pseudomonas species, pig feaces, owo, resistance, organisms

## Introduction

Pig (*Sus scrofa)* is one of the most important farm animals both in numbers and biomass (Magnusson *et al*., 2019) [19], even though there are some religious restrictions in some cultures. It has been predicted by the UN's Food and Agriculture Organization that pig farming will be one of the animal industries that will grow faster and stronger, and the expected increase rate is likely to be about 8.6% and 12.7% by 2030 and 2050 respectively. (Magnusson *et al*.,2019) [19]. The swine industry plays a key role in the food supply chain and has a high economic impact. In the whole worldwide, Magnusson *et al*., 2019) [19] also reported that the production value of pork was around $94 billion USD in the 2018.

It has been pinpointed that *Pseudomonas* spp. Is predominantly psychrotrophic bacteria, and an important bacteria when it comes to food spoilage. (Marchand *et al*.,2009) [20]. The *Pseudomod* spp., genus *Pseudomonas*, is very much available in our surroundings for example in soil, water,and sediment (Devarajan *et al*., 2016) [7]. Members of this genus inhabit in a wide variety of environments, due to their metabolic capacity and their ability to adapt to numerous conditions (Moradali *et al*., 2017) [23]. They are Gram-negative bacteria that are always present and have a wide metabolic skillfulness and this enable them to adjust to different habitats with temperature around $42^0$C (Quigley *et al*., 2013) [32]. *Pseudomonas* tent to reproduce at low temperatures and are usually responsible for more than half of bacterias in milk (Munsch- Alatossava and Alatossava, 2006) [24]. They are not identified as one of the usual flora of humans and are often implicated in opportunistic infections (Wisplinghoff, 2017) [43]. *Pseudomonas* spp. has a wide variety of species, including the opportunistic pathogen *P. aeruginosa* which is of increasing medical and veterinary importance, causing infections usually in patients that has compromised immune systems, or in people with cystic fibrosis (Moradali *et al*., 2017) [23]. *Basically Pseudomonas* are large genome sizes from 3 to 7 Mbp (Hesse *et al*., 2018) [12], and it contain numerous genetic active elements such as megaplasmids as well as the whole acquired resistance mechanisms (Lister *et al*., 2009). These properties enable the survival of *Pseudomonas* spp. in saveral environments, e.g community reservoirs such as soil and water, and rhizosphere in a large environment. (Nadimpalli *et al*.,2020) [26].

Lipases that spoil raw milk, heat-stable extracellular peptidases and some other strains are been generated by *Pseudomonas* strains and are opportunistic pathogens that existed, such as *Pseudomonas* aeruginosa (Jeukens *et al*., 2017) [14]. *Pseudomonas* spp. Can also protect other organisms by covering them from conditions that are not unfavorable within biofilm formations (Puga *et al*., 2018) [31], same in the adoption of mechanisms such as quorum sensing (Venturi *et al*., 2010) [42]. Usually, livestock rneeds serious management of infection

and it includes the use of antibiotic therapy (Timothy *et al*., 2012) [39]. Antibiotics in livestock is a therapeutic agents, prophylactic agents and at the same time act as growth promoters in feed (McEwen and Fedorka-Cray, 2009) [22]. There is a scientific evidence that pointed to the fact that the use of antibiotics in animal feed can lead to the building up of resistance to pathogenic bacteria which migrates to human body through food chain (Van Looveren *et al*., 2010) [40].

A great interest has been developed in *Pseudomonas* because of its activities in both plant and human diseases, and also by their potential in biotechnological applications (Silby *et al*., 2011) [37].

A large quantity is also used as feed additives for the purpose of enhancing animal growth (Graham *et al*., 2007; Chattopadhyay, 2014) [11] and prophylaxis. Bacteria also pass resistance genes back and forth, creating another mechanism by which antibiotic resistance could be transferred to human pathogens (Rushton *et al*., 2014) [33]. The veterinary use of antimicrobial agents in edible animals to promote growth and prevent diseases is pointed out as one of the major risk factors that cause antibiotic resistant-bacteria in animals (Scott *et al*., 2018) [34]. As a result of this efficient availability of antibiotics reduces and also the tendency of transmission of AMR pathogen is increased. Additionally, the existence of multi-drug resistant (MDR) bacteria poses an increasing challenge for veterinarians to administer good treatment to sick farm animals (Sharma *et al*., 2018) [35].

The aim of this study is to isolate, identify and determine the antimicrobial susceptibility profile of *Pseudomonas* species isolated from pig feaces in Owo metropolis. Isolation, identication and determination of the antimicrobial susceptibility profile of *Pseudomonas species* from pig feaces are of paramount concern in this study as they are opportunistic pathogens which pose a threat not only to pig health but to humans who consume them.

## Materials and methods

### Sample collection

Between October and November 2022, freshly passed pig feacal samples were aseptically collected at privately owned pig farm at Owo in Ondo State from 6 apparently healthy pigs into appropriately labelled sterile capped universal bottles with sterile spatula, preserved in ice packs and transported to Microbiology unit of the Department of Science Laboratory Technology, Rufus Giwa Polytechnic Owo (RUGIPO) for immediate analyses.

### Ethical approval and informed consents

No ethical approval was required; however, during the collection of samples; verbal permission was taken from the farm owners and farm workers.

### Isolation of *Pseudomonas* species

1g of the pig feacal samples was weighed into 10ml of de-ionized water to make a stock solution. From the stock tenfold serial dilution was carried out. 1ml each of the serial diluents ($10^{-2}$, $10^{-4}$ and $10^{-6}$) was dispensed into appropriately labeled sterile Petri dishes. Aseptically, Nutrient Agar, MacConkey Agar, Eosin Methylene Blue (EMB) Agar and Centrimide Agar respectively cooled to about $50^0$C was separately dispensed into the aliquots of samples in the three petri dishes and swirled gently, allowed to solidified and incubated in an inverted position at $37^0$C for 24 hours (Egea *et al*., 2012) [8]. The production of yellowish-green fluorescent pigment on centrimide agar is commonly associated with Pseudomonads (Lamonth and Martins, 2003) [17]. Distinct 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 further identified using standard microbiological techniques (Cheesbrough, 2010) [4].

### Morphological characterization of isolates

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

### Gram staining

A smear was made on a clean microscope slide using 24 hours old culture of the test isolate and heat-fixed. The slide was stained with crystal violet solution for 1minute and rinsed with water. Lugol's iodine solution was applied on the slide for 1minute, drained off and thereafter rinsed with water. The slide was then decolorized with a few drops of 95% ethanol for 20 seconds. The slide was counter-stain with safranin and allowed to dry. The stained slide was viewed using oil immersion magnification. Gram-negative cells are decolorized by alcohol and appear pink to red in colour while Gram-positive cells will appear purple in colour (Sohani and Sanjeeda, 2012) [38].

### Biochemical characterization of the isolates

The isolates were further identified via 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 carbohydrate utilization which include glucose, lactose and sucrose.

### Antimicrobial susceptibility test of the *Pseudomonas* species

This was done using the standard Kirby-Bauer disk diffusion (Jayabarath, 2015) [13]. The *Pseudomonas* species inoculum was prepared by suspending the freshly grown bacteria in 5 ml sterile nutrient broth and its turbidity was brought to 0.5. The antimicrobial susceptibility testing was performed on Mueller-Hinton agar using the following antibiotics; beta-lactam combination agent (augmentin 20/10µg), cephem (cefotaxime 30µg, ceftazidime 30µg, cefuroxime 5µg), carbapenem (imipenem 10µg), aminoglycosides (gentamicin 10µg), fluoroquinolone (ciprofloxacin 5µg, ofloxacin, 5µg), monobactam (aztreonam 30µg) and nitrofurans (nitrofurantoin 300µg). The plates were incubated aerobically at about 37°C for not less than 24 hours. The zones of inhibition were measured with a metre rule and the results were recorded and interpreted according to the Clinical and Laboratory Standards

Institute (CLSI) guidelines (2020). The control was *Pseudomonas aeruginosa* strain ATCC 27853

## Results and discussion

### Results

### Morphological, cultural and staining characteristics of the isolates recovered from pig feaces

Identification of the isolates was done by colony characteristics on different bacteriological media presented in table 4.1.1.

### Gram reaction and biochemical characterization of the isolates recovered from pig feaces

All the isolates were Gram-negative rod shaped bacteria. They were motility, catalase and citrate positive but were indole, methyl red, Voges-Proskauer, glucose, lactose and sucrose negative (table 4.1.2).

### Antibiotic susceptibility patterns of all the *Pseudomonas* species isolated from pig feaces

Table 4.1.3 showed the level of resistances and susceptibilities exhibited by the *Pseudomonas* species isolated from the pig feaces to the test antibiotics. The *Pseudomonas* species isolates from the pig feaces were highly resistant to cefuroxime 20 (66.7%) but were totally susceptible (100.0 %) to Imipenem, aztreonam and nitrofurantoin.

### Antibiotype of the isolated *Pseudomonas* species

The Antibiotypes of the isolated *Pseudomonas* species showed that 20 (66.7%) of the isolates showing resistance to antibiotics were multidrug resistance. In addition, one *Pseudomonas* species were resistant to a combination of two different classes of antibiotics (CIP-AUG); two to three different classes of antibiotics (CTX-GEN-AUG); three, two and three to four different classes of antibiotics respectively (CIP-CTX-OFX-AUG), (CIP-CAZ-GEN-AUG) and (NIT-CRX-IMP-AUG); two, two and two to five different classes of antibiotics respectively (AZT-IMP-CTX-GEN-OFL), (CIP-CRX-GEN-OFL-AUG) and (NIT-CIP-CTX-GEN-AUG); one, one and one respectively to six different classes of antibiotics respectively (AZT-CIP-CAZ-GEN-OFL-AUG), (AZT-CIP-IMP-GEN-CRX-AUG) and (NIT-CIP-IMP-CAZ-GEN-AUG) (Table 4.1.5).

**Table 1:** Morphological, cultural and staining characteristics of the isolates recovered from pig 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 |
| 2 | Eosin Methylene Blue Agar | Circular, raised, mucoid, milky, smooth, translucent | |
| 3 | MacConkey Agar | Circular, raised, mucoid, smooth colourless, transparent | |
| 4 | Nutrient Agar | Circular, raised, mucoid, smooth yellowish-green, translucent-opaque | |

**Key:** S/N = Serial number

**Table 2:** Biochemical Characteristics of the Isolates Recovered from Pig Feaces

| S/N | Isolates | MOT | CAT | CIT | IND | MR | VP | TSI | | | Probable Organism |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Slant/Butt | Gas | $H_2S$ | |
| 1-48 | 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/Alkalin

**Table 3:** Antibiotic susceptibility patterns of all the *Pseudomonas* species (n=30) isolated from pig feaces

| Family 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 | 5 (16.7) | 25 (83.3) |
| Cephem | Cefotaxime | CTX | 30 | ≥ 21 | ≤ 14 | 9 (30.0) | 21 (70.0) |
| | Ceftazidime | CAZ | 30 | ≥ 18 | ≤ 14 | 10 (33.3) | 20 (66.7) |
| | Cefuroxime | CRX | 5 | ≥ 16 | ≤ 20 | 5 (16.7) | 25 (83.3) |
| Carbapenem | Imipenem | IMP | 10 | ≥ 19 | ≤ 15 | 25 (83.3) | 5 (16.7) |
| Aminoglycosides | Gentamicin | GEN | 5 | ≥ 15 | ≤ 12 | 12 (40.0) | 18 (60.0) |
| Fluoroquinolones | Ciprofloxacin | CIP | 5 | ≥ 21 | ≤ 15 | 11 (36.6) | 19 (63.3) |
| | Ofloxacin | OFL | 5 | ≥ 16 | ≤ 12 | 12 (40.0) | 18 (60.0) |
| Monobactam | Aztreonam | AZT | 30 | ≥ 22 | ≤ 15 | 18 (60.0) | 12 (40.0) |
| Nitrofuran | Nitrofurantoin | NIT | 300 | ≥ 17 | ≤ 14 | 20 (66.7) | 10 (33.3) |

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

**Table 4:** Antibiotype of *Pseudomonas* species isolated from pig 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 | 0 (0.0) |
| 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 | 1 (3.3) |
| 5 | AZT-IMP-CTX-GEN-OFL | 0 (0.0) |
| 4 | NIT-CRX-IMP-AUG | 2 (6.7) |
| 4 | CIP-CAZ-GEN-AUG | 1 (3.3) |
| 4 | CIP-CTX-OFX-AUG | 2 (6.7) |
| 4 | CIP-CAZ-GEN-AUG | 0 (0.0) |
| 3 | CAZ-OFX-AUG | 0 (0.0) |
| 3 | CTX-GEN-AUG | 2 (6.7) |
| 2 | CAZ-AUG | 2 (6.7) |
| 2 | CIP-AUG | 1 (3.3) |
| 1 | NIT | 10 (33.3) |
| 1 | IMP | 5 (16.7) |
| Total | | 30 |

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

## Discussion

Antibiotic resistance has been causing serious problems to human medicine and also to animal, livestock, and veterinarians (Lawson, 2008). The heavy and off-label use of antibiotics has been reported to be a risk factor for the development and spread of beta-lactamase producing organisms (Chishimba *et al*., 2016; Vangelis and Panagiota, 2015) [5, 41]. Therefore, the present study was aimed at isolating, identifying and determining the antibiotic resistance pattern of *Pseudomonas* species isolated from pig feaces.

The colony morphology of the isolated *Pseudomonas* species showed circular, raised, mucoid, smooth, yellowish-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. This observation correspond with the characteristics of *Pseudomonas* species as previously suggested by other researchers (Abedin *et al*., 2020) [3].

All the isolates were positive to motility, catalase and citrate but were negative to indole methyl red; voges Prauskaur test and gas ($CO_2$ and $H_2S$) production thus were identified as *Pseudomonas* species. This finding correspond with the specific biochemical characters of *Pseudomonas* species previously suggested by other researchers (Abedin *et al*., 2020; El-Barbary, 2010) [3, 9-10]. All *Pseudomonas* species recovered from pig feaces in the present study were subjected to ten antibiotics belonging to seven classes: beta-lactam combination agent (augmentin), cephem (cefotaxime, ceftazidime, cefuroxime), carbapenem (imipenem), aminoglycosides (gentamicin), fluoroquinolone (ciprofloxacin, ofloxacin), monobactam (aztreonam) and nitrofurans (nitrofurantoin). The reason for choosing this antimicrobial was their wide use in the hospital as antipseudomonal agents. *Pseudomonas* is one of the major pathogens in healthcare associated infections (HAI) (Olayinka *et al.*, 2009) [29]. This is not only because they cause infections that are associated with significant morbidity and mortality but also because of their increasing rates of resistance which make them more difficult to be treated with inexpensive antibiotics. Emerging and increasing resistance to newer and otherwise efficacious antibiotics may compound the whole problem (Okeke and Sosa, 2003) [28].

In this present study, 83.3% susceptibility shown by the isolated *Pseudomonas* species to imipenem is parallel to results achieved in previous studies carried out in Turkey by Shenoy *et al*. (2002) [36] and Deniz Yilmaz *et al*. (2016) [6] and in Kenya by Mwinyikombo (2018) [25], who revealed that *Pseudomonas* isolates demonstrated higher degrees of susceptibility to imipenem. In addition, the high resistance level shown by the isolates in the present study to gentamicin (60.0%) and ceftazidime (66.7%) respectively is similar but lower than the 100% resistance shown by same isolates from same sample reported by Matjuda and Aiyegoro, (2016) [21]. Furthermore, the 33.3% resistance to nitrofurantoin shown by the isolates in the present study is far lower than the 100% resistance shown by same isolate from same source to same antibiotic.

Also, the high resistance of the isolates from pig feaces observed in this present study to augmentin (83.3%) is similar but higher than the 74.1% reported by Olufemi *et al*. (2017) [2] against same antibiotic and same isolate but now from cattle feaces. Furthermore, the high resistance to ciprofloxacin (63.3%) observed in this study is lower than the 66% resistance observed against same antibiotic by Kumari *et al*. (2019) [15] who isolated same microorganism but now from cattle feaces.

The observed multidrug resistance (50.0%) of the *Pseudomonas* species in the present study is high and is comparatively similar to the 61.5% and the 75.8% respectively reported in previous studies (Abd El-Baky *et al*., 2013) [1]. Such multiple antibiotics resistant in *Pseudomonas* spp. has been attributed to combination of acquisition of resistance gene through genetic exchange and mutation, as well as physiological mechanism such as the possession of specific protein, poor membrane permeability, biofilm formation and efflux pumps (Livermore, 2002; Lambert, 2002; Olsen, 2015) [18, 16, 30].

## Conclusion and recommendation

The fact that the *Pseudomonas* species recovered from the pig feaes in this study showed varying levels of susceptibility and resistance to different classes of antibiotics is suggestive of misuse and abuse of the antibiotics in the pig production. This study calls for concern and urgent intervention on antibiotic

stewardship among pig farmers. Therefore, there is a need to create awareness about the danger inherent in indiscriminate use of antibiotics in rearing pig and thereby Strict antibiotic policy is required urgently to protect and sustain the efficient availability of antibiotics. Equally, there is need to create enough attention on alternative control strategies for *Pseudomonas* infections in pigs without the use of antibiotics, in same vain, characterization of the isolated *Pseudomonas* species including pathogenicity studies is urgently required in order to understand their implication on pigs and human health

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