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Antibiotic-resistant *Pseudomonas aeruginosa* from abattoir and aquaculture environment in Abakaliki, Ebonyi State, southeast Nigeria

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Abstract:

Background: *Pseudomonas aeruginosa* is frequently identified as the predominant bacterial pathogen in abattoir and aquaculture settings. In Ebonyi State, Nigeria there has been a lack of thorough investigation on the impact of the organism in the environment on public health. Therefore, it was necessary to investigate the occurrence of *P. aeruginosa* and determine its resistance characteristics to antimicrobial agents in selected abattoirs and aquaculture facilities in Abakaliki, Ebonyi State, southeast Nigeria.
Methodology: Wastewater samples from randomly selected abattoirs (n=25) and aquaculture (n=25) sites in various locations in Abakaliki, Ebonyi State, Nigeria, were collected into sterile universal bottles and transported to the microbiology laboratory of Ebonyi State University, Abakaliki, for microbiological analysis. For heterotrophic colony count, measured in colony forming unit/ml (CFU/ml), to estimate the microbial load of the samples, a 1-in-10 dilution of the samples were prepared and cultured on nutrient agar, incubated at 37°C for 24 hours. Colonies from the culture plate were then sub-cultured on *Pseudomonas* agar and incubated aerobically at 37°C for 24 hours. *Pseudomonas aeruginosa* was phenotypically confirmed on the culture plate by conventional morphological characteristics and biochemical tests. Antibiotic susceptibility testing of the *P. aeruginosa* isolates was performed by the disk diffusion test and results interpreted using the Clinical Laboratory Standards Institute (CLSI) zone diameter breakpoints. Multiple antibiotic resistance index (MARI) was calculated for each isolate.
Results: The microbial load varied from 3.0±2.8 to 33.4±23.5x10^5 CFU/ml for abattoir samples and 0.00 to 26.0±2.8x10^5 CFU/ml for aquaculture samples. For the abattoir, wastewater samples from the butcher table had the highest frequency of *P. aeruginosa* (50.0%) isolation, followed by wastewater from the drainage (26.7%), while the lowest frequency was wash water (23.3%). For the aquaculture, wastewater from earth pond had a higher frequency (63.6%) of *P. aeruginosa* isolation than concrete pond (36.4%). The antibiotic susceptibility result showed that *P. aeruginosa* exhibited high resistance rate to amoxicillin-clavulanic acid (80.0%) and cefotaxime (80.0%). Additionally, the bacteria showed resistance rate of 50.0% to tobramycin. On the other hand, the isolates demonstrated high sensitivity rates of 90.0% to imipenem and cefepime, while sensitivity rates of 60.0% were observed for meropenem and ceftazidime. The multiple antibiotic resistance index (MARI) ranged from 0.2 to 0.7, with a mean MARI of 0.6.
Conclusion: The results of this study highlight the importance of close monitoring abattoir and aquaculture settings, as they may serve as major sources for the environmental dissemination of antibiotic resistant bacteria such as *P. aeruginosa*.

Keywords: Antibiotic resistance, *Pseudomonas aeruginosa*, Environment; Waste waters; Ebonyi State

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Pseudomonas aeruginosa résistant aux antibiotiques dans les abattoirs et les environnements d'aquaculture à Abakaliki, État d'Ebonyi, sud-est du Nigéria

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Résumé:

Contexte: *Pseudomonas aeruginosa* est fréquemment identifié comme le pathogène bactérien prédominant dans les abattoirs et les environnements d'aquaculture. Dans l'État d'Ebonyi, au Nigéria, il n'y a pas eu d'enquête approfondie sur l'impact de l'organisme dans l'environnement sur la santé publique. Français Par conséquent, il était nécessaire d'étudier la présence de *P. aeruginosa* et de déterminer ses caractéristiques de résistance aux agents antimicrobiens dans des abattoirs et des installations d'aquaculture sélectionnés à Abakaliki, dans l'État d'Ebonyi, au sud-est du Nigéria.
Méthodologie: Des échantillons d'eaux usées provenant d'abattoirs (n=25) et de sites d'aquaculture (n=25) sélectionnés au hasard dans divers endroits d'Abakaliki, dans l'État d'Ebonyi, au Nigéria, ont été collectés dans des bouteilles universelles stériles et transportés au laboratoire de microbiologie de l'Université d'État d'Ebonyi, à Abakaliki, pour analyse microbiologique. Pour le dénombrement des colonies hétérotrophes, mesuré en unités formant colonie/ml (UFC/ml), afin d'estimer la charge microbienne des échantillons, une dilution 1:10 des échantillons a été préparée et cultivée sur gélose nutritive, incubée à 37°C pendant 24 heures. Les colonies de la boîte de culture ont ensuite été repiquées sur gélose *Pseudomonas* et incubées en aérobiose à 37°C pendant 24 heures. Français *Pseudomonas aeruginosa* a été confirmé phénotypiquement sur la plaque de culture par des caractéristiques morphologiques conventionnelles et des tests biochimiques. Le test de sensibilité aux antibiotiques des isolats de *P. aeruginosa* a été réalisé par le test de diffusion sur disque et les résultats ont été interprétés à l'aide des points de rupture du diamètre de zone du Institut de normalisation des laboratoires cliniques (CLSI). L'indice de résistance multiple aux antibiotiques (MARI) a été calculé pour chaque isolat.
Résultats: La charge microbienne variait de 3,0±2,8 à 33,4±23,5x10^5 UFC/ml pour les échantillons d'abattoir et de 0,00 à 26,0±2,8x10^5 UFC/ml pour les échantillons d'aquaculture. Pour l'abattoir, les échantillons d'eaux usées de la table de boucherie présentaient la fréquence la plus élevée d'isolement de *P. aeruginosa* (50,0%), suivis des eaux usées du drainage (26,7%), tandis que la fréquence la plus faible était l'eau de lavage (23,3%). Pour l'aquaculture, les eaux usées des bassins en terre présentaient une fréquence plus élevée (63,6%) d'isolement de *P. aeruginosa* que celles des bassins en béton (36,4%). Le résultat de la sensibilité aux antibiotiques a montré que *P. aeruginosa* présentait un taux de résistance élevé à l'amoxicilline-acide clavulanique (80,0%) et à la céfotaxime (80,0%). De plus, les bactéries présentaient un taux de résistance de 50,0% à la tobramycine. D'autre part, les isolats présentaient des taux de sensibilité élevés de 90,0% à l'imipénème et à la céfépime, tandis que des taux de sensibilité de 60,0% ont été observés pour le méropénème et la céftazidime. L'indice de résistance multiple aux antibiotiques (MARI) variait de 0,2 à 0,7, avec un MARI moyen de 0,6.
Conclusion: Les résultats de cette étude soulignent l'importance d'une surveillance étroite des abattoirs et des bassins d'aquaculture, car ils peuvent servir de sources majeures de dissémination environnementale de bactéries résistantes aux antibiotiques telles que *P. aeruginosa.*

Mots clés: Résistance aux antibiotiques; *Pseudomonas aeruginosa*; Environnement; Eaux usées; État d'Ebonyi

Introduction:

Pseudomonas species are Gram-negative non-fermentative bacteria that inhabit various terrestrial and aquatic environments (1). *Pseudomonas* is widely distributed and also responsible infectious diseases in man, animals, and plants (2). *Pseudomonas* encompasses diverse range of species, among them is the opportunistic pathogen, *Pseudomonas aeruginosa*, which is gaining significance in the fields of medicine and veterinary science (1). *Pseudomonas aeruginosa* is a zoonotic pathogen that can infect both animals and humans, particularly those with weakened immune systems are very vulnerable to *P. aeruginosa* infection, where it may cause disease with significant damage to the lungs (3). The pathogen employs several tactics to initiate and sustain infection, such as the formation of biofilms, resistance to multiple drugs, and the ability to tolerate antibiotics (4).

Antibiotic resistant (AMR) microorganisms present in the environment can potentially be transmitted to human through several routes. These bacteria can transfer their antibiotic resistance genes to humans through the food chain (5). The increasing prevalence of multidrug resistance in *P. aeruginosa*, as well as in other bacterial species, is a significant cause for concern (4). As a result, treatment of infections caused by *P. aeruginosa* is becoming more challenging due to the emergence of high levels of antibiotic resistance. This resistance is manifested by the existence of numerous strains that are resistant to antibiotics, as well as the rapid development of resistance during the course of treatment (6).

Abattoir wastes possess the capacity to pollute both surface and groundwater (7). The release of abattoir effluents into water bodies carries significant health consequences (8). Abattoir effluents have the capacity to transport resistant pathogenic bacteria, potentially playing a role in the worldwide dissemination of these strains in various habitats (9).

Aquaculture is a worldwide practice that involves the reproduction of many species of fish (10). The management of wastewater from fish ponds is a significant environmental concern. A significant number of fish farmers release their wastewater directly into water bodies or into drainage systems that ultimately run into the water bodies. This practice poses a threat to both human health and the environment at large (11). Significant quantities of antibiotics and disinfectants are utilized in the cultivation of ornamental fish to prevent and address bacterial infections. This phenom-

enon can significantly influence the selection and spread of genes that confer resistance to antibiotics (12).

In Nigeria, the establishment and functioning of several private and government abattoirs, including those in Ebonyi State, are lacking in regulation. Typically, they are situated in close proximity to bodies of water to ensure reliable access to water for processing. Abattoirs, aquaculture environments, and the wastewaters associated with them have the potential to be sources of pathogenic bacteria. These bacteria can operate as reservoirs and propagate antibiotic resistance genes within bacterial populations, affecting both animals and humans that consume food from these sources. This study aimed to identify and determine the antimicrobial susceptibility of *P. aeruginosa* in aquaculture and abattoir environments in Abakaliki, Ebonyi State, Nigeria.

Materials and method:

Study location and selection of sites:

The study was conducted from March to June 2020 at the Microbiology Laboratory of Ebonyi State University, Abakaliki, Nigeria. Ebonyi State is located in southeastern Nigeria within longitude 7.30' and 8.30'E and latitude 5.40' and 6.45'N and was created on October 1, 1996 from the former Abia and Enugu States, with Abakaliki as its capital. It is bounded to the north by Benue State, to the west by Enugu State, to the east by Cross River State and to the south by Abia State.

There are thirteen Local Government Areas (LGAs) in the State namely; Abakaliki, Ebonyi, Ishielu, Ohaukwu, Izzi, Ikwo, Ezza North, Ezza South, Afikpo North, Afikpo South, Ivo, Ohaozara and Onicha LGAs. For the purpose of this study, a total of 25 abattoir and 25 aquaculture farms were randomly selected from various locations in Abakaliki.

Ethical consideration:

Approval to survey the farms were obtained from the owners of the various fish ponds and abattoirs.

Sample collection and transportation:

Fresh environmental water samples were collected from the selected abattoirs (n=25) and aquaculture farms (n=25) in Abakaliki aseptically into universal sterile container and immediately transported to the laboratory in ice packs for microbiological analysis.

Culture isolation and bacterial identification:

Approximately 1 ml of each sample was aseptically dispensed into test tubes containing 9ml of sterile distilled water and tubes were thoroughly shaken for even distribution of organisms. Ten-fold serial dilutions of the samples were then carried out and subsequently inoculated onto freshly prepared nutrient agar plates. The culture plates were incubated aerobically at 37℃ for 24 hours. Microbial load was estimated by heterotrophic colony count which was performed in triplicate and expressed as mean colony forming unit per milliliter (CFU/ml).

Colonies from the culture plates were transferred to freshly prepared *Pseudomonas* agar and incubated for 24 hours. Representative colonies on culture growth were selected, sub-cultured and identified as *P. aeruginosa* using conventional biochemical tests (13).

Antibiotic susceptibility testing:

Antibiotic susceptibility testing was performed using the disk diffusion method according to Clinical and Laboratory Standards Institute guideline (14) against selected antibiotics that were chosen for their use in both medicine and human veterinary practice and from previous studies that reported microbial resistance to them. The antibiotics used included amoxicillin-clavulanic acid (30µg), cefotaxime (30µg), ceftazidime (30µg), cefepime (30µg), tobramycin (10µg), imipenem (10µg), and meropenem (10µg).

Colonies of confirmed *P. aeruginosa* were collected using sterile wire loop and dispensed into test tubes containing 5ml distilled water. The inoculum was adjusted to 0.5 MacFarland standard and them streaked on freshly prepared Mueller-Hinton agar plates (Oxoid, UK) plates). The plates were allowed to stand for 15 minutes after which antibiotic disks were placed on the inoculated plates, 5mm apart, and incubated at 37^{o}C for 24 hours. The diameter of zone of inhibition was measured and interpreted as sensitive, intermediate or resistant according to CLSI guideline (14).

Multiple antibiotic resistance (MAR) index:

The MAR index was determined as the number of antibiotics to which isolate is resistant divided by the total number of antibiotics against which isolate was tested, as previously described by Osundiya et al., (15).

Statistical analysis:

The microbial load (expressed as CFU/ml) was presented as mean±standard deviation and one-way ANOVA was used to determine statistical differences in the data generated. The frequency of isolation of *P. aeruginosa* (in percentage) was calculated as the number of *P. aeruginosa* isolated from each wastewater sample point divided by the total number of *P. aeruginosa* from the abattoir or aquaculture sites multiplied by 100.

Results:

The microbial load from the abattoir samples as shown in Table 1, revealed that

waste water samples from abattoir drainage with sample code AB_5 had the highest microbial load (33.4±23x10^5 CFU/ml), followed by AB_4 (22.6±24.6x10^5 CFU/ml), AB_3 (15.8± 8.2x 10^5 CFU/ml), AB_1 (15.4±1.9x10^5 CFU/ml) and AB_2 (15.4±6.5x10^5 CFU/ml). The butcher table with sample code AB_4 had the highest microbial load (15.4±1.9x10^5 CFU/ml), followed by AB_2 (10.6±8.7x10^5 CFU/ml), AB_1 (9.8±11.1x 10^5 CFU/ml) and AB_5 (7.6±0.5x10^5 CFU/ml), while AB_4 had the least microbial load (6.4± 0.5x10^5 CFU/ml). From the wash water, AB_5 had the highest microbial load (22.6±0.9x10^5 CFU/ml), followed by AB_3 (15.0±8.7x10^5 CFU/ml), and AB_2 (3.0±2.8x10^5 CFU/ml).

The highest microbial load (26.0 ± 2.8 x10^5 CFU/ml) was observed in concrete pond water with the sample code PW_1, followed by concrete pond water PW_4 (23.6±1.9x10^5 CFU/ml), earthen pond PW_4 (22.0±21.8x10^5 CFU/ml), while earthen pond PW_1 had no microbial load (Table 2). The result of frequency of *P. aeruginosa* isolation as represented in Table 3, showed that waste water samples from the butcher table had the highest frequency of isolation of *P. aeruginosa* (50.0%), followed by wastewater from drainage (26.7%), while wash water had the least frequency (23.3%). On the basis of sample code, AB_5 had highest frequency of *P. aeruginosa* isolation (26.7 %), followed by AB_5 (23.3%), AB_1 (20.0%) and AB_3 (20.0%), while AB_2 (10.0%) recorded the least as indicated in Table 3.

Table 1 : Microbial load of abattoir samples

Sample code	Wash Water	Drainage	Butcher Table
AB_1	13.4 ± 3.6	15.4 ± 1.9	9.8 ±11.1
AB_2	3.0 ± 2.8	15.4 ± 6.5	10.6 ± 8.7
AB_3	15.0 ± 8.7	15.8 ± 8.2	14.2 ±11.1
AB_4	8.6 ± 0.8	22.6 ± 24.6	6.4 ± 7.3
AB_5	22± 0.9	33.4± 23.5	7.6± 0.5

AB= Abattoir; Values were mean ± standard deviation (SD)

Table 2: Microbial load of aquaculture samples

Sample code	Earth Pond	Concrete Pond
PW_1	0.0	26.0 ± 2.8
PW_2	16.0 ± 5.6	15.8 ± 1.4
PW_3	21.7 ± 12.0	20.4 ± 1.3
PW_4	22.0 ± 21.5	23.6 ± 11.9

PW= Pond water, Values were mean ± standard deviation (SD)

Table 3: Frequency of *Pseudomonas aeruginosa* isolates from waste water samples from abattoir

Sample code	Wash water (%)	Drainage (%)	Butcher Table (%)	Total (%)
AB_1	1 (14.3)	2 (25.0)	3 (20.0)	6 (20.0)
AB_2	1 (14.3)	2 (25.0)	0	3 (10.0)
AB_3	2 (28.6)	0	4 (26.7)	6 (20.0)
AB_4	2 (28.6)	2 (25.0)	3 (20.0)	7 (23.3)
AB_5	1 (14.3)	2 (25.0)	5 (33.3)	8 (26.7)
Total	**7 (23.3)**	**8 (26.7)**	**15 (50.0)**	**30 (100.0)**

Table 4: Distribution of the *P. aeruginosa* isolates from waste water samples from aquaculture

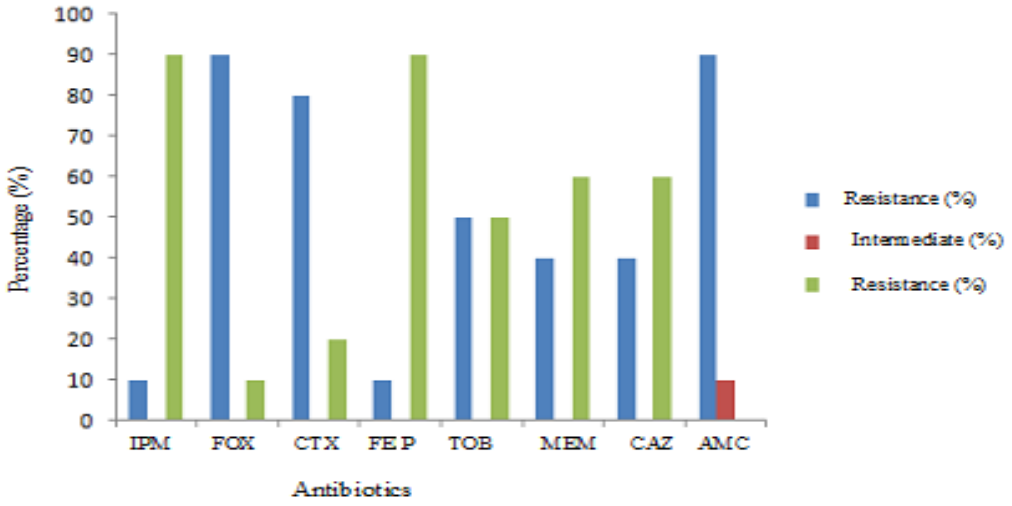
Sample code	No in Concrete Pond (%)	No in Earth Pond (%)	Total No (%)
PW_1	1 (25.0)	2 (28.6)	3 (27.3)
PW_2	0	2 (28.6)	2 (18.2)
PW_3	1 (25.0)	0	1 (9.1)
PW_4	1 (25.0)	1 (14.4)	2 (18.2)
PW_5	0	2 (28.6)	2 (18.2)
PW_6	1 (25.0)	0	1 (9.1)
Total	**4 (36.4)**	**7 (63.6)**	**11 (100.0)**

PW = Pond water, % = Percentage.

Aquaculture samples had the highest frequency of *P. aeruginosa* isolation from earthen pond (63.6%) while concrete pond had the least frequency (36.4%) as shown in Table 4. Pond water PW_1 had the highest frequency of *P. aeruginosa* (27.3%) isolation, followed by PW_2 (18.2%), PW_4 (18.2%) and PW_5 (18.2%), while PW_3 (9.1%) and PW_6 (9.1%) as shown in Table 4.

Fig 1 shows the result of the antibiotic susceptibility of *P. aeruginosa* to the respective antibiotics used. The isolates had highest resistance to amoxicillin-clavulanic acid (90%) followed by cefotaxime (80.0%), tobramycin (50.0%), imipenem (10.0%) and cefepime (10.0%). The highest susceptibility rate was observed with imipenem (90.0%) and cefepime (90.0%), followed by meropenem (60%) and ceftazidime (60.0%), while amoxicillin-clavulanic (10.0 %) acid showed the lowest susceptibility.

From the aquaculture samples, the highest MAR index of 0.7 was obtained with PW_3, followed by PW_1 (0.6), PW_2 (0.5), while the lowest MA index was obtained with PW_4 (0.2) as indicated in Table 5. From the abattoir samples obtained, highest MAR index of 0.6 was obtained with AB_3, followed by AB_4 (0.6) and AB_5, while the lowest MAR index was obtained with AB_1 (0.2) and AB_2 (0.2) as indicated in Table 6.



CTX = Cefotaxime, MEM = Meropenem, CAZ = Ceftazidime, AMC = Amoxicillin-Clavulanic acid, TOB = Tobramycin, IPM = Imipenem

Fig 1: Antibiotic susceptibility of *Pseudomonas aeruginosa* to selected antibiotics

Table 5: Multiple antibiotic resistance indices (MARI) of *Pseudomonas* species from aquaculture samples

Sample Code	MARI	Antibiotics
PW_1	0.6	CTX, AMC, TOB, CAZ
PW_2	0.4	CTX, AMC, TOB
PW_3	0.7	CTX, AMC, TOB, CAZ, MEM
PW_4	0.2	CTX, AMC
PW_5	0.4	CTX, AMC, MEM

PW= Pond water; CTX = Cefotaxime; MEM = Meropenem; CAZ = Ceftazidime; AMC = Amoxicillin-Clavulanic acid; TOB = Tobramycin
IPM = Imipenem; MARI = Multiple antibiotic resistance index

Table 6: Multiple antibiotic resistance indices (MARI) of *Pseudomonas aeruginosa* from abattoirs samples

Sample Code	MARI	Resistance profile
AB_1	0.2	CTX, AMC
AB_2	0.2	CTX, AMC
AB_3	0.6	CTX, AMC, TOB, CAZ
AB_4	0.4	TOB, CAZ, MEM
AB_5	0.4	CTX, AMC, MEM

AB = Abattoir; CTX = Cefotaxime; MEM = Meropenem; CAZ = Ceftazidime; AMC = Amoxicillin-Clavulanic acid
TOB = Tobramycin; IPM = Imipenem; MARI = Multiple antibiotic resistance index

Discussion:

In many underdeveloped nations, like Nigeria, the butchering of animals and the production of meat for human consumption result in significant amounts of waste that are often inadequately handled (16). The microbial condition of water is crucial in the transmission of diseases among farmed fish (17). The bacterial load serves as a significant indicator for assessing the potential existence of coliform and potential pathogens like *Pseudomonas* in water samples (18).

The findings of our study indicate that the total bacterial counts obtained exceeded the limit of <100 CFU/ml set by the Environmental Monitoring Agency (EMA) (19). The high bacteria count reported in this study is a definitive indication of exceedingly elevated levels of contamination that present a significant risk to both human and aquatic life. This may be attributed to the distinctive properties of abattoir effluent across Ebonyi State. According to the study by Onuoha et al., (20), abattoir effluent contains significant amounts of pathogens due to the inadequate sanitation and hygiene practices of the abattoir management, the health condition of the animals being slaughtered, and the lack of skilled workers. Furthermore, recent findings have indicated a significant presence of bacteria in the wastewater originating from abattoirs, as reported by Odjadjare and Ebowemen (18), Asibor et al., (21), Gufe et al., (9), and Joseph et al., (8).

Reports have indicated that fish ponds had significantly elevated microbial load that exceed the recommended safety threshold (22). The findings of our study align with that of Okafor et al., (17), who reported that the elevated bacterial levels in fish farms are a result of the introduction of organic matter from the surrounding areas into the water intake source. The high microbial load in fish ponds can be related to the fertilization of the ponds with animal manure, which is directly discharged into the ponds, or the excretion of waste by the fish into the ponds (23).

The wastewater samples from the butcher's table had the highest frequency of *P. aeruginosa* isolation of 50.0%, while the wash water had the lowest frequency of 23.3%. Samples from abattoir AB_5 also had the highest frequency *P. aeruginosa* isolation of 26.7%

while AB_2 had the lowest frequency of 10.0%. The isolation of *P. aeruginosa* in abattoir wastewater in our study aligns with previous findings of Odjadjare and Ebowemen (18), Gufe et al., (9), Savin et al., (23), Hosu et al., (7) and Homeier-Bachmann et al., (24). The frequency of *P. aeruginosa* isolation was highest in the earthen pond (63.6%), while the concrete pond had the lowest frequency of 36.4%. Pond water sample PW1 had the highest frequency of *P. aeruginosa* isolation of 27.3%, followed by PW2, PW4, and PW5, each at 18.2%, and PW3 and PW6 had the lowest frequency of 9.1% each. These findings align with the study by Adekanmbi and Falodun (25) who proposed the concept that aquaculture environments act as focal points or reservoirs for harmful *Pseudomonas* strains.

The presence of harmful bacteria reduces the quality of the fish and elevates the chance of mortality. Consuming these bacteria through contaminated water or food can lead to disease condition in humans that may be associated with mortality or ill health (26). Consumers may be at risk if they ingest undercooked fish caught from ponds infested with these pathogenic bacteria (25). Accurate diagnosis, prediction of outbreaks, and execution of preventive and/or prophylactic treatments in aquaculture need the identification of *Pseudomonas* species (28). *Pseudomonas* species are frequently identified as prevalent bacterial pathogens in cultured fish, known to induce stress-related illnesses in freshwater fish, particularly in farming settings (29,30). Consumers could be at risk if they ingest fish from ponds infected with dangerous pathogens especially if the fish is not properly cooked.

Antibiotic resistance has emerged as a significant challenge for the human population (31). Abattoir wastewater is recognized as a source of antibiotic-resistant microorganisms and antibiotic residues, which are not effectively eliminated by traditional treatment methods (24). The use of antimicrobials in the rapidly growing aquaculture business is expected to contribute to the increase in antimicrobial resistance. This has the potential to negatively impact the health of animals, humans, and ecosystems (31). *Pseudomonas* are predominantly known to cause invasive or opportunistic infections for man and other organisms. Additionally, this genus has become significant in the context of antibiotic resistance (29). The improper use of antibiotics in veterinary medicine has led to the proliferation of antibiotic-resistant bacteria in the effluent of abattoirs and aquaculture environments. This poses a significant risk to human health, aquatic life, and the whole ecosystem.

The highest susceptibility rates of the *P. aeruginosa* isolates in our study were observed with imipenem (90.0%) and cefepime (90.0%), followed by meropenem (60.0%) and ceftazidime (60.0%) while the lowest susceptibility rates were seen with amoxicillin-clavulanic acid (0.0%). These findings clearly demonstrate that imipenem or cefepime, either alone or in combination, can effectively be utilized for treating *P. aeruginosa* infection. According to the study by Ahmad et al., (32), *P. aeruginosa* from an abattoir in Ibadan showed the maximum susceptibility (100.0%) to imipenem and meropenem. Similarly, Ahmad et al., (32) found that *P. aeruginosa* had relatively high susceptibility to imipenem (74.0%) and meropenem (74.0%) and recent studies (33,34) showed high susceptibility of *P. aeruginosa* to cefepime, ranging from 85.0% to 95.35%. However, our findings contradicted the reported low susceptibility rates of 8.57%, 15.7%, and 20.8% to imipenem in *P. aeruginosa* from abattoir wastewater, clinical, and clinical samples, respectively (35,36, 37). This clearly demonstrates that *P. aeruginosa* possesses both innate resistance and the ability to acquire resistance during the course of an infection, resulting in a poor susceptibility to imipenem and other antibiotics.

Pseudomonas aeruginosa isolates in our study were highly susceptible to cefepime, with a rate of 90.0%, which contrasts the relatively low susceptibility (42.7%) reported by Jia *e*t al., (38) and in a recent study that demonstrated resistance to carbapenems (imipenem) in *P. aeruginosa* (39). The study by Mahfoud et al., (41) in Syria reported that *P. aeruginosa* were remarkably resistant (97.7%) to amoxicillin-clavulanic acid, which aligns with the findings of our study. A previous study (42) reported a significant level of resistance of *P. aeruginosa* to amoxicillin-clavulanic. The reported high resistance of *P. aeruginosa* to amoxicillin-clavulanic acid and cefotaxime may be the result of mutations driven by repeated exposure of *P. aeruginosa* to high concentrations of these antibiotics.

Multiple antibiotic resistance (MAR) index has been employed to distinguish bacteria from various origins by utilizing antibiotics that are frequently prescribed for treatment in human (15). The MAR index serves as a valuable indicator for assessing the potential life-threatening risk of pollution (44). The aquaculture samples analyzed showed a MAR index for *P. aeruginosa* ranging from 0.4 to 0.7. When the MAR index is above the 0.20 threshold, it strongly suggests that the bacteria originated from sources that are potentially hazardous, where antibiotics are commonly employed. It is likely that the bacteria were introduced through contamination of human or animal faeces.

Several studies (44,45) have reported MARI values above 0.2 for various bacterial isolates found in aquaculture environment. Our study agrees with previous research indicating that there is a significant presence of

antibacterial compounds in catfish that are raised and sold in Abakaliki metropolis and in other areas in southeast Nigeria. The MAR index of isolates from abattoir samples ranged from 0.4 to 1.0. Unprocessed waste from slaughterhouses can serve as a possible source for the spread of antibiotic-resistant bacteria that are capable of causing diseases in humans (45). One method to address antibiotic-resistant strains of bacteria is by employing synergistic pharmacological action (26). Multiple reports have indicated a MAR index of over 0.2 (23) from bacterial isolates from abattoir effluent, which strongly suggests that the bacteria obtained originate from sources that are possibly highly contaminated.

Conclusion:

While it is known that abattoir effluents are significant environmental sources *of P. aeruginosa,* there is a lack of information in the literature regarding the antibiotic susceptibility patterns of *P. aeruginosa* in aquaculture and abattoir effluents in Abakaliki, Ebonyi State. The isolates in the present study demonstrated high susceptibility to imipenem, meropenem, cefotaxime, and cefepime. The MAR index revealed a significant level of resistance in *P. aeruginosa* to routinely prescribed antibiotics. This finding strongly suggests that these pathogens have acquired resistance to the antibiotics commonly employed in treating infections caused by them.

Contributions of authors:

OSC was involved in study conceptualization, methodology, formal analysis, resources, writing, reviewing, editing, and supervision; NRN was involved in resources and visualization, editing, project administration, formal analysis and laboratory investigation. All authors read and approved the manuscript submitted for publication.

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References:

1. Moradali, M., Ghods, S., and Rehm, B. H. A. *Pseudomonas aeruginosa* lifestyle: A paradigm for adaptation, survival, and persistence. Front Cell Infect Microbiol. 2017; 7: 39. doi:10.3389/fcimb.2017.00039
2. Ruiz-Roldan, L., Rojo-Bezares, B., Toro, M., et al. Antimicrobial resistance and virulence of *Pseudomonas* species among healthy animals: Concern about exolysin ExlA detection. Sci Rep. 2020; 10: 1-11. doi:10.1038/s41598-020-68575-1.
3. El-Ghany, W. A. A. *Pseudomonas aeruginosa* infection of avian origin: Zoonosis and one health implications. Vet World. 2021; 14(8): 2155–2159. doi:10.14202/vetworld.2021.2155-2159
4. Sindeldecker, D., and Stoodley, P. The many antibiotic resistance and tolerance strategies of *Pseudomonas aeruginosa.* Biofilm. 2021; 3: 1-7. doi:10.1016/j.bioflm.2021.100056
5. Ahmad, S., Alotaibi, M. A., and Alamri, M. S. Antibiotic sensitivity pattern of clinical isolates of *Pseudomonas aeruginosa* at a Tertiary Care Hospital in Saudi Arabia. Dhaka University J Pharmaceut Sci. 2020; 19 (1): 77-82. https://doi.org/10.3329/dujps.v19i1.47821
6. Dégi, J., Moțco, O., Dégi, D. M. Antibiotic susceptibility profile of *Pseudomonas aeruginosa* canine Isolates from a multicentric Study in Romania. Antibiotics. 2021; 10 (7): 1-12. doi:10.3390/antibiotics10070846
7. Hosu, M., Vasaikar, S., Okuthe, G. E., and Apalata, T. Molecular detection of antibiotic-resistant genes in *Pseudomonas aeruginosa* from non-clinical environment: public health implications in Mthatha, Eastern Cape Province, South Africa. Int J Microbiol. 2021; 1: 1-9. doi:10.1155/2021/8861074
8. Joseph, M. O., Ibrahim, B., Zaky, S. K., Abdulkadir, S., and Auta, I. K. Bacterial assessment of effluents from selected abattoirs into adjoining water bodies in Kaduna metropolis. Sci World J. 2021; 16 (1): 1-6.
9. Gufe, C., Ndlovu, M. N., Sibanda, Z., Makuvara, Z., and Marumure, J. Prevalence and antimicrobial profile of potentially pathogenic bacteria isolated from abattoir effluents in Bulawayo, Zimbabwe. Sci Afr. 2021; 14: 1-10. doi:10.1016/j.sciaf.2021.e01059
10. Adebami, G. E., Fasiku, S. A., Solomon, O. D., and Babalola, B. A. Physicochemical and microbial evaluations of different fish ponds' wastewaters and the antibiotics profiles of isolated bacteria. Ethiop J Environ Stud Manag. 2020; 13 (4): 509-521.
11. Niyi-David, C. C., Wemedo, S. A., Akani, N. P., and Douglas, S. I. Culture-based characterization of bacteria associated with fish pond wastewater undergoing treatment using plants. J Adv Microbiol. 2021; 21(5): 1-10. doi:10.9734/jamb/2021/v21i530346
12. Liu, X., Wang, H., and Zhao, H. Prevalence of antibiotic resistance genes in wastewater collected from ornamental fish market in northern China. Environ Pollution. 2021; 271: 1-11. doi:10.1016/j.envpol.2020.116316
13. Cheesbrough, M. District laboratory practice in tropical countries, Part 2. Cambridge University Press, Cambridge, UK. 2006; 23-78, 137-159.
14. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute, 27th edition, Wayne, USA, 2017
15. Osundiya, O. O., Oladele, R. O., and Oduyebo, O. O. Multiple antibiotic resistance (MAR) indices of *Pseudomonas* and *Klebsiella* species isolates in Lagos University Teaching Hospital. Afr J Clin Exper Microbiol. 2013; 14 (3): 164-168. doi:10.4314/ajcem.v14i3.8
16. Ebong, G. A., Ettesam, E. S., and Dan, E. U. Impact of abattoir wastes on trace metal accumulation, speciation, and human health–related problems in soils within Southern Nigeria. Air, Soil and Water Research. 2020; 13: 1–14. https://doi.org/10.1177/1178622119898430
17. Okafor, U. C., Ezeanochie, P. E., and Obubu, M. Microbial assessment of some selected fish ponds in Awka, Anambra State: Comparative study and Modelling. Agric Biol Sci J. 2020; 6 (2): 91-99.
18. Odjadjare, E. E. O., and Ebowemen, M. J. Antibiogram of *Pseudomonas* isolates and potential public health impact of an abattoir effluent in

Benin-City, Nigeria. Afr J Clin Exper Microbiol. 2020; 21: 240-249. https://doi.org/10.4314/ajcem.v21i3.10

19. Manhokwe, S., Zvidzai, C., Mareesa, W., and Marume, P. Wastewater treatment strategies of selected Zimbabwean food industries. Int J Water Res Environ Eng. 2018; 10(4): 45–53. doi:10.5897/ijwree2015.0587
20. Onuoha, S. C., Eluu, S. C., and Okata, M. O. In-vitro antimicrobial resistance of *Shigella* and *Salmonella* species recovered from abattoir effluent in Afikpo, South Eastern Nigeria. Int J Curr Microbiol Appl Sci. 2016; 5 (4): 488–497. http://dx.doi.org/10.20546/ijcmas.2016.504.058
21. Asibor, G., Edjere, O., and Azubuike, G. Status of discharged abattoir effluent and its effects on the physico-chemical characteristics of Orogodo river Delta State, Nigeria. Water Pollution. 2020; 15: 51-61.doi:10.2495/WP200051
22. Amuneke, K. E., Igbodiegwu, G. C., Okeke, P. A., and Adibe, A. C. Bacteriological profile of selected fish species and water sample from Otuocha river Anambra State. J Agric Food Sci. 2020; 18 (1): 11-26. doi:10.4314/jafs.v18i1.2
23. Savin, M., Alexander, J., Bierbaum, G., et al. Antibiotic resistant bacteria, antibiotic resistance genes, and antibiotic residues in wastewater from a poultry slaughterhouse after conventional and advanced treatments. Sci Rep. 2021; 11:1-11. doi:10.1038/s41598-021-96169-y
24. Homeier-Bachmann, T., Heiden, S. E., Lübcke, P. K., et al. Antibiotic-resistant *Enterobacteriaceae* in wastewater of Abattoirs. Antibiotics. 2021; 10 (5): 568. doi:10.3390/antibiotics10050568
25. Adekanmbi, A. O., and Falodun, O. I. Heavy metals and antibiotics susceptibility profiles of *Staphylococcus aureus* isolated from several points receiving daily input from the Bodija abattoir in Ibadan, Oyo State, Nigeria. Adv Microbiol. 2015; 871-880. doi:10.4236/aim.2015.513091
26. Ogbonna, D. N., and Inana, M. E. Characterization and Multiple Antibiotic Resistance of Bacterial Isolates Associated with Fish Aquaculture in Ponds and Rivers in Port Harcourt, Nigeria. J Adv Microbiol. 2018; 10(4): 1-14. doi:10.9734/jamb/2018/41073
27. Fadel, A., Mabrok, M., and Aly, S. Epizootics of *Pseudomonas anguilliseptica* among cultured seabream (*Sparus aurata*) populations: Control and treatment strategies. Microb Pathog. 2018; 121:1–8.doi:10.1016/j.micpath.2018.04.021
28. Derome, N., Gauthier, J., Boutin, S., and Llewellyn, M. Bacterial opportunistic pathogen in fish. In: Hurst, C. J. (ed). The rasputin effect: When commensals and symbionts become parasitic. Adv Environ Microbiol. 2016: 81-108 doi:10.1007/978-3-319-28170-4
29. Duman, M., Mulet, M., Altun, S., et al. The diversity of *Pseudomonas* species isolated from fish farms in Turkey. Aquaculture. 2021; 1: 1-14.
30. Ginovyan, M., Hovsepyan, V., Sargsyan, M., Grigoryan, K., and Thrchunyan, A. Antibiotic resistance of *Pseudomonas* species isolated from Armenian fish farms. Aquaculture. 2017; 1: 163-173 doi:10.1016/j.aquaculture.2021.736369
31. Schar, D., Klein, E. Y., Laxminarayan, R., Gilbert, M., and Boeckel, T. P. V. Global Trends in Antimicrobial Use in Aquaculture. Sci Rep. 2020; 10:1-9. doi:10.1038/s41598-020-78849-3.
32. Ahmad, S. I., Malak, H. A., and Abulreesh, H. H. Environmental antimicrobial resistance and its drivers: a potential threat to public health. J Glob Antimicrob Resist. 2021; 27: 101-111. doi:10.1016/j.jgar.2021.08.001
33. Litwin, A., Rojek, S., Gozdzik, W., and Duszynska W. *Pseudomonas aeruginosa* device associated-healthcare associated infections and its multidrug resistance at intensive care unit of University Hospital: polish, 8.5-year, prospective, single-centre study. BMC Infect Dis. 2021; 21: 1- 8. doi:10.1186/s12879-021-05883-5
34. Pfaller, M. A., Shortridge, D., Harris, K. A., et al. Ceftolozane-tazobactam activity against clinical isolates of *Pseudomonas aeruginosa* from ICU patients with pneumonia: United States, 2015-2018. Int J Infect Dis. 2021; 112: 321–326 doi:10.1016/j.ijid.2021.09.064
35. Igbinosa, E. O., Odjadjare, E. E., Igbinosa, I. H., Orhue, P. O., Omoigberale, M. N. O., and Amhanre, N. I. Antibiotic synergy interaction against multidrug-resistant *Pseudomonas aeruginosa* isolated from an abattoir effluent environment. Sci World J. 2012; 1: 1-16. doi:10.1100/2012/308034
36. Hassuna, N. A., Darwish, M. K., Sayed, M., and Ibrahem, R. A. Molecular epidemiology and mechnisms of high-level resistance to meropenem and imipenem in *Pseudomonas aeruginosa.* Infect Drug Resist. 2020; 13: 285- 293. doi:10.2147/IDR.S233808
37. Mirzaei, B., Bazgir, Z. N., Goli, H. R., Iranpour, F., Mohammadi, F., and Babaei, R. Prevalence of multi-drug resistant (MDR) and extensively drug resistant (XDR) phenotypes of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolated in clinical samples from Northeast of Iran. BMC Res Notes. 2020; 13: 1-6. doi:10.1186/s13104-020-05224-w
38. Jia, X., Ma, W., He, J., et al. Hetero-resistance to cefepime in *Pseudomonas aeruginosa* bacteraemia Int J Antimicrob Agents. 2020; 55 (3): 1-9. doi:10.1016/j.ijantimicag.2019.10.013
39. Kousovista, R., Athanasiou, C., Liaskonis, K., Ivopoulou, O., and Karalis, V. Association of Antibiotic Use with the Resistance Epidemiology of *Pseudomonas aeruginosa* in a Hospital Setting: A Four-Year Retrospective Time Series Analysis. Sci Pharmaceut. 2021; 13: 1-12. doi:10.3390/scipharm89010013
40. Badulla, W. F. S., Alshakka, M., and Ibrahim, M. I. M. Antimicrobial resistance profiles for different isolates in Aden, Yemen: A cross-sectional study in a resource-poor setting. Biomed Res Int. 2020; 1:1-8.doi:10.1155/2020/1810290.
41. Mahfoud, M., Al-Najjar, M., and Hamzeh, A. R. Multidrug resistance in *Pseudomonas aeruginosa* isolated from nosocomial respiratory and urinary infections in Aleppo, Syria. J Infect Dev Ctries, 2015; 9 (02): 210-213. doi:10.3855/jidc.5643.
42. Ladadweh, H. E., Falana, H. H., Ma'ali, J. M., Aweis, P. A., Nofal, H. N., and Naseef, H. A. Antimicrobial resistance pattern of *Pseudomonas aeruginosa* from different clinical specimens: Survey article. Am J Pharmacol Toxicol. 2021; 16:1-9.doi:10.3844/ajptsp.2021.1.8
43. Kathleen, M. M., Samuel, L., and Felecia, C. Antibiotic resistance of diverse bacteria from aquaculture in Borneo. Int J Microbiol. 2016; 20:169. doi:10.1155/2016/2164761
44. Lihan, S., Lee, S. Y., Toh, S. C., and Leong, S. S. Plasmid-mediated antibiotic resistant *Escherichia coli* in Sarawak Rivers and aquaculture farms, Northwest of Borneo. Antibiotics. 2021; 10: 1-16. doi:10.3390/antibiotics10070776
45. Chibuike, K. U., Iroha, I. R., Moses, I. B., et al. Phenotypic screening of multidrug-resistant *E. coli* from water and fish collected from different fish farms within Abakaliki metropolis, Nigeria. Sci Res Essay. 2021; 16 (2):15-19 https://doi.org/10.5897/SRE2020.6705