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Prevalence and antibiogram of pseudomonas species isolated from selected African catfish (Clarias Gariepinus) ponds in Owo Metropolis

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Abstract
One of the most common sources of protein globally is catfish which are artificially reared in Nigeria. *Pseudomonas* species are opportunistic in nature, pathogenic Gram negative bacteria and may build multidrug resistant which can cause a serious threat to public health when present in environmental samples. This research was meant to determine the antibiogram of *Pseudomonas* species and its prevalence isolated from catfish ponds in Owo metropolis. The pond's water samples were aseptically collected from three ponds at three different locations in Owo. Centrimide Agar was used for the Isolation of *Pseudomonas* species, as well as Nutrient Agar, MacConkey Agar, and Eosin Methylene Blue Agar were used for their morphological and cultural characteristics, and the isolates were therefore characterized. Kirby Bauer disc diffusion method was adopted to carry out Antibiogram of the isolates. The isolates indicated different morphological and cultural characteristics. Prevalence of the *Pseudomonas* species reported were: 43%, 33% and 23% accordingly. The isolates were susceptible to the carbapenem (imipenem, 100%), monobactam (aztreonam, 96%), nitrofuran (nitrofurantoin, 93%), aminoglycosides (gentamicin, 90%), cephems (cefotaxime, 90%, ceftazidime, 93%, cefuroxime, 86%), fluoroquinolones (ciprofloxacin, 70% and ofloxacin, 63%) respectively. meanwhile, the susceptibility of the isolates to beta-lactam combination appeared to be low (augmentin, 30%), while carbapenem (Imipenem) was the most highly active antibiotic, followed by the monobactam (aztreonam). This research revealed that *Pseudomonas* species were common in catfish ponds and were differently resistant to some of the tested antibiotics, thus poses threat to public health which raises the need for serious monitoring of catfish ponds.

Keywords: *Pseudomonas* species, catfish ponds, prevalence, antibiogram, public health

Introduction

Fish is considered highly nutritional which contains fat, high level of proteins and minerals commonly sodium calcium, potassium, magnesium and phosphorous (Aliyu *et al*., 2016) [7]. In Nigeria, fish gives more protein than other animals. The fact that it is accepted without any religious bias globally, increases its level of consumption. (Aliyu *et al*., 2016) [7]. Therefore, different researches on fish safety triggered consumers fear as regards fish and fish products, for example, fish-borne diseases by pathogenic microorganisms. (Li *et al*., 2019) [32]. Fishes are commonly raised in different water confinement e.g. earthen pots, concrete, and plastics ponds leaving the Concrete and earthen ponds as the most established method of fish culture in Nigeria. Fishes raised using these methods are mostly affected by pathogenic and opportunistic microorganisms. This contamination has been attributed to poor water quality, stock densities and contaminated animal manure feeds (Abdel-Wahed *et al*., 2018) [1]. farmers usually use animal manure as supplement in order to reduce cost of feeding, (Abdel-Wahed *et al*., 2018) [1].

Pseudomonads are one of the pathogens that induce hemorrhagic septicemia and ulcerative syndrome (Eissa *et al*., 2010) [19], which are present in aquatic animals environments. (Austin and Austin, 2007). They are present in fish tissues as well as gills, skin and intestine of fishes (Lavilla *et al*. 2014) [31]. They are common opportunistic pathogens in fish ponds and can be a causative of various fish diseases (Tripathy *et al*. 2007) [44]. Report shows that it is one of the most common infectious agents of reared fish and has been known for its stress-related diseases in freshwater fish. (Derome *et al*., 2016) [17]. *Pseudomonads* are responsible for economic losses worldwide as one of the bacterial pathogens affecting a large number of aquatic animals. The economic losses are majorly related to high death rate, poor growth and poor flesh quality. (El-Sayed *et al*., 2019) [20]. *Pseudomonas* species are commonly use in aquaculture as probiotic organisms, due to their ability to suppress mycological and bacterial agents (Liu *et al*., 2015). pigment production is the major phenotypic characteristics of the pseudomonas such as fluorescein or pyocyanin, and oxidase-positivity, but the identification of non-pigmented or oxidase-negative species by biochemical methods is difficult (Palleroni, 2005) [38]. The identification of *Pseudomonas* spp. is important for correct diagnosis in aquaculture (Fadel *et al*., 2018) [21]. Even though *Pseudomonas* spp. has been described as opportunistic pathogens, many other species have also been described as part of the primary pathogen of several diseases in cultured fish, including *P. putida, P. aeruginosa, P. baetica, P. chlororaphis, P. koreensis, P. luteola, P. plecoglossicida, P. fluorescens, P. pseudoalcaligenes* and *P. anguilliseptica* (Derome *et al*., 2016) [17].

The use of Antibiotics to prevent and treat animal diseases in most of the livestock production systems in order to improve the efficiency of animal production is almost endemic. (Abdul *et al*., 2014) [2]. Antibiotics are quickly excreted from the animal, others are not readily metabolized. Therefore, their residues will continue to exist in the animal tissues and then enter the human body through consumption, causing health challenges (Abdul *et al*., 2014) [2]. Antibiotic resistance became more challenging to human health since late 20th century (Ginovyan *et al*. 2015) [24]. Antimicrobial resistance can surface as a result of administration of antibiotics in human therapy and animal agriculture (CDC, 2013) [13] leading to the emphasis of the necessity to monitor and control its usage, (World Health Organisation, 2006).

Aquaculture is widely practiced in the whole wide world; mostly in the developing countries where most farmers lack information about precautionary measures of antibiotics usage and Government regulation (FAO, 2016) [22]. The release of wastewater into the environment has been reported to be a source of contamination. The spread of resistance agents to aquatic environment and soil organisms, have been reported as a result of release of untreated waste water. (Adelowo *et al.,* 2009) [5]. The major risk is the widening of storage of the antibiotic genes and bacteria resistant when wastewater penetrates into groundwater.

Prevalence and antibiogram of Pseudomonas species from fish ponds are of paramount concern in this study as they are opportunistic pathogens which pose a threat not only to catfish health but to humans who consume them. Currently in Owo metropolis, there is paucity of information on the prevalence and antibiogram of *Pseudomonas* species from fish ponds and its possible transmission to human hence, the need to determine the prevalence and antibiogram of *Pseudomonas* species isolated from catfish ponds in Owo metropolis.

Materials and methods

Sample collection

Between October and November 2022, three catfish ponds (one concrete, one plastic tank and one earthen) water samples were aseptically collected (four samples were collected per catfish pond making a total of 12 samples) early in the morning (between 7:00am and 9:00am) at approximately 30cm below the fish ponds water surface (CLSI, 2018) [16] into appropriately labeled sterile glass bottles protected with an aluminium seal and/ or a teflon cap, completely filled, and kept at 4^{0}C in icebox containing ice during transportation to the Microbiology Unit laborato, department of Science Laboratory Technology, Rufus Giwa Polytechn Owo for immediate processing.

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

Iml of the homogenized collected catfish pond water sample was drawn and tenfold serial dilution was carried out. 1ml each of the serial diluents (10^{-2} and 10^{-4}) was dispensed into appropriately labeled three 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) [18]. The production of yellowish-green fluorescent pigment on Centrimide agar is commonly associated with Pseudomonads (Lamonth and Martins, 2003). 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, 2005) [14].

Maintenance of bacteria stock culture

Each of the isolated *Pseudomonas* species isolates were maintained as stock cultures for further studies on nutrient agar (Oxoid, UK) slants in Bijou bottles. This was done by streaking the isolates on the agar slants, and incubating at 37^{0}C for 24 hrs. After incubation, the inoculated slants were stored in the refrigerator at ambient temperature and these stock cultures served as source of *Pseudomonas* species isolates for further bacteriological studies.

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

This was carried out according to the method of (Becerra *et al*, 2016) [10]

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, oxidase, citrate, indole, Methyl Red, Voges-Proskauer and carbohydrate utilization which include glucose, lactose and maltose.

Preparation and standardizing inoculum suspension

The inoculum suspension was prepared by picking 2-3 colonies of a 24-hour culture with asterile wire loop and was suspended in 5M normal saline, the suspension was mixed with a vortex mixer. The turbidity of the suspension was standardized to match the 0.5 McFarland's standard which corresponds to approximately 1.5x 108 cfu/ml and this was done by comparing the test suspension with barium sulphate suspension by placing the tubes in front of a white paper with black lines.

Antimicrobial susceptibility test of the *Pseudomonas* species

This was done using the standard Kirby-Bauer disk diffusion (Jayabarath, 2015) [28]. The *Pseudomonas* species inoculum was prepared by suspending the freshly grown bacteria in 5 ml sterile nutrient broth and its turbidity adjusted to 0.5 McFarland standards. 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 37°C for 24 h. 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 (2018) [16]. *Pseudomonas aeruginosa* strain ATCC 27853 was used as controls.

Results

Table 1: Morphological, cultural and staining characteristics of the isolates from the catfish ponds

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 catfish ponds

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 catfish ponds

Sampling site	No. of samples collected	No. of *Pseudomonas* species isolated	Prevalence of *Pseudomonas* species (%)
C	4	10	33.3
E	4	13	43.3
T	4	7	23.3
Total	16	30	100.0

KEY: C = Concrete pond, E = Earthen pond, T = Plastic tank pond

Table 4: Antibiotic susceptibility patterns of all the *Pseudomonas* species (n = 30) isolated from catfish ponds

Group 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	9 (30.0)	21 (70.0)
Cephem	Cefotaxime	CTX	30	≥ 21	≤ 14	27 (90.0)	3 (10.0)
	Ceftazidime	CAZ	30	≥ 18	≤ 14	28 (93.3)	2 (6.7)
	Cefuroxime	CRX	5	≥ 16	≤ 20	26 (86.7)	4 (13.3)
Carbapenem	Imipenem	IMP	10	≥ 19	≤ 15	30 (100.0)	0 (0.0)
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	19 (63.3)	11 (36.7)
Monobactam	Aztreonam	ATM	30	≥ 22	≤ 15	29 (96.7)	1 (3.3)
Nitrofuran	Nitrofurantoin	NIT	300	≥ 17	≤ 14	28 (93.3)	2 (6.7)

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

Table 5: Antibiotype of pseudomonas species isolated from catfish ponds

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

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

Discussion

Fish farming business in artificial ponds is a common practice in Owo environs as a means of maintaining regular supply. Bacterial infection also attacks Fish in their natural home but the rate of this infestation is highly dependent on some factors such as environmental and climatic factors and the species of fish (Hossain *et al*., 2013) [27]. This study was aimed at determining the prevalence and the antibiogram of *Pseudomonas* species isolates recovered from catfish ponds in Owo metropolis, Ondo State, Nigeria.

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 is the same with the reports suggested previously by other researchers such as (Ogbukagu *et al*., 2021) [36]

All the used isolates were positive to motility, citrate and catalase but negative to indole methyl red and voges Prauskaur test, gas (CO_2 and H_2S) production were therefore categorized as *Pseudomonas* species. This report is in conformity with the specific biochemical characters of *Pseudomonas* species that was reported by other researchers (Abedin *et al*., 2020) [3]. Certain variation was observed in the prevalence of *Pseudomonas* species isolated from the three ponds used: concrete (33%), earthen (43%) and plastic tank (23%). Usually, this may be as a result of different pond used, the water and management practices. This report is in line with the report of Adah *et al*. (2020) [4] that suggested higher bacterial load in the gills of fish (*C. gariepinus)* reared in earthen ponds compared to plastic tanks. The sources of water used could be a determing factior for the highest prevalence of *Pseudomonas* species found in earthen pond (43%). Dams, streams, rivers, lakes and runoff water which serves as sources of earthen ponds are usually untreated, while, the sources of concrete and plastic tanks are underground water (Mustapha, 2017) [34]. Therefore the rate at which the water is be changed in different holding facilities also affects the microbial load, mostly in concrete and plastic tanks where water can be drained completely and replaced with new water. Meanwhile, in earthen ponds more water is being added to maintain a certain volume of water. More so, over feeding and faeces also increase bacteria load in fish holding facilities (Olojo *et al*., 2010) [37].

All the *Pseudomonas* species isolated from catfish ponds in this research were subjected to ten antibiotics belonging to seven classes: beta-lactam combination agent (augmentin), cephem (cefotaxime, ceftazidime, and cefuroxime), carbapenem (imipenem), aminoglycosides (gentamicin), fluoroquinolone (ciprofloxacin, ofloxacin), monobactam (aztreonam) and nitrofurans (nitrofurantoin)

Among the ten antibiotics, complete susceptibility (100%) of all the *Pseudomonas* species was found with imipenem. This is in conformity with the earlier report of (Nasreen *et al.* (2015) [35]. all the *Pseudomonas* species was highly susceptible to aztreonam (96%), nitrofurantoin and ceftazidime respectively (93%), gentamicin and cefotaxime respectively (90%), cefuroxime (86%) and ciprofloxacin (70%). This study is in conformity with other report which reported high level of susceptibility to *Pseudomonas* species tofluoroquinolones (ciprofloxacin and ofloxacin), cephem (ceftazidime) and aminoglycosides (gentamicin) respectively (Senbadejo and Fagade, 2017) [41].

The high resistance to augmentin (70%) in this study, is lower compared with the report of Adewoye and Lateef (2004) [6] both of whom reported high resistance (100%) to augmentin. The moderate resistance of the isolates of this study to ciprofloxacin (30%) is in concordance with 33% resistance to same antibiotic reported by Adah *et al*. (2020) [4].

The observed resistance of the isolated *Pseudomonas* species in this study to combinations of antibiotics are similar to the previous report by Amos *et al.* (2023) [8] who isolated identified and determined the antimicrobial susceptibility pattern of *Pseudomonas* species isolated from pig feaces in Owo metropolis. As reported by Piddock (1996) [39], the consumption of products with MDR bacteria could be risky to consumers.

Conclusion and recommendation

This present study reported varying prevalence of *Pseudomonas* species in catfish ponds sample which suggested that the isolates could lower catfish yield, and equally endanger the ultimate consumers (humans) hence the needs to give attention to catfish ponds. This in such is suggestive of misuse, abuse of the antibiotics in the catfish production. This study also suggested that imipenem could be used to treat catfish infected with *Pseudomonas* species. Consequently, Improving the sanitary conditions of the catfish ponds among catfish workers is urgently needed as well focus on alternative control strategies for *Pseudomonas* infections in catfish without the use of antibiotic should be encouraged. Government at all tiers should endeavor to sponsor researchers on development of new antibiotics that could be relevant in the treatment of severe infections caused by antibiotic resistant *Pseudomonas* species.

References

1. Abdel Wahed RK, Shaker IM, Elnady MA, Soliman MAM. Impact of Fish farming Management on Water Quality, Plankton Abundance and Growth Performance of Fish in Earthen Ponds. Egyptian Journal of Aquatic Biology and Fisheries,2018:22:49–63.
2. Abdul SM, Muhammad K, Aijaz HS, Muhammad GS. Antibiotic residues detection in raw beef meat sold for human consumption in sindh, Pakistan. International Journal of Research in Applied, Natural and Social Sciences,2014:2:15-20.
3. Abedin MZ, Aktar MB, Zaman MSU, Hasan R, Jarin L, Karim MR, *et al*. Occurrence and antimicrobial susceptibility profiling of bacteria isolated from cultured Pangas Catfish (*Pangasiuspangasius*) and Climbing Perch (*Anabas testudineus*) Fishes. Journal of Marine Biology and Aquaculture,2020:6(1):7-12.
4. Adah DA, Saidu L, Oniye SJ, Adah SA, Daodu OB, David SM, *et al*. Microbiota of gills and antibiotic susceptibility patterns of bacteria isolates from *Clarias gariepinus* in different holding facilities. Sokoto Journal of Veterinary Sciences,2020:18(3):119-128.
5. Adelowo OO, Ojo FA, Fagade OE. Prevalence of multiple antibiotic resistances among bacterial isolates from selected poultry waste dumps in South-western Nigeria. World Journal of Microbiology and Biotechnology,2009:25:713–719.

6. Adewoye SO, Lateef A. Assessment of the microbiological quality of Clariasgariepinus exposed to an industrial effluent in Nigeria. Environmentalist,2004:24:249-254.
7. Aliyu A, Ibrahim YK, Oyi RA. Bacteriological and elemental quality of *Clarias gariepinus* (cat fish) samples from River Lavun, Bida Niger State, Nigeria. Nigerian Journal of Pharmaceutical Research,2016:12(2):139-147.
8. Amos AAO, Temitope AA, Elizabeth OA. Isolation, identification and antimicrobial susceptibility profile of Pseudomonas species isolated from pig (Susscrofa) feces in owo metropolis. Journal of Advanced Education and Sciences,2023:3(2):44-49.
9. Austin B, Austin DA, Austin B, Austin DA. Pseudomonads. Bacterial fish pathogens: disease of farmed and wild fish, 2016, 475-498.
10. Becerra SC, Roy DC, Sanchez CJ, Christy RJ, Burmeister DM. An optimized staining technique for the detection of Gram positive and Gram negative bacteria with in tissue. BMC research notes,2016:9(1):1-10.
11. Buschmann AH, Tomova A, López A, Maldonado MA, Henríquez LA, Ivanova L, *et al*. Salmon aquaculture and antimicrobial resistance in the marine environment, 2012.
12. Cabello FC. Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. Environmental microbiology,2006:8(7):1137-1144.
13. Centers for Disease Control and Prevention (CDC). Antibiotic resistance threats in the United States. Current, 2013, 114.
14. Cheesbrough M. District laboratory practice in tropical countries, part 2. Cambridge university press, 2005.
15. Chuah LO, Effarizah ME, Goni AM, Rusul G. Antibiotic application and emergence of multiple antibiotic resistance (MAR) in global catfish aquaculture. Current environmental health reports,2016:3:118-127.
16. Clinical and Laboratory Standard Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. Twenty-eighth Information Supplement CLSI document M100-S26. Wayne, P.A,2018:38(3):1-296.
17. Derome N, Gauthier J, Boutin S, Llewellyn M. Bacterial opportunistic pathogens of fish. The Rasputin effect: When commensals and symbionts become parasitic, 2016, 81-108.
18. Egea P, López Cerero L, Torres E, del Carmen Gómez Sánchez M, Serrano L, Sánchez Ortiz MDN, *et al*. Increased raw poultry meat colonization by extended spectrum beta-lactamase-producing *Escherichia coli* in the south of Spain. International journal of food microbiology,2012:159(2):69-73.
19. Eissa NME, El Ghiet EA, Shaheen AA, Abbass A. Characterization of Pseudomonas species isolated from tilapia "Oreochromisniloticus" in Qaroun and Wadi-El-Rayan lakes, Egypt. Global Veterinaria,2010:5(2):116-121.
20. El Sayed M, Algammal A, Abouel Atta M, Mabrok M, Emam A. Pathogenicity, genetic typing, and antibiotic sensitivity of Vibrio alginolyticus isolated from Oreochromisniloticus and Tilapia zillii. Rev Med Vet,2019:170:80-86.
21. Fadel A, Mabrok M, Aly S. Epizootics of *Pseudomonas anguilliseptica* among cultured seabream (Sparusaurata) populations: Control and treatment strategies. Microbial pathogenesis,2018:121:1-8.
22. Food and Agriculture Organization (FAO). The State of World Fisheries and Aquaculture 2016; Food and Agriculture Organization of the United Nations: Rome, 2016.
23. Ganguly NK, Arora NK, Chandy SJ, Fairoze MN, Gill JPS, Gupta Ul. Rationalizing antibiotic use to limit antibiotic resistance in India. Indian Journal of Medical Resource,2011:134(3):281–94.
24. Ginovyan M, Keryan A, Bazukyan I, Ghazaryan P, Trchounian A. The large scale antibacterial, antifungal and anti-phage efficiency of Petamcin-A: new multicomponent preparation for skin diseases treatment. Annals of clinical microbiology and antimicrobials,2015:14(1):1-7.
25. Heuer OE, Kruse H, Grave K, Collignon P, Karunasagar I, Angulo FJ. Human health consequences of use of antimicrobial agents in aquaculture. Clinical Infectious Diseases,2009:49(8):1248-1253.
26. Holmström K, Gräslund S, Wahlström A, Poungshompoo S, Bengtsson BE, Kautsky N. Antibiotic use in shrimp farming and implications for environmental impacts and human health. International journal of food science & technology,2003:38(3):255-266.
27. Hossain MG, Saha S, Rahman MM, Singha JK, Mamun AA. Isolation Identification and Antibiogram Study of *Pseudomonas Aeruginosa* from Cattle in Bangladesh. Journal of veterinary advances,2013:3(7):180-185.
28. Jayabarath J, Joseline JJ, Gobika K. Isolation and Characterization of Antibiotic Resistant Bacteria from Lutjanuscampechanus. World Journal of Pharmaceutical Research,2015:5:4-8.
29. Kümmerer K. Antibiotics in the aquatic environment–a review–part I. *Chemosphere*,2009:75(4):417-434.
30. Lamont IL, Martin LW. Identification and characterization of novel pyoverdine synthesis genes in *Pseudomonas aeruginosa*. Microbiology,2003:149(4):833-842.
31. LavillaLerma L, Benomar N, Casado Munoz MDC, Gálvez A, Abriouel H. Antibiotic multi-resistance analysis of mesophilic and psychrotrophic Pseudomonas spp. isolated from goat and lamb slaughterhouse surfaces throughout the meat production process. Applied and Environmental Microbiology,2014:80(21):6792-6806.
32. Li Y, Pei X, Yan J, Liu D, Zhang H, Yu B, *et al*. Prevalence of foodborne pathogens isolated from retail freshwater fish and shellfish in China. Food Control,2019:99:131-136.
33. Marshall BM, Levy SB. Food animals and antimicrobials: impacts on human health. Clinical microbiology reviews,2011:24(4):718-733.
34. Mustapha MK. Comparative assessment of the water quality of four types of aquaculture ponds under different culture systems. Advanced research in life sciences,2017:1(1):104-110.
35. Nasreen M, Sarker A, Malek MA, Ansaruzzaman MD, Rahman M. Prevalence and resistance pattern of Pseudomonas aeruginosa isolated from surface water. Advances in Microbiology,2015:5(01):74.

36. Ogbukagu CM, Anaukwu CG, Ekwealor CC, Mba AN, Ekwealor IA. Bacteriological Assessment and Antibiotic Susceptibility Profile of Bacteria Recovered from *Clarias gariepinus* Selected from Various Fish Farms in Anambra North Senatorial Zones in Anambra State, Nigeria. Advances in Microbiology,2021:11:243-256.
37. Olojo EAA, Amusa NA, Osho A, Badejo VO. Commensal Bacterial Flora of *Synodontis nigrita* and *Ciarias gariepinus* from River Osun, Southwest Nigeria, Nigeria. Res J Appl Sci,2010:5(3):231-235.
38. Palleroni NJ. Genus I *Pseudomonas Migula* 1894, 237. Bergey's manual of systematic bacteriology, 2005, 323-378.
39. Piddock LJ. Does the use of antimicrobial agents in veterinary medicine and animal husbandry select antibiotic-resistant bacteria that infect man and compromise antimicrobial chemotherapy?. The Journal of antimicrobial chemotherapy,1996:38(1):1-3.
40. Romero J, Feijoó CG, Navarrete P. Antibiotics in aquaculture–use, abuse and alternatives. Health and environment in aquaculture,2012:159:159-198.
41. Senbadejo TY, Fagade OE. Antibiotic susceptibility patterns of Pseudomonas species isolated from clinical and environmental samples in Awba Dam, Ibadan. Fountain Journal of Natural and Applied Sciences, 2017, 6(2).
42. Shah SQ, Colquhoun DJ, Nikuli HL, Sørum H. Prevalence of antibiotic resistance genes in the bacterial flora of integrated fish farming environments of Pakistan and Tanzania. Environmental science & technology,2012:46(16):8672-8679.
43. Sorum H, Aarestrup FM. Antimicrobial resistance in bacteria of animal origin. Antimicrobial drug resistance in fish pathogens, 2006, 213-238.
44. Tripathy S, Kumar N, Mohanty S, Samanta M, Mandal RN, Maiti NK. Characterization of *Pseudomonas aeruginosa* isolated from freshwater culture systems. Microbiological Research,2007:162(4):391-396.
45. World Health Organization. World Health Organization. Antimicrobial Resistance—Global Report on Surveillance, 2014.
46. World Health Organization. Report of a joint FAO/OIE/WHO Expert Consultation on antimicrobial use in aquaculture and antimicrobial resistance, Seoul, Republic of Korea, 2006, 13–16.