TESTING THE EFFICACY OF TWO ANTIBIOTICS, (TETRACYCLINE AND AMOXICILLIN) ON BACTERIA ISOLATED FROM THE INTESTINE OF WILD AFRICAN CATFISH (CLARIAS GARIEPINUS BURCHELL, 1822) FROM AJIWA RESERVOIR, KATSINA STATE, NIGERIA

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ABSTRACT

Antibiotics are powerful medicines that fight certain infections. However, illegal dumping of human and livestock wastes into water bodies has severe consequences, including the development of antibiotic-resistant bacteria. This study isolated and identified bacteria from the intestine of wild Clarias gariepinus (African catfish) from the Ajiwa reservoir. Nine fish samples (W1-W9) yielded five different bacteria species: Pseudomonas aeruginosa, Escherichia coli, Cytrobacter freundii, Acinetobacter baumannii, and Klebsiella pneumoniae. Antibiotic effectiveness was determined using Kirby-Bauer disc diffusion. All isolated bacteria showed 100% effectiveness to Tetracycline, except one Pseudomonas spp (W3), which showed 13.5% effectiveness. However, all isolates were 100% resistant to Amoxicillin. These results suggest that Tetracycline has minimal interference, while Amoxicillin has abundant resistance. The introduction of multi-antibiotic-resistant bacteria from terrestrial environments poses a significant threat to aquatic ecosystems and human health.

KEYWORDS

Antibiotic resistance, Bacteria isolation, Tetracycline, Amoxicillin, Clarias gariepinus

1. INTRODUCTION

Aquatic ecosystems, particularly those around farmlands in Nigeria, face significant threats from bacterial pathogens that affect fish populations (Lipp & Ross, 1997; Olufemi & Akinwumi, 2020). *Clarias gariepinus* (African catfish), is a vital food source, and is susceptible to various bacterial infections (Austin, 2011; Adeogun et al., 2022). These infections can lead to significant economic losses, reduced fish production, and increased risk of antibiotic resistance transfer to humans (FAO/NACA/WHO, 1997; CDC, 2022). The misuse of antibiotics has accelerated the emergence of antibiotic-resistant bacteria, posing a significant threat to aquaculture and public health (HHS, 1999; WHO, 2020). Antibiotic-resistant bacteria have been isolated from fish, including catfish (De Paola et al., 1995; Ibrahim et al., 2022). The development of antibiotic resistance is further complicated by factors such as poor water quality, inadequate sanitation and hygiene practices, and lack of effective regulations (Okoro et al., 2020). Tetracycline and Amoxicillin are widely used antibiotics in animal husbandry and aquaculture (Kuhne et al., 2000; Soge et al., 2022). However, their indiscriminate use has led to the development of resistant bacterial strains (Van den Bogaard & Stobberingh, 2000; Okoro et al., 2020). Understanding the antibiotic resistance profile of bacteria in wild *Clarias gariepinus* is crucial for developing effective treatment strategies and mitigating the risk of antibiotic resistance transfer to humans. This study aims to investigate the efficacy of Tetracycline and Amoxicillin against bacteria isolated from the intestine of wild *Clarias gariepinus* from Ajiwa Reservoir, Katsina State, Nigeria. The findings will contribute to the development of informed antibiotic use policies in aquaculture and promote sustainable aquatic ecosystem management in the state and Nigeria at large.

2. MATERIALS AND METHODS

2.1. Study Area

Ajiwa reservoir is located in the Batagarawa local government area of Katsina state, Nigeria, at latitude and longitude 12°54'69" - 12°57'58" N and 7°42'53" - 7°47'50" E, respectively (Parkman and Haskoning, 1996).

2.2. Sample Collection

Nine African catfish (*Clarias gariepinus*) specimens were collected from Ajiwa Reservoir, Kadaji landing site, Batagarawa Local Government Area, Katsina State. The collection was conducted in June, 2024 and in the morning to minimize stress on the fish. Immediately after collection, the fish were transported to the Microbiology Laboratory, Aminu Kano Teaching Hospital (AKTH), Kano State, in a plastic bucket containing reservoir water to maintain their natural environment.

2.3. Bacterial Isolation

The African catfish (*Clarias gariepinus*) specimens were dissected using a sterile scalpel, and the contents were collected and stored in sterile containers labeled W1-W9. Each intestinal sample was inoculated onto nutrient agar plates using a sterile wire loop. The plates were incubated at 37 °C for 18-24 hours to facilitate bacterial growth.

2.3.1.Bacterial Identification

Bacteria isolated from the collected fish were identified using morphological characteristics and biochemical tests (Fish and Service, 2010; MacFaddin, 2000). Colony morphology was examined, noting; shape, color, pigmentation, hemolytic activity, size, edges, and elevation. Gram staining was performed to differentiate isolates into Gram-negative and Gram-positive bacteria. Isolates requiring further identification were observed under a binocular microscope at ×100 magnification. Selective media, MacConkey agar, was used for subculturing. Biochemical tests, including urease, citrate oxidase, and triple sugar iron (TSI), were conducted for additional differentiation.

2.4. Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the disc diffusion method, as described by Hudzicki (2009). Twelve isolates (W1, W2-1, W2-2, W3, W4, W5-1, W5-2, W6, W7-1, W7-2, W8, and W9) were tested against tetracycline (30 µg) and amoxicillin (10 µg).

2.4.1. Preparation of Inoculum

Colonies from each isolate were emulsified in 0.85% sodium chloride to create a suspension matching the 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL).

2.4.2. Disc Diffusion Assay

The suspension was inoculated onto Mueller-Hinton agar and spread using a sterile loop. After drying (2-5 minutes), antibiotic discs were applied using a sterile forcep. Plates were incubated at 37 °C for 18-24 hours.

2.4.3. Interpretation of Results

Zones of inhibition were measured using a ruler. Isolates were classified as resistant (R),

intermediate (I), or **susceptible (S)** based on CLSI guidelines (CLSI, 2006; CLSI, 2015):

Amoxicillin: ≥17 mm (susceptible), 14-15 mm (intermediate), ≤13 mm (resistant)

Tetracycline: ≥15 mm (susceptible), 12-14 mm (intermediate), ≤11 mm (resistant

3. Results and Discussion

A total of 12 bacteria had been isolated and identified from nine samples collected. they are as follows, (W1, W2-1, W3, W4, W5-1, W6, W7-2, W9) *Pseudomonas aeruginosa* as appeared in eight (8) samples and (W2-2) *Escherichia coli,* (W5-2) *Cytrobacter freundii,* (W7-1) *Acinetobacter baumannii,* and (W8) *Klebsiella pneumoniae*. As shown in table 1.

Table 1. Biochemical Characterization of the Bacterial Isolates

S/N	MAC	Gram	Oxidase	Citrate	Urease	Triple Sugar Irons				Isolates
						Butt	Slant	H2S	Gas	
W1	NLF	GNB	+	+	+	R	R	-	-	*Pseudomonas aeruginosa*
W2-1	NLF	GNB	+	+	+	R	R	-	-	*Pseudomonas aeruginosa*
W2-2	LF	GNB	-	-	-	Y	Y	-	+	*Escherichia coli*
W3	NLF	GNB	+	+	+	R	R	-	-	*Pseudomonas aeruginosa*
W4	NLF	GNB	+	+	+	R	R	-	-	*Pseudomonas aeruginosa*
W5-1	NLF	GNB	+	+	+	R	R	-	-	*Pseudomonas aeruginosa*
W5-2	NLF	GNB	-	-	+	Y	Y	-	+	*Cytrobacter freundii*
W6	NLF	GNB	+	+	+	R	R	-	-	*Pseudomonas aeruginosa*
W7-1	LLF	GNCB	-	+	-	R	R	-	-	*Acinetobacter baumannii*
W7-2	NLF	GNB	+	+	+	R	R	-	-	*Pseudomonas aeruginosa*
W8	LF	GNB	-	+	+	Y	Y	+	-	*Klebsiella pneumoniae*
W9	NLF	GNB	+	+	+	R	R	-	-	*Pseudomonas aeruginosa*

Key: W= Wild fish, LF=Lactose fermenter, NLF = Non-Lactose Fermenter, LLF = Late Lactose Fermenter, GNB=Gram Negative Bacilli, R=Red, Y=Yellow, +=positive, -=Negative. Out of the twelve bacterial species obtained, *Pseudomonas aeruginosa* has the highest prevalence rate of 66.7%, with the rest of the isolates, *Escherichia coli, Cytrobacter freundii, Klebsiella pneumoniae,* and *Acinetobacter baumannii,* all having 33.3%. This study isolated a total of (five) 5 different bacteria species which also inline with the findings of Wamala *et al.,* (2018) who conducted similar research and also isolated *Klebsiella spp, Pseudomona florescence, Aeromonas hydrophila, Aeromonas sobria, Proteus spp, and Plesiomonas shigelloides* were isolated from intestine of wild *Clarias gariepinus* in Uganda. This research is also inline the work of Adekunle *et al.,* (2022) who isolated *Aeromonas caviae, Proteus mirabilis, Acinetobacter gerneri, Serratia rubidaea, Pseudomonas mosselii, Acinetobacter soli, and Klebsiella variicola* from milt of cultured *clarias gariepinus* in Lagos State. All the bacteria isolated in this study were gram negative bacteria, which is similar with the findings of Akaniro *et al.,* (2020) in Enugu state, which showed majority of the bacteria species were gram negative, which is in line with the finding of this study. Gram negative bacteria are known to cause serious disease in fish and those of varying significance as fish pathogens. (Joh *et al*. 2013)**.** ***Pseudomonas aeruginosa was*** the most dominant bacteria isolated in this study, which occurred in all samples, with the exception of sample W8, this differ from a research conducted by Wamala *et al.,* (2018) in Uganda where Aeromonas *spp* was the most common bacteria isolated. This Differences in the bacteria composition may be due to differences in the microbial composition of the aquatic habitat of the two different study area.

3.1. Antibiotic Effectiveness Test

Tetracycline showed the great activities (100.0%) against all the isolated bacteria species except in one specie (W3) of *Pseudomonas aeruginosa* that showed a sensitivity rate of (13.5%), while the same species, showed (100%) resistance to Amoxicillin.

Table 2. Antimicrobial Profile of Bacterial Species

S/N (30µg)	Isolates	NO.	Amoxicillin (10µg)			Tetracycline		
			S	I	R	S	I	R
1- 1(13.5%)	*P. aeruginosa*	8		0	0	8(100%)	7(87.5%)	0
2- 0	*E. coli*	1		0	0	1(100%)	1(100%)	0
3- 0	*K. pneumoniae*	1		0	0	1(100%)	1(100%)	0
4- 0	*C. freundii*	1		0	0	1(100%)	1(100%)	0
5- 0	*A. baumannii*	1		0	0	1(100%)	1(100%).	0

Resistant (R), Intermediate (I) or Susceptible (S).

All isolated bacteria from this study showed 100% effectiveness to tetracycline except one *Pseudomonas spp* that was isolated from sample (W3) Which Showed 13.5% level of effectiveness, Such high effective level of fish bacteria against antibiotics has been reported by (Wamala *et al.,* 2018) in Uganda where both *Aeromonas spp, and P.shigelloides* showed 100% effectiveness against Tetracycline Sulphamethoxazole-trimethoprim, Gentamycin, Streptomycin, Imipenem, Ceftriaxone, Chloramphenicol, Aztreonam, Nalidixic acid and Enrofloxacin, However, the same isolates in this study were 100% resistant to amoxicillin, which correspond with different authors reporting up to 100% resistance against various antibiotics such as Penicillin, Gentamycin, Streptomycin, Imipenem, Erythromycin, Ampicillin in fish farms (Newaj-Fyzul *et al.,* 2008; Sarter *et al.,* 2007; Castro-Escarpulli *et al.,* 2003). This high level of resistance of bacteria against antibiotics may be due to the transfer of resistant bacteria strains from human sewages and livestock waste to the water body or the naturally occurring resistant bacteria found in the aquatic environment and soils (Cantas *et al.,* 2013).

4. CONCLUSION

This study identified the possible bacteria in wild *Clarias gariepinus* in the Ajiwa reservoir and determined the effectiveness of antibiotics (Tetracycline and Amoxicillin) against the possible bacteria isolated. The bacteria isolates were highly effective against tetracycline, and totally resistant to amoxicillin, therefore Tetracycline is more effective than Amoxicillin for fish bacteria treatment in the Ajiwa reservoir. This information is vital for fish disease management in Ajiwa reservoir, as it provides baseline data for future reference in Katsina state and Nigeria at large.

REFERENCES

[1] Adekunle, F.A., Ayofe Mutalib, A.H., Akintade, O.A., Olufemi, O.W., Modupe, O.O., Olarinde, G.M., Joseph, J.H., Adeolu, A.A., and Adebimpe, O.O. (2022). Isolation and Identification of Bacteria Found in the Milt of Cultured Clarias gariepinus. Department of Fisheries, Faculty of Science, Lagos State University, Ojo, Lagos State, Nigeria. DOI: https://doi.org/10.30564/jfs.v4i2.4418.

[2] Adeogun, A. O., et al. (2022). Bacterial pathogens associated with Clarias gariepinus in Nigeria. Journal of Fish Diseases, 45(2), 257-265.

[3] Akaniro, I. R., Anumudu, O.H., Oweredaba, C.I., Koledowo, A.K., Egboka, K.C., and Ofonegbu, M.N. (2020). Isolation, Characterization and Antibiotic Resistance Profile of Bacteria from the Gut of African Catfish; Clarias Gariepinus. Journal of Scientific and Engineering Research, 7(3):188-195.

[4] Austin, B. (2011). Taxonomy of Bacterial Fish Pathogens. Vet. Res., 42 (1): 20.

[5] Cantas, L., Shah, S.Q., Cavaco, L.M., Manaia, C.M., Walsh, F., Popowska, M., Garelick, H., Burgmann, H., and Sorum, H. (2013). A brief multi-disciplinary review on antimicrobial resistance in medicine and its linkage to the global environmental microbiota. Front Microbial.

[6] Castro-Escarpulli, G., Figueras, M.J., Aguilera, G., Soler, L., Fernandez- Rendon, E., Aparicio, G.O., Guarro, J., and Chacon, M.R. (2003). Characterisation of Aeromonas spp. isolated from frozen fish intended for human consumption in Mexico. Int J Food Microbial. 84(1):41–9

[7] CDC (2022). Antibiotic Resistance Threats in the United States.

[8] CLSI, (2006). Methods for dilution of antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard M7-A7. 7th ed. Wayne: Clinical and Laboratory Standards Institute.

[9] CLSI, (2015). Methods for antimicrobial dilution and disk susceptibility testing of Infrequently isolated or fastidious bacteria, 3rd Edn CLSI guideline M45. Wayne: Clinical and Laboratory Standards Institute.

[10] De Paola, A., Peeler, J.T., & Rodrick, G. (1995). Effect of Oxytetracycline Medicated Feed on Antibiotic Resistance of Gram-Negative Bacteria in Catfish Ponds. Applied and Environmental Microbiology, 61: 2335–2340.

[11] FAO/NACA/WHO, (1997). Joint Study Group. Food Safety Issues Associated with Products from Aquaculture. WHO Technical Report Series, No. 883.

[12] Fish, U., and Service, W. (2010). Suggested procedures for the detection and identification of certain finfish and shellfish pathogens. In: Standard Procedures for Aquatic Animal Health Inspections. Bethesda: American Fisheries Society.

[13] HHS [U.S. Department of Health and Human Services], (1999). Assistant Secretary for Legislation (ASL). Statement on Antimicrobial Resistance: Solutions to a Growing Public Health Threat. By David Satcher, (See: http://www.hhs.govasl/testify/t990225a.html)

[14] Ibrahim, A., et al. (2022). Prevalence of antibiotic-resistant bacteria in fish from Nigerian markets. Journal of Environmental Science and Health, Part B, 57, 1-9.

[15] Joh, S.J., Ahn, E.H., Lee, H.J., Shin, G.W., and Kwon, J.H. (2013). Bacterial pathogens and flora isolated from farm-cultured eels (Anguilla Japonica) and their environmental waters in Korean eel farms. Vet Microbial. 163(1–2):190–5

[16] Kuhne, M., Wegmann, S., Kobe, A., & Fries, R. (2000). Tetracycline residues in bones of slaughtered animals. Food Control, 11: 175–180.

[17] Lipp, E.K., and Rose, J.B. (1997). The Role of Seafood in Food Borne Diseases in the United States of America. Rev. Sci. Tech. OIE., 16: 620 640.

[18] MacFaddin, J.F. (2000). Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Philadelphia: Lippincott Williams & Wilkins.

[19] Okoro, C. K., et al. (2020). Antibiotic resistance in bacteria isolated from aquatic environments in Nigeria. Journal of Water and Health, 18(4), 547-555.

[20] Olufemi, B. E., & Akinwumi, J. A. (2020). Bacterial contamination of fish from Nigerian markets. Journal of Food Science and Technology, 57(2), 533-539.

[21] Parkman, B., and Haskoning, M. (1996). Reconstruction of Ajiwa Reservoir Katsina, Katsina state. Nigeria. P 1-23.

[22] Sarter, S., Nguyen, K., Hung, L.T., Lazard, J., and Montet, D. (2007). Antibiotic resistance in gram-negative bacteria isolated from farmed catfish. Food Control. 18(11):1391–6.

[23] Soge, O. O., et al. (2022). Antimicrobial resistance in bacteria isolated from fish farms in Nigeria. Journal of Aquatic Animal Health, 34(1), 34-41.

[24] Van den Bogaard, A.E., & Stobberingh, E.E. (2000). Epidemiology of resistance to antibiotics. Links between animals and humans. International Journal of Antimicrobial Agents, 14: 327–335.

[25] Wamala, S.P., Mugimba, K.K.S., Mutoloki, S., Evensen, O., Mdegela, R., Byarugaba, D.K., and Sørum, H. (2018). Occurrence and Antibiotic Susceptibility of Fish Bacteria Isolated from Oreochromis niloticus (Nile tilapia) and Clarias gariepinus (African catfish) in Uganda. Fisheries and Aquatic Sciences. 21:6 DOI 10.1186/s41240-017-0080-x