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Occurrence of multi-drug resistant *Enterobacteriaceae* in cultured *Clarias gariepinus* (African catfish) in Kano metropolis, Nigeria

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Abstract

Multi-drug resistant *Enterobacteriaceae* were isolated from cultured African catfish (*Clarias gariepinus*) from ten different fish farms located in Kano Metropolis, Nigeria using conventional methods of bacterial isolation, phenotypic characterization and antimicrobial susceptibility test. This study seeks to document the occurrence of *Enterobacteriaceae* isolates in cultured African catfish from ten registered fish farms, determine possible resistance to some antimicrobials and the fish safety for human consumption. Isolation and identification of microorganisms were carried out based on the standard procedures and antimicrobial susceptibility to 8 commonly used antimicrobials were conducted using the Kirby-Bauer disc diffusion method. Out of the 400 fish liver sampled, 370 (92.5%) were positive for *Enterobacteriaceae* isolates, these included 277 (69.25%) *E. coli,* 13 (3.25%) *Salmonella* spp, 36 (9%) *Klebsiella* spp*,* 21 (5.25%) *Proteus* spp and 23 (5.75%) *Enterobacter* spp*.* The prevalence of the multi-drug resistance was 97.5% for *E. coli*, 100% for *Salmonella* spp, 100% for *Klebsiella* spp*,* 90.5% for *Proteus* spp and 82.6% for *Enterobacter* spp. This study establishes the presence of some *Enterobacteriaceae* and the development of multi-drug resistance by these microorganisms. More studies like molecular characterization need to be carried out to determine the resistant genes in these organisms, also to assess antimicrobial use among fish farmers and the drug residue levels in the edible tissues of cultured African catfish in Kano Metropolis, Nigeria.

Keywords: African-catfish, *Enterobacteriaceae*, Multi-drug-resistance, Kano, Nigeria

Introduction

As far back middle of the 1990s, catfish has been the dominant fish cultured in Nigeria and is currently responsible for the major aquaculture output of the country (FAO, 2017). Adewumi *et al.* (2010) opined that *Clarias gariepinus* gave Nigeria a niche in the global aquaculture production, and Nigeria is

currently the highest producer of aquaculture products in Africa and the highest producer of African catfish in Africa as well as the world (FAO, 2017). However, infectious and non-infectious diseases constitute a major constraint to aquaculture productivity (Bagumire *et al.*, 2010).

Antimicrobial regimens are being employed prophylactically and therapeutically to combat these challenges as well as for growth promotion. Extensive use of these antimicrobials for prophylaxis or additives may translate to more risk of pathogenic bacteria developing resistance (Maciej *et al.,* 2020).

The antimicrobials used either for therapy, as prophylactic or for growth promotion purposes below therapeutic doses can lead to the transfer of resistance genes from the aquatic animals to humans, thereby establishing a source of resistant microbes. Consequently, contaminated fish with these antibiotic-resistant bacteria can be a major threat to public health, as the resistant genes can be transferred to other bacteria of clinical significance (Sérgio *et al.*, 2018).

Smith *et al.* (2013) and the World Organization for Animal Health (OIE), aquatic animal health code (2018) recommended continuous monitoring and surveillance of antimicrobial resistance in microorganisms associated with aquatic animals (OIE, 2018).

The multi-drug resistance survey in food animals will help to develop guidelines for the prudent use of antimicrobials (Geidam *et al.*, 2012).

This study aimed at determining the frequency and antimicrobial susceptibility pattern of *Enterobacteriaceae* isolated from cultured African catfish (*Clarias gariepinus*) in Kano Metropolis, Nigeria. Members of the family *Enterobacteriaceae* are small Gram negative, non-spore forming straight rods. Some genera are motile; others (*Tatumella*, *Shigella* and *Klebsiella* species) are non-motile. They are facultative anaerobe and most species grow well at 37°C. They grow well on peptone and meat extract media. Some strains grow on D- glucose as the sole source of carbon and energy, but other strains require vitamins and or amino acids. Acid is produced during the fermentation of D- glucose and other carbohydrates. They are oxidase negative and catalase reactions vary among *Enterobacteriaceae*. Nitrates are reduced to nitrites except by some strains of *Erwinia.* They are distributed worldwide and may be found in soil, water, plants, humans and animals (PHC, 2013).

Multi-drug resistance here is defined as being resistant to at least three antimicrobials from different classes as defined by Shimaa & Raghda (2020).

Materials and Methods

Sample collection

Convenient sampling was carried out; 400 Catfish samples were sourced from 10 registered commercial farms (40 samples per farm) spread within Kano metropolis. The culture system in all the farms sampled is pond fish culture, raising only African catfish of the same age. All the fish sampled were between the age of 22 to 25 weeks with an average mass and length of 800 ± 79.16g and 32 ± 2.67cm respectively. All were killed by positioning two hands and holding firmly the head and upper trunk, forcefully bending it to immobilized, incised longitudinally using a sterile scissors from the anal opening to the operculum. Liver samples were aseptically removed, labeled in a small sterile nylon and transported to the Veterinary Microbiology Diagnostic Laboratory, Department of Veterinary Microbiology Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria for the analysis.

Isolation and identification of Enterobacteriaceae

Isolation and identification were carried out based on the procedure described by Quin *et al.* (2002). The convex surface of a hot sterile spatula was used to sear the surface of the liver. The seared surface was then cut using a sterilized scissors. A swab stick was then placed deep into the liver tissue through the cut surface to make a primary smear. Using a sterilized wire loop, secondary and tertiary streaks were made. Briefly, each sample was inoculated on MacConkey agar plate and incubated aerobically at 37^{o}C for 24h. The growth was then sub-cultured on Eosin Methylene blue (EMB) agar and incubated at 37^{o}C for 24h. Colonial morphology was then studied and the growth were subjected to Gram staining and biochemical tests (Urease test, Citrate test, Methyl Red Voges-Proskauer Test (MR-VP), Motility and Indole test, Triple Sugar Iron) for identification. All identified microorganisms were preserved in a nutrient agar slant for antimicrobial susceptibility test.

Gram staining

All the isolated organisms were subjected to Gram staining techniques for identification as described by Quin *et al.* (2002).

Biochemical tests

The biochemical tests that were carried out include: triple sugar iron (TSI), Indole, Urea, Citrate, MRVP

(Methyl red Voges Proskeur) and motility. The media were prepared based on the manufacturer's instructions. The test tubes containing the test media were labeled and arranged properly in a test tube rack, each was inoculated with the test organism and incubated aerobically at 37°C for 24hours, after which Kovac's reagent, VP1 & VP2 were added to the incubated peptone water for both Indole and MRVP tests respectively. Table 1 shows the criteria adopted to classify the isolates to be either *Enterobacteriaceae* or not.

Antimicrobial susceptibility testing (disc diffusion)

The Antimicrobial susceptibility testing was carried out on Mueller Hinton agar using Kirby-Bauer disc diffusion method and results were interpreted according to Clinical laboratory standards Institute guide (CLSI, 2018). Susceptibility to the following antimicrobials from Oxoid™ was tested: Gentamicin, Amoxicillin, Erythromycin, Tetracycline, Penicillin, Streptomycin, Nitrofurantoin and Doxycycline. Out of the 8 antimicrobials tested, any isolate resistant to at least 3 of them from different classes was considered multi-drug-resistant.

Results

Table 2 shows the isolation rate of the *Enterobacteriaceae* isolated from the liver of cultured African catfish from 10 different farms, 1 in Dala, 1 in Fagge, 1 in Nasarawa, 2 in Ungoggo, 2 in Tarauni, 1 in Municipal, 1 in Gwale and 1 in Kumbotso Local Government Areas distributed within Kano Metropolis, Nigeria. Out of 400 samples, 277 (69.25%), 13 (3.25%), 36 (9%) 21 (5.25%), 23 (5.75%) were positive for *E. coli, Salmonella* spp, *Klebsiella* spp*, Proteus* spp and *Enterobacter* spp, respectively, 30 (7.5%) were negative (No microbial growth was observed). Table 3 shows the multi-drug resistance pattern of *Enterobacteriaceae* isolated from the liver of African catfish from Kano Metropolis, Nigeria.

As shown in Table 4, only 7 *E. coli* isolates were resistant to 1 antimicrobial, 21 isolates were resistant to 2 antimicrobials; other *E. coli* isolates were resistant to 3 or more antimicrobials. Only 2 isolates of *Klebsiella* spp were resistant to 2 antimicrobials, other *Klebsiella* spp isolates were resistant to 3 or more antimicrobials. Only 1 isolate

Table 1: Biochemical test for identification of *Enterobacteriaceae* isolated from the liver of African catfish from Kano Metropolis, Nigeria

S/N	Test	*Salmonella* spp	*E. coli*	*Klebsiella* spp	*Proteus* spp	*Enterobacter* spp
1.	Grams reaction	-	-	-	-	-
2.	Oxidase	-	-	-	-	-
3.	TSI	K/A	A/A	A/A	K/A	A/A
4.	Indole	-	+	-	+	-
5.	Methyl red	+	+	-	+	-
6.	Voges-proskauer	-	-	+	-	+
7.	Citrate	+	-	+	-	+
8.	Urease	-	-	+	+	+
9.	Motility	+	+	-	+ (Swarming)	+

Key: TSI = Triple sugar iron; K/A = Alkaline/Acid; A/A = Acid/Acid; + = positive; - = Negative; spp = species

Table 2: Frequency isolation rate of the *Enterobacteriaceae* isolated from the liver of cultured African catfish from ten (10) different farms distributed within Kano Metropolis, Nigeria

S/No.	Farms (N=400)	*E. coli*	*Salmonella* spp	*Klebsiella* spp	*Protesus* spp	*Enterobacter* spp	*Samples negative*
1.	K	8	3	4	4	8	13
2.	D	34	3	2	0	0	1
3.	O	36	0	0	2	2	0
4.	J	24	0	3	6	0	7
5.	L	28	3	2	0	2	5
6.	N	27	0	7	4	0	2
7.	R	34	0	1	0	5	0
8.	A	33	1	4	2	0	0
9.	X	32	0	2	0	4	2
10.	Z	21	3	11	3	2	0
	Total	277	13	36	21	23	30

Table3: Multi-drug resistance pattern of *Enterobacteriaceae* isolated from the liver of African Catfish in some selected farms from Kano Metropolis, Nigeria

Antimicrobials	*Disc Conc. (*µg)	*E. coli*	*Salmonella* spp	*Klebsiella* spp	*Protesus* Spp	*Enterobacter* spp	Cumulative	**%**
Gentamicin (CN)	10	136	11	23	11	13	194	52.4
Amoxycilin (AML)	10	197	7	25	14	13	256	69.2
Erythomycin (E)	15	149	9	18	7	8	191	51.6
Tetracycline (TE)	30	152	7	25	11	8	203	54.9
Penicillin (P)	10 units	238	13	33	18	18	320	86.5
Streptomycin (S)	10	131	7	18	9	9	174	47.0
Nitrofuramtoin (F)	50	103	8	9	8	3	133	35.9
Doxycycline (DO)	30	123	11	15	6	7	162	43.8

Table 4: Isolates resistant to 1 or 2 antimicrobials

S/N	Isolate (n)	Frequency of resistance to antimicrobials	Antimicrobial-resistant pattern
1	*E. coli* (7)	1	5P, 1E & 1S
2	*E. coli* (21)	2	5P & S, 4P & TE, 4P & CN, 2P & DO, 1E & TE, 1AML & DO, 1CN & DO, 1DO & F, 1E & P and 1CN & AML
3	*Klebsiella species* (2)	2	1P & DO and 1P & TE
4	*Proteus species* (1)	1	1F
5	*Proteus species* (3)	2	1P & DO and 2P & S
6	*Enterobacter species* (4)	1	2P and 2CN
7	*Enterobacter species* (5)	2	1P & S, 1CN & AML, 2CN & P and 1P & F

Key: CN=Gentamicin, AML=Amoxycilin, E=Erythromycin, TE=Tetracycline, P=Penicillin, S=Streptomycin, F=Nitrofurantoin, DO=Doxycycline

of *Proteus* spp was observed to be susceptible to all antimicrobials tested, 1 isolate was resistant to 1 antimicrobial, 3 isolates were resistant to 2 antimicrobials, other *Proteus* spp isolates were resistant to 3 or more antimicrobials.

Only 4 isolates of *Enterobacter* spp were resistant to 1 antimicrobial, 5 isolates were resistant to 2 antimicrobials; other *Enterobacter* spp isolates were resistant to 3 or more antimicrobials. All isolates resistant to more than two antimicrobials were considered multi-drug resistant as defined by Shimaa & Raghda (2020).

Discussion

Members of the group *Enterobacteriaceae* of human origin survive and multiply in the GIT and tissues of fish (Udeze *et al.*, 2012). In this study, *E. coli* was the predominant species found in the liver of fish. The presence of *E. coli* suggests it as the possible cause of frequent gastrointestinal illness in humans and may constitute potential danger of antibiotic resistance to humans, Grema *et al.* (2015). The high number of *E. coli* isolates in this study agrees with the findings of Grema *et al.* (2015) that reported 75.7% multi-drug resistant *E. coli* isolates from fish. In Malaysia, Wan & Gerald (2017) reported 100% Erythromycin and Penicillin, 23.2% Tetracycline and 14.5% Streptomycin resistant *E. coli* strains in fish. This however, contradicts the findings in this study, perhaps this is attributed to exposure, non-exposure to the drugs or different types of strains involved.

Multi-drug resistance in fish was also reported in *Klebsiella* spp and *Proteus* spp with most isolates resistant to 4 to 8 antibiotic agents (Grema *et al.*, 2015), this also agrees with the results obtained in this study.

All the isolated microorganisms in this study were found to be present in fish livers, and in other studies were isolated in water and fish samples from market environments (Da Costa *et al.*, 2013). These microorganisms may carry the genes of multi-drug resistance that are transferable to other humans or animals pathogens through environment and or consumption in food (Da Costa *et al.*, 2013; Grema *et al.*, 2015).

The findings in this study also agree with earlier reports of Overdevest *et al.* (2011) that antibiotic resistance in *Enterobacteriaceae* has increased dramatically during the past decade. Also these results provide evidence that there is an increased emergence of antibiotic resistance from bacterial isolates of fish. This agrees with the reports of

Albuquerque *et al.* (2007) who found increasing emergence of antibiotic resistance in bacterial isolates originating from fish and fish handlers.

This study established the presence of some *Enterobacteriaceae* and the development of multi-drug resistance by these microorganisms in some selected fish farms in Kano Metropolis, Nigeria. The findings from this study should serve as an indicator for the likelihood of consuming *Enterobacteriaceae* resistant to the tested antimicrobials and that may give room for the transfer of the resistant genes to other organisms. There is a need to carry out the molecular characterization of the enteric microorganisms in the study area, for a detailed understanding of these microorganisms and the arrays of conditions they do cause in aquaculture and fish farms. More studies need to be carried out to assess antimicrobial use among fish farmers and determine drugs residues level in the edible tissues of cultured African catfish in Kano Metropolis, Nigeria.

Conflicts of Interest

The authors declare no conflict of interest.

References

Adewumi AA & Olaleye VF (2010). Catfish culture in Nigeria: Progress, prospects and problems. *African Journal of Agricultural Research,* **15**(4): 1281-1285.

Albuquerque WF, Macrae A, Sousa OV, Vieira GHF & Vieira RHSF (2007). Multiple drug resistant *Staphylococcus aureus* strains isolated from a fish market and from fish handlers. Brazilian *Journal of Microbiology,* **38**(1): 131-134.

Bagumire A, Todd EC, Nasinyama GW & Muyanja C (2010). Food safety-related control measures in emerging aquaculture enterprises in Sub-Saharan Africa: Compliance of Ugandas operations against international market requirements. *African Journal of Food Science*, **4**(7): 444-457.

CLSI (2018). Performance standards for antimicrobial disk susceptibility tests; approved standard— eleventh edition. Clinical and Laboratory Standards Institute, M02-A13. Clinical and Laboratory Standards Institute, Wayne.

Da Costa PM, Loureiro L, Augusto J & Matos F (2013). Transfer of multi-drug-resistant bacteria between intermingled ecological niches: The interface between humans, animals and the environment. *International Journal of Environmental Research and Public Health*, **10**(1): 278-294.

Endimiani A, Luzzaro F, BriganteG, Perilli M, Lombardi G, Amicosante G, Rossolini GM & Toniolo A (2005). *Proteus mirabilis* bloodstream infections: Risk factors and treatment outcome related to the expression of extended-spectrum beta-lactamases. *Antimicrobial Agents Chemotherapy*, **49**(7):2598-2605.

FAO (2017). Fishery and Aquaculture Statistics. Global aquaculture production 1950-2015 (Fish statJ). In: FAO Fisheries and Aquaculture Department. Rome.

Geidam YA, Zunita Z, Saleha AZ, SitiKB, Jalila A & Sharina O (2012). High prevalence of multi-drug resistant bacteria in selected poultry farms in Selengor, Malaysia. *Asian Journal of Animal and Veterinary Advances,* **7**(9): 891-897.

Grema HA, Geidam YA, Suleiman A, Gulani IA & Birma RB (2015). Multi-drug Resistant Bacteria Isolated from Fish and Fish Handlers in Maiduguri, Nigeria. *International Journal of Animal and Veterinary Advances*, **7**(3): 49-54.

Maciej Z, Beata W & Andrzej G (2020). Phages as a cohesive prophylactic and therapeutic approach in aquaculture systems. *Antibiotics,* doi.10.3390/antbiotics9090564.

Overdevest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P, Heck M, Savelkoul P, Vandenbroucke-Grauls C, Vanderzwaluw K, Huijsdens X & Kluytmans J (2011). Extended spectrum β-lactamase genes of *Escherichia coli* in chicken meat and humans, the Netherlands. *Emerging Infectious Diseases,* **17**(7): 1216-1222.

PHE (2013). Standards for Microbiology investigations, identification of *Enterobacteriaceae. Standards Unit, Microbiology Services.* Public Health England, UK.

Quin PJ, Carter ME, Markey B & Carter GR (2002). Clinical Veterinary Microbiology. Sixth edition. Mosby and imp. Wolf London. 66-85.

Sérgio HM, Graziela CR, Moura A, Fabiana G & Fabiana P (2018). Antibiotic Residues and Resistant Bacteria in Aquaculture (Review article). *The Pharmaceutical and Chemical Journal*, **5**(4): 127-147.

Shimaa AA & Raghda H (2020). Colistin susceptibility among multi-drug resistant Gram-negative

bacilli isolated from Tertiary hospital in Egypt. *Novel Research in Microbiology Journal*, **4**(5): 968-978.

Smith P, Alday-Sanz V, Matysczak J, Moulin G, Lavilla-Pitogo CR & Prater D (2013). Monitoring and surveillance of antimicrobial resistance in microorganisms associated with aquatic animals. *Revue Scientific Et Technique De L Offlce International Des Epizootic*, **32**(2): 583-593.

Udeze AO, Talatu M, Ezediokpu MN, Nwanze JC, Onoh C & Okonko IO (2012). The effect of *Klebsiella pnemoniae* on catfish (*Claria sgariepinus)*. *Researcher,* **4**(4): 51-59.

Vass M, Hruska K & Franek M (2008). Nitrofuran antibiotics: a review on the application, prohibition and residual analysis. *Veterinarni Medicina,* **53**(9): 469–500.

Wan Norhana MN & Gerald NM (2017). Country Report on Antimicrobial Resistance in Fisheries: Malaysia. *Food and Agriculture Organization.* Fisheries and Aquaculture circular, report no. 1215: 18-19.

World Organisation for Animal Health (OIE) Aquatic Animals health code. (2018). Twenty-first edition*.* Pp 113-124.