MULTIDRUG RESISTANCE BACTERIA ISOLATES FROM FISH FARMS IN IGBOORA, OYO STATE.

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ABSTRACT

This study was targeted at evaluating the resistance pattern of bacteria isolates from gut and blood of adult *Clarias gariepinus*. Gut contents and blood samples of 50 adult *Clarias gariepinus* from Igboora, Oyo State were randomly sampled and analysed bacteriologically. The bacteria isolated were subjected to antimicrobial sensitivity test using Kirby-Bauer disk diffusion method. Antibiotics used for the antimicrobial test were Ampicillin (10 µg), Chloramphenicol (30 µg), Ciprofoxacin (5 µg), Doxycycline (30 µg), Enrofloxacin (10 µg), Fosfomycine (50 µg), Nitrofurantoin (300 µg), Norfloxacin (10 µg), Oxytetracycline (30 µg), and Sulphamethozaxole (25 µg). Seven bacteria genera were isolated from the blood and gut contents. These bacteria were *Citrobacter freundi, Edwardsiella tarda, Escherichia coli, Klebsiella sp, Salmonella sp, Staphylococcus* and *Streptococcus Sp.* Cumulative prevalence for all the antimicrobial agents were 59% resistance, 39% susceptibility and 2% intermediate, while the differential prevalence for individual antibiotic is as follows: susceptibility was between 6 to 61%, intermediate was between 6 to11% while resistance was between 39 to 83%. The MARI i.e. multiple antibiotic resistance index ranges from 0.4 to 0.9, while the prevalence of frequency of MARI ranges from 5.5 to 28%. The highest resistance prevalence pattern (83%) was seen in chloramphenicol and oxytetracycline, the highest susceptibility prevalence pattern was seen in enrofloxacin while the highest intermediate prevalence pattern was seen in chloramphenicol. *Salmonella sp* had the highest MARI i.e. 0.9 while *Citrobacter freundi* had the lowest MARI i.e. 0.4. Necessary actions should be taken to prevent further damage by this pending plague.

Key words: Antibiotics, Bacteria, *Clarias gariepinus,* Resistance, Susceptibility.

INTRODUCTION

Antibacterial resistance (AR) is currently one of the greatest threats to mankind and animals as it constitutes health crisis. Extensive use of antibacterial agents in human and veterinary medicine, and farm crops have resulted in emergence of antibacterial-resistant organisms in different environmental settings including aquaculture (Anyanwu and Chah, 2016). The issue of antimicrobial resistance is a great concern in aquaculture because the aquatic systems serve as the hotspots for genetic exchange, recombination of antibacterial resistance genes (ARGs) and these genes are transferred from aquaculture system to other ecosystems and food chains which can shape the evolution of future resistance profiles (Banquero *et al.,* 2008; Anyanwu and Chah, 2016). This study was targeted at evaluating the antimicrobial profile of bacteria isolates of blood and gut of *Clarias gariepinus* in commercial farms in Igboora, Oyo State.

MATERIALS AND METHODOLOGY

Fifty *Clarias gariepinus* were randomly sampled from ten ponds in Igboora community. Nutrient agar, MacConkey agar and Salmonella Shigella agar were used to conduct bacteriological analysis on gut contents and blood samples (collected from the heart) at 37^0C for 24 hours. Isolated bacteria were identified according to Bergy's Manual of Systematic Bacteriology (Brenner *et al*., 2005) base on Gram reactions, colony formation and biochemical characterization. Antibiotic susceptibility test was performed according to Kirby-Bauer disk diffusion method (Bauer *et al*., 1996) on bacteria which were isolated from samples collected. Growth inhibition zones diameters were measured according to NCCLS, 2001. Multiple Antibiotic Resistance Index (MARI) was analysed according to Tenover, 2006.

RESULTS

Seven bacteria genera were isolated in this experiment. Differential prevalence (DP) of susceptibility was between 6 to 61%, DP for intermediate was between 6-11%, while DP for resistance was 39-

83%. Antimicrobial agent with the highest resistance prevalence was oxytetracycline and chloramphenicol, enrofloxacin has the highest susceptibility prevalence. The cumulative prevalence (CP) for susceptibility was 39%, 2% for intermediate and 59% for resistance. MARI for all the isolate was between 0.4 and 0.9. Organism with the highest MARI was *Salmonella*, while *Citrobacter freundi* had the lowest MARI i.e. 0.4. All the organisms were involved within the highest prevalence of occurrence of MARI (i.e. 28%, with MARI value of 0.6 and 0.7) except *Klebsiella sp* and all antimicrobial agents were involved.

Table 1: Resistance pattern, MARI and prevalence of Sensitivity, intermediate and resistance of bacteria isolated from blood and gut of *Clarias gariepinus* in commercial farms of Igboora, Oyo State.

	Amp	Chl	Cip	Dox	Enro	Fos	Nit	Nor	Oxy	Sul	MARI
Citrobacter freundi	R	I	S	R	S	S	S	S	R	R	0.4
Citrobacter freundi	R	R	S	R	S	R	R	S	R	I	0.6
Edwardsiella tarda	R	R	R	S	S	S	R	S	R	R	0.6
Escherichia coli	R	R	S	R	S	R	R	S	R	R	0.7
Escherichia coli	R	R	S	R	S	S	S	S	R	R	0.5
Klebsiella sp	R	R	R	R	S	S	R	R	R	R	0.8
Salmonella sp	R	R	R	R	R	R	S	R	R	R	0.9
Salmonella sp	R	R	R	R	S	S	R	S	R	R	0.7
Salmonella sp	R	R	R	R	S	S	R	S	R	R	0.7
Salmonella sp	R	R	R	R	S	S	R	S	R	R	0.7
Salmonella sp	S	R	R	S	S	R	R	S	R	R	0.6
Salmonella sp	R	R	S	R	R	R	S	R	R	R	0.8
Staphylococcus 1	R	I	S	R	R	R	R	R	R	R	0.8
Staphylococcus 2	R	S	S	R	R	R	R	R	R	R	0.8
Staphylococcus Sp	S	R	R	S	R	R	R	S	S	R	0.6
Staphylococcus Sp	S	R	R	S	S	R	R	R	S	S	0.5
Streptococcus sp	R	R	S	R	R	R	I	R	R	S	0.7
Streptococcus Sp	S	R	R	R	R	S	R	R	S	S	0.6
	DP										CP
S (%)	22	6	44	22	61	44	22	56	17	17	39
I (%)	-	11	-	-	-	-	6	-	-	6	2
R (%)	78	83	56	78	39	56	72	44	83	77	59

Key: Amp – Ampicillin (10 µg), Chl – Chloramphenicol (30 µg), Cip – Ciprofoxacin (5 µg), Dox – Doxycycline (30 µg), Enro – Enrofloxacin (10 µg), Fos – Fosfomycine (50 µg), Nit – Nitrofurantoin (300 µg), Nor – Norfloxacin (10 µg), Oxy – Oxytetracycline (30 µg), Sul – Sulphamethozaxole (25 µg), S (%) – prevalence of susceptibility (%), I (%) – prevalence of intermediate (%), R (%) – prevalence of resistance (%), DP – differential prevalence (prevalence of resistance, susceptibility and intermediate of individual microbial agent), CP – cumulative prevalence (prevalence of resistance, susceptibility and intermediate of all the microbial agents), R – resistance, S – susceptibility, I – intermediate, MARI – multiple antibiotic resistance index.

Table 2: MARI, frequency of occurrence of MARI, prevalence of occurrence of MARI and antibiotics bacteria were resistance to.

M	F	P (%)	Bacteria affected	Antibiotics bacteria were resistance to
0.4	1	5.5	*Citrobacter freundi*	Amp, Dox, Oxy, Sul
0.5	2	11	*Escherichia coli*,	Amp, Chl, Dox, Oxy, Sul
			Staphylococcus Sp	Amp, Dox, Enro, Oxy, Sul
0.6	5	28	*Citrobacter freundi*,	Amp, Chl, Dox, Fos, Nit, Oxy
			Edwardsiella sp,	Amp, Chl, Cip, Nit, Oxy, Sul
			Salmonella sp,	Chl, Cip, Fos, Nit, Oxy, Sul

			Staphylococcus Sp	Chl, Cip, Enro, Fos, Oxy, Sul
			Streptococcus Sp	Chl, Cip, Dox, Enro, Nit, Nor
0.7	5	28	*Escherichia coli,*	Amp, Chl, Dox, Fos, Nit, Oxy, Sul
			3- Salmonella sp,	Amp, Chl, Cip, Dox, Nit, Oxy, Sul
			Streptococcus sp	Amp, Chl, Dox, Enro, Fos, Nor, Oxy
0.8	4	22	*Klebsiella sp,*	Amp, Chl, Cip, Dox, Nit, Nor, Oxy, Sul
			Salmonella sp,	Amp, Chl, Dox, Enro, Fos, Nor, Oxy, Sul
			2-Staphylococcus Sp,	Amp, Dox, Enro, Fos, Nit, Nor, Oxy, Sul
0.9	1	5.5	*Salmonella sp*	Amp, Chl, Cip, Dox, Enro, Fos, Nor, Oxy, Sul
T	**18**	**100**		

Key: Amp – Ampicillin, Chl – Chloramphenicol, Cip – Ciprofoxacin, Dox – Doxycycline, Enro – Enrofloxacin, Fos – Fosfomycine, Nit – Nitrofurantoin, Nor – Norfloxacin, Oxy – Oxytetracycline, Sul – Sulphamethozaxole, MARI – multiple antibiotic resistance index, M – MARI, F – frequency of MARI, P – prevalence of frequency of MARI.

DISCUSSION

The bacteriological analysis of this study showed that *Citrobacter freundi, Edwardsiella tarda, Escherichia coli, Klebsiella sp, Salmonella sp, Staphylococcus* and *Streptococcus Sp* were isolated from the gut and blood of adult *Clarias gariepinus*. All these bacteria isolates are either pathogenic to fish or human. This study is similar to what was reported by Obisesan *et al.*, 2021 and Adah *et al.*, 2022. All these isolates were reported by these authors in tissue and organs of fish subjected to bacteriological studies with the exception of *Klebsiella sp* and *Edwardsiella tarda* by the authors respectively. All these organisms subjected to antimicrobial sensitivity test showed high resistance to all the antibiotics ranging from 56 to 83% differential prevalence except enrofloxacin with 39% resistance. This trend of resistance was reported by Efuntoye *et al*., 2012 and Adah *et al.*, 2022. Multiple antibacterial resistance index (MARI) which has been defined as resistance to three or more classes of antibacterial agents by an isolate (Tenover, 2006) was shown to be between 0.4 to 0.9 in this study, Salmonella had the highest MARI, while *Citrobacter freundi* had the lowest. Obisesan *et al*., 2017 and Adah *et al.*, 2022 had similar results for similar isolates whose MARI also ranges from 0.5 to 0.7 and 0.2 to 0.8 respectively. Prevalence of occurrence of MARI was analysed, all the organisms were involved within the highest prevalence of occurrence of MARI (i.e. 28%, with MARI value of 0.6 and 0.7) except *Klebsiella sp*.

CONCLUSION

Antimicrobial resistance is present in aquaculture industry in Igboora, Oyo State Nigeria, necessary actions should be taken to avoid great economic loss in future by stakeholders.

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