



Bacteria Isolates of Moribund *Clarias Gariepinus* and their Resistance Pattern

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Abstract

The scope of bacterial infections and resistance pattern of organisms causing infection in aquaculture is amplifying at any given opportunity. Bacteriological investigation was carried out on 128 moribund *Clarias gariepinus* (having superficial lesion on their body) using the standard conventional bacteriological method and identified accordingly. Antimicrobial sensitivity test was also conducted using Kirby-Bauer disk diffusion method with fourteen antimicrobial agents: amoxicillin– clavulanic acid, ampicillin, ceftriaxone, doxycycline, tetracycline, oxytetracycline, chloramphenicol, streptomycin, gentamicin, metronidazole, ciprofloxacin, enrofloxacin, colistin sulfate and furaltadone on the bacteria isolates. Three bacteria belonging to the family Enterobacteriaceae were identified (i.e. *Escherichia coli*, *Enterobacter cloacae*, and *Klebsiella aerogenes*) plus a Gram-positive bacterium, *Enterococcus faecalis*. Frequency of resistance and MARI (Multi drug Antibiotics Resistance Index) for the bacteria isolates were between 64.3 to 85.7% and 0.6 to 0.8 bacteria isolates were 100% resistance to seven drugs, 75% resistance to four drugs and 25% resistance to three drugs. Bacteriological diagnosis and antimicrobial sensitivity test should not be under emphasized when dealing with cases of infection in aquaculture; these should be done promptly to avoid catastrophic economic loss in the industry. Aquatic farmers should strictly adhere to the recommended antimicrobial agents during outbreaks of infection.

Key words: Diagnosis, *Clarias gariepinus*, Moribund, Resistance

Introduction

Clarias gariepinus (Burchell, 1822) is a species of great economic importance in Africa and South-East Asia, especially as a food fish and vital in the local sustainability of the aquaculture activity Teugels (1986). Aquaculture are being threatened by infectious diseases which causes mortality and economic losses (Pachade *et al.*, 2014). Bacterial infection of fish constitutes a huge menace for aquaculture, leading to disastrous economic loss (Lau *et al.*, 2007). *Aeromonas*, *Edwardsiella*, *Pseudomonas*, *Flavobacterium*, *Vibrio* and *Streptococcus* are major genera of fish pathogens causing diseases in different tropical freshwater fishes (Plumb, 1997). In recent years, some opportunistic bacterial fish pathogens have been identified as the causal agents for severe outbreaks in aquaculture facilities. Among them, *Enterococcus* sp., (Rahman *et al.*, 2017) *Escherichia coli*, *Enterobacter cloacae* and *Klebsiella aerogenes* (Akande and Onyedibe, 2019; Nair *et al.*, 2021; Cao *et al.*, 2017). Antimicrobial agents can be used as a tool to maintain the health and disease prevention of cultured animals

(Bischoff *et al.*, 2005). The use of antibiotics for the control of bacterial diseases in aquaculture has been proven unsustainable and ineffective due to development of antibiotic resistance in pathogens (Cabello, 2006). Antibiotic resistance is a great concern in the management of bacterial diseases worldwide (Arias and Murray, 2012). The aim of this study is to identify organisms responsible for infection in *C. gariepinus* and conduct antimicrobial sensitivity test to all organisms isolated from commercial fish farms in Oyo State.

Materials and methods

Swabs were collected from 128 diseased fish (fry to adult *Clarias gariepinus*) exhibiting red spots, hemorrhages, and lesions near the operculum, dorsal fin, and intraperitoneal region. The samples were collected from six commercial fish farms and one Fish Diseases Diagnostic Clinics in Oyo State and transported to the laboratory for bacteriological analysis. Swabs were immersed into 10ml of buffered peptone water and streaked on nutrient agar plates (HiMEDIA®, USA) which were



incubated for 24 hours at 30° C. Dominant colonies were sub-cultured repeatedly on MacConkey and Trypticase Soy Agar (TSA) for evaluation of colonial morphology and purity according to Tilahun and Engdawork (2020). The bacteria isolates were subjected to biochemical and physiological reactions including Gram staining, oxidase, catalase, motility, Simon citrate, Indole, Glucose (gas), Mannitol, Hydrogen sulfide, methyl red, Vogues Proskauer test, Triple sugar Iron agar slant culture and fermentation of sugars for purpose of identification (Tilahun and Engdawork, 2020; Oliveira *et al.* 2014). Differential diagnosis was drawn from the biochemical test according to Brenner *et al.* (2005), while confirmation was concluded by imputing differential diagnosed data into www.gideononline.com (a software for bacteria confirmation) (Gideon, 2016). Kirby-Bauer disk diffusion method according to CLSI, (2020, 2006) and Hartantyo *et al.* (2020) was used to conduct antimicrobial sensitivity test for the bacteria isolates of which the following antimicrobial agents were used: amoxicillin– clavulanic acid, ampicillin, ceftriaxone, doxycycline, tetracycline, oxytetracycline, chloramphenicol, streptomycin, gentamicin, Metronidazole, ciprofloxacin, Enrofloxacin, Colistin sulfate and Furaltadone.

Results

Table 1 expressed detailed bacteriological classifications of *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella aerogenes* and *Enterococcus faecalis* isolated from moribund *Clarias gariepinus*, three of these (i.e. of *Escherichia coli*, *Enterobacter cloacae* and *Klebsiella aerogenes*) isolates belong to the family Enterobacteriaceae while *Enterococcus faecalis* belongs to the Enterococcaceae family. Table 2 displayed frequency of resistance (FR) and frequency of susceptibility (FS) by each isolate to all antimicrobial agents used are as follows: *E. coli* (78.6% and 21.4%) *E. cloacae* (78.6% and 21.4%), *K. aerogenes* (64.3% and 35.7%) *Enterococcus faecalis* had (85.7% and 14.3%), respectively and MARI (Multidrug Antibiotics Resistance Index) for the isolates were 0.7, 0.7, 0.6 and 0.7 respectively. Frequency of resistance of all isolates to individual antimicrobial agent (FRIAA-%) were also stated in Table 2. Ciprofloxacin, enrofloxacin and ceftriaxone had 75% susceptibility i.e. 25% resistance; amoxicillin–clavulanic acid, ampicillin, gentamicin and streptomycin had 75% resistance, while doxycycline, tetracycline, oxytetracycline, chloramphenicol, Metronidazole, Colistin sulfate and Furaltadone had 100% resistance.

Table 1: The identification and biochemical characterization of bacteria isolated from moribund *Clarias gariepinus*

Biochemical Characteristics	<i>Escherichia coli</i>	<i>Enterobacter cloacae</i>	<i>Klebsiella aerogenes</i>	<i>Enterococcus faecalis</i>
Gram Reaction	Gram -ve rod	Gram -ve short rod	Gram -ve short rod	Gram +ve cocci
Catalase	+ve	+ve	+ve	-ve
Oxidase	-ve	-ve	-ve	-ve
Motility	+ve	+ve	-ve	-ve
Methyl-red	+ve	-ve	-ve	-ve
Hydrogen Sulfide production	-ve	-ve	-ve	-ve
Indole production	+ve	-ve	-ve	-ve
Voges-Proskauer	-ve	+ve	+ve	+ve
Urea Hydrolysis test	-ve	-ve	+ve	-ve
OF (Oxidative- Fermentative)	Fermentative	Facultative anaerobes	Fermentative	Fermentative
Citrate	-ve	+ve	+ve	-ve
MacConkey Agar	Dry pinkish colonies	Mucoid and pinkish colonies	Large mucoid and pinkish colonies	Small and red-purple colonies
TSI Agar	Alkaline slant Acidic butt Presence of gas	Alkaline slant Acidic butt Presence of gas	Alkaline slant Acidic butt Presence of gas	Alkaline slant Acidic butt absence of gas
Sugar Fermentation				
Glucose	+ve	+ve	+ve	+ve
Maltose	+ve	+ve	+ve	+ve
Lactose	+ve	-ve	+ve	+ve
Mannitol	+ve	+ve	+ve	+ve
Sucrose	-ve	+ve	+ve	+ve
Arabinose	+ve	+ve	+ve	-ve
Xylose	+ve	+ve	+ve	-ve

Keys: TSI Agar +ve – positive, -ve – negative



Table 2: The antimicrobial sensitivity of bacterial isolated from moribund *C. gariepinus*

AA	Disc content	<i>E. coli</i>	<i>E. cloacae</i>	<i>K. aerogenes</i>	<i>E. faecalis</i>	FRIAA (%)
Ampicillin	10 µg	R	R	R	S	75
AC	30 µg	R	R	R	S	75
Ceftriaxone	30 µg	S	S	S	R	25
Chloramphenicol	30 µg	R	R	R	R	100
Ciprofloxacin	5 µg	S	S	S	R	25
Colistin sulfate	10 µg	R	R	R	R	100
Doxycycline	30 µg	R	R	R	R	100
Enrofloxacin	5 µg	S	S	S	R	25
Furaltadone	30 µg	R	R	R	R	100
Gentamicin	10 µg	R	R	S	R	75
Metronidazole	5 µg	R	R	R	R	100
Oxytetracycline	30 µg	R	R	R	R	100
Streptomycin	10 µg	R	R	S	R	75
Tetracycline	30 µg	R	R	R	R	100
FR (%)		78.6	78.6	64.3	85.7	
FS (%)		21.4	21.4	35.7	14.3	
MARI		0.7	0.7	0.6	0.8	

Keys: AA – Antimicrobial Agent, AC – Amoxicillin– Clavulanic acid, FRIAA (%) – frequency of resistance of all isolates to individual antimicrobial agent, FR (%)– Frequency of Resistance by each organism to all antimicrobial agents, FS (%) – Frequency of Susceptibility by each organism to all antimicrobial agents (%), *Escherichia coli*, *Enterobacter cloacae*, *Klebsiella aerogenes*, MARI– Multidrug Antibiotics Resistance Index.

Discussion

Escherichia coli, *Enterobacter cloacae* and *Klebsiella aerogenes* isolated in this study are bacteria belonging to the family Enterobacteriaceae. They are widely distributed in water bodies and frequently isolated from apparently healthy fish in aquatic environments (Rawash *et al.*, 2019; Ribeiro *et al.*, 2015). *E. cloacae* and *K. aerogenes* (previously known as *E. aerogenes* (Mann *et al.*, 2021) have been reported to be associated with infections in fish by Nair *et al.* (2021) and Cao *et al.* (2017) who respectively isolated and characterized these organisms from moribund *Etiopplus maculatus* fish and diseased channel catfish. Akande and Onyedibe (2019) reported isolating enteropathogenic and entero-invasive *Escherichia coli* from *Clarias gariepinus*. Adeyemi *et al.* (2013) reported *E. coli* infectivity when *Clarias gariepinus* was experimentally challenged with it. *Enterococcus faecalis* which was also isolated is a Gram-positive bacterium, is known to be responsible for infection in tilapia in Egypt and Thailand and has been isolated from healthy and infected fish in Bangladesh (Rahman *et al.*, 2017).

High multidrug resistance pattern was seen in these isolates ranging from 64 to 85% and MARI (multiple antibiotic resistance index) from 0.6 to 0.8 (Table 2). Seven out of fourteen antimicrobial drugs showed 100% resistance to the four bacteria isolates, four showed 75% resistance while three drugs

showed 75% susceptibility. This result corroborates with Obisesan *et al.* (2023), who conducted a study on resistance pattern of bacteria isolates from gut and blood of *C. gariepinus*. MARI for *E. coli* and *Klebsiella spp* was between 0.5 to 0.8. Cao *et al.* (2017) reported resistance of *Klebsiella aerogenes* to the β -lactam, chloramphenicol, tetracyclines antibiotics which was also reported in this study. Akande and Onyedibe (2019) reported similar result to this study, i.e. *E. coli* isolated from catfish in Jos, Nigeria to be resistance to amoxicillin– clavulanic and susceptible to ceftriaxone. However, contrary result by Akande and Onyedibe (2019) was that *E. coli* was resistance to ciprofloxacin and susceptible to gentamicin. Report of Nair *et al.* (2021) on resistance pattern of *E. cloacae* isolated from moribund *E. maculatus* was slightly different from this study he reported sensitivity of *E. cloacae* to gentamicin and chloramphenicol where we had resistance. Al-Niaeem *et al.* (2021) reported resistance of *E. cloacae* isolated from infected common carp to ampicillin and ceftriaxone and intermediate inhibition to ciprofloxacin, whereas *E. cloacae* in this study was susceptible to ceftriaxone and ciprofloxacin. Rahman *et al.* (2017) reported resistance of *E. faecalis* isolated moribund tilapia (*Oreochromis niloticus*) and catfish (*Clarias batrachus* and *Heteropneustes fossilis*) to be resistance to ampicillin and susceptible to gentamicin which is contrary to the result in this study.



Conclusion

Four bacteria were identified i.e. *Enterococcus faecalis*, *Escherichia coli*, *Enterobacter spp.* and *Klebsiella spp.* as the pathogens responsible for lesions in the moribund catfish and resistance pattern of the bacteria isolates to antimicrobial agents was very high. Farmers should endeavour to invite veterinary personnel during outbreaks of diseases so that proper laboratory diagnosis is conducted. Survey on the rate at which antimicrobial agents are been used in aquaculture need to be conducted in Oyo State, Nigeria, so as to ascertain what is responsible for this high resistance pattern.

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