International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 05 Issue 01 January 2025

Page No : 45-54

DOI: https://doi.org/10.47191/ijpbms/v5-i1-09, Impact Factor:7.792

Antibiotic Susceptibility Profile of Gram-Negative Bacteria Isolated From Pond Catfish in Ketu Adie-Owe, Ado-Odo Local Government Area of Ogun State, Nigeria

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ABSTRACT

The increasing number of fish ponds in Nigeria is driven by the demand for fish and fishery products. Catfish, specifically *Clarias gariepinus*, is popular for cultivation due to its economic significance. However, bacterial infections pose a threat to the health of catfish in these ponds, resulting in the need for antibiotic usage. This study is aimed to assess the antibiotic susceptibility pattern of Gram-negative bacteria isolated from pond catfish. This study involved isolating bacteria from the catfish skin and intestine, analyzing their biochemical characteristics, and conducting antimicrobial susceptibility testing. Fungi present in the catfish samples were also identified. The Gram-negative isolates were subjected to antibiotic susceptibility testing, revealing the prevalence of multidrug-resistant strains. The findings contribute to the understanding of antibiotic resistance in aquaculture and highlight the need for appropriate management strategies. The bacterial load in different catfish samples was examined through total plate counts and dilution effects. The results revealed variations in colony counts and CFU/ml values among the samples, with the highest bacterial load observed in the skin of large catfish. The bacterial load observed in the skin of catfish ranged from 4.7 X 10^3 CFU/ml to 9.8 X 10^3 CFU/ml, while the bacterial load observed in the intestine of catfish ranged from 3.1 X 10^3 CFU/ml to 5.4 X 10^3 CFU/ml. Biochemical testing identified specific organisms, including *Enterobacter cloacae, Aeromonas hydrophila, Klebsiella pneumoniae, Citrobacter brakii, Citrobacter freundii,* and *Enterobacter aerogenes*. Varying resistance patterns emphasize the need for careful antibiotic use and infection control in catfish aquaculture. Therefore, continuous monitoring and preventive measures are recommended.

ARTICLE DETAILS

Published On:
17 January 2025

KEYWORD: Clarias gariepinus, Enterobacter cloacae, Aeromonas hydrophila, Klebsiella pneumoniae, Citrobacter brakii, Citrobacter freundii, Enterobacter aerogenes

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https://ijpbms.com/

INTRODUCTION

The surging demand for fish in Nigeria has prompted the establishment of numerous fish ponds, constituting over 70% of the country's fish supply, especially in Oyo State. Fish ponds, varying from small to large commercial systems, are gaining global popularity. Notably, catfish species such as *Clarias anguillaris*, *Clarias gariepinus*, *Heterobranchus congifilis* and *Heterobranchus bidorsalis* are cultivated, with *Clarias gariepinus* being the predominant choice in Nigeria **[1]**. Globally, fish farming which is a critical subsector of aquaculture has experienced rapid growth. Half of the world's total fish consumption is now sourced from fish farming, with exports from developing countries steadily increasing over the past two decades **[2]**. The breeding and rearing of catfish and other finfish in ponds, known as pisciculture have historical roots marked by infectious diseases posing threats to animals, including humans. Alexander Fleming's 1928 discovery of antibiotics had a profound global impact. However, within pisciculture the confined environments and sanitation challenges of finfish in ponds have resulted in the extensive use of diverse antimicrobials **[3]** and non-biodegradable antibiotics used in human antibiotics **[4]**.

Antibiotic resistance stands as a formidable global health challenge, with intricate combinations of factors driving resistome expansion **[5]**. Established relationships now link antibiotic usage in pisciculture environments to the escalation of drug-resistant bacteria and genes. The incorporation of growth-promoting antimicrobials in feeds, exposing catfish gut microbiomes to sub-therapeutic doses of these antimicrobials is identified as a major cause of drug resistance in aquaculture **[6]**. Misuse and abuse of antibiotics, particularly in regions lacking stringent control measures contribute to the emergence of drug-resistant bacteria and genes in aquaculture systems **[7]**. The reservoir of resistance genes within pathogenic and nonpathogenic bacteria in aquaculture environments can through horizontal gene transfer in bacteria **[8]**. The intensification of aquaculture to meet the protein demands of the growing human population is severely hindered by the issue of drug resistance, particularly in regions with lax regulatory controls **[9]**. The bacterial selection of antibiotics reserved for human therapy due to the misuse by farmers lacking proper knowledge of prudent antibiotic use in aquaculture operations raises serious concerns **[10]**. While Europe and the United States have implemented control strategies, many developing countries including Nigeria, lack such measures leading to poor standards of hygiene and inadequate monitoring of antibiotic usage in catfish farms **[11]**. Stressed fish in aquaculture are susceptible to bacterial diseases, causing high mortalities and economic losses **[12]**. To mitigate such losses, fish farmers resort to antibiotics and antimicrobial agents for prevention, treatment of diseases and control of external parasites, fungi, aquatic weeds and mollusks. However, this practice poses a risk to human health as resistant bacteria in fish farms could be transferred to other bacteria or directly to human pathogens **[13]**.

Antibiotics play a pivotal role in controlling infectious diseases in cultured fish farms. Commonly used antibiotics in fish farms include sulphamethoxazole, chloramphenicol, tetracycline, flucloxacillin, gentamicin, ciprofloxacin and ampicillin usually added to fish feed or applied directly to pond water. Aside from disease control, antibiotics are used to disinfect fish eggs, improve water quality, and promote growth **[14]**. Antibiotic use in aquaculture significantly contributes to the global increase in antibiotic resistance **[15]**. Despite substantial interventions, the emergence and spread of antimicrobial-resistant bacteria persist **[16]**. The water environments of cultured fish serve as well-known reservoirs and routes of transmission of antibiotic resistance **[17]**. Zoonotic pathogens can infect humans who come into contact with aquaculture facilities or eat contaminated food, as well as the aquatic animals themselves. Fish act as reservoirs for zoonotic pathogens **[18]**. Human diseases can be caused by a variety of bacterial species found in fish and aquatic habitats, including *Aeromonas*, *Pseudomonas*, *Enterobacteriaceae*, *Vibrio* and other Gram-negative bacteria.

The mechanisms by which antimicrobial resistance genes infiltrate open aquaculture farms remain poorly understood, but these genes originate either endogenously, such as through the natural microbiota of fish or exogenously, through the introduction of antimicrobial-resistant bacteria or genetic determinants via wastewater or organic fertilizers like animal manure.

The antibiotic susceptibility pattern of Gram-negative organisms isolated from pond catfish is a critical aspect of managing infections in aquaculture. Several studies have investigated the susceptibility of various bacteria to antibiotics, providing valuable insights into the resistance patterns and susceptibility of different bacterial strains. A study by **[19]** demonstrated that 90.8% of Gram-negative organisms were susceptible to a specific antibiotic. Similarly, **[20]** found that most Gram-positive cocci were susceptible to aminoglycoside (86%), fluoroquinolone (81%), and cephalosporin (79%). Furthermore, **[21]** reported that over 90% of gram-negative isolates maintained susceptibility to test antibiotics over 15 years. Additionally, **[22]** highlighted the high sensitivity of Gram-negative bacteria to amikacin. Moreover, the presence of antibiotic resistance in fish farming environments has been documented. **[23]** discussed the isolation and identification of tetracycline-resistant *A. veronii* isolates from farm-raised catfish, indicating resistance to multiple antibiotics. This study aimed to assess the antibiotic susceptibility pattern of Gram-negative bacteria isolated from pond catfish, addressing the need for prudent antibiotic use and effective infection control measures in catfish aquaculture.

METHODOLOGY

STUDY LOCATION

Three male *Claria gariepinus* specimens were collected from a concrete fish pond located in Apena Village, Ketu, Igbesa, Ogun State. The coordinates of the location are latitude 6° 32' 0.9672" N and longitude 3° 8' 2.9796" E.

SAMPLE COLLECTION

The live samples consisting of a small fish aged approximately 8 months, a Medium-aged fish of 1.5 years old, and a matured fish aged 2 years old were collected after obtaining permission from the relevant authorities in charge of the pond. The three live fish samples, collected in water directly from the pond were carefully transported to the Microbiology Laboratory at Crawford University in Faith City, Ketu Adie-Owe, Lusada-Igbesa, Ogun State. To ensure the well-being of the fish during transportation, a plastic container which had been covered with a net to maintain adequate airflow and prevent any potential escape or damage was used. This transportation process from collection to arrival at the laboratory was completed within an hour.

SAMPLE & MEDIA PREPARATION

The workstation was sterilized with 70% ethanol. For media preparation, Nutrient Agar, Salmonella-Shigella agar and MacConkey Agar was prepared in accordance with the manufacturer's specifications. The skin and intestine of each fish sample were individually weighed and aseptically macerated to achieve a weight of 1 gram each. Subsequently, these fish tissues (skin and intestine) were separately placed into test tubes, each containing 9 ml of sterile distilled water, to establish a stock solution. This stock solution was then serially diluted up to a five-fold dilution (10^{-5}).

BACTERIAL COLONY COUNT

Plating (pour plate method) was done by pipetting 0.1 ml of the 10^{-1}, 10^{-3}, 10^{-5} of both the skin and intestine each at the center of three different sterile Petri dishes by using sterile syringes. Cooled but molten Nutrient agar media were poured into the Petri dishes containing the inoculums and swirled to evenly distribute the agar and organisms.

After the solidification of the media, the plates were inverted and incubated at 37 °C for 24 hours. The plates were examined after incubation and the number of colonies forming units (CFU) that developed, were counted and recorded.

The fishes were assessed for Total Bacterial Counts (TBC) and dilutions (10^{-1}, 10^{-3}, and 10^{-5}) were selected.

Calculation of colony forming unit (CFU) per ml for the bacteria was based on the formula:

$$\frac{\text{CFU}}{\text{ml}} = \frac{\text{Number of colonies x dilution factor}}{\text{Volume of Sample inoculated}}$$

CULTURE AND ISOLATION

A sterile inoculating loop was inserted into the diluents (10^{-1} and 10^{-5}) of the skin and intestine test tubes, a loop full was taken and streaked onto the surface of MacConkey Agar and Salmonella-Shigella Agar plate. The agar plates were then incubated for 24 hours at 37°C. To acquire pure cultures, the bacterial isolates were sub-cultured.

IDENTIFICATION OF ISOLATED BACTERIA

In order to identify the isolates, Distinct colonies on the culture media were examined for phenotypic characteristics (colonial morphology, pigmentation, shape). Further characterization of the isolates was carried out using the API 20E identification kit; as described by the manufacturer (BioMérieux Marcy-l'Étoile, France). The API20E kit was used to identify Gram-negative bacteria.

ANTIBIOTIC SUSCEPTIBILITY TESTING

Identified isolates were evaluated for antibiotic susceptibility using the (Kirby-Bauer disc diffusion technique). Tetracycline (10µg), Cotrimoxazole (25µg), Gentamicin (10µg), Cefuroxime (30µg), Chloramphenicol (10µg), Ceftriaxone (30µg), Cefotaxime (30µg), Ciprofloxacin (5µg), Amikacin (30µg), Vancomycin (30µg), Ceftazidime (30µg), Meropenem (10µg) were the antibiotic discs used for this test.

A 24-hour pure culture of the identified isolates was inserted into a McCartney tube with 2ml of normal saline, the mixture in the tube was then compared to a 0.5 McFarland standard.

A sterile swab stick is inserted into the McCartney tube and streaked all over a Muller Hinton agar plate, which was incubated at 37°C for 18-24 hours. Following incubation, the plate was examined for zones of inhibition surrounding the antibiotic discs. The results were interpreted according to the Clinical and Laboratory Standards Institute guidelines **[24]** to establish antibiotic susceptibility or resistance.

RESULTS

The results obtained from the Small Catfish (skin) sample showed that the total number of organisms ranged from 4.7 x 10^3 CFU/ml, combining the counts from dilutions 10^{-1}, 10^{-3} and 10^{-5}. The Small Catfish (intestine) sample had a total of 3.1 x 10^3 CFU/ml when the counts from the three dilutions were added. For the Medium catfish (skin) sample, the total organism count was 7.2 x 10^3 CFU/ml, taking into account the counts from dilutions (10^{-1}, 10^{-3}, and 10^{-5}). The Medium Catfish (intestine) sample had a total of 3.8 x 10^3 CFU/ml. In the case of the Large Catfish (skin) sample, the total organism count was 9.8 x 10^3 CFU/ml, combining the counts from dilutions (10^{-1}, 10^{-3}, and 10^{-5}). The Large Catfish (intestine) sample had a total of 5.4 x 10^3 CFU/ml.

A total of 9 isolates were obtained from diluents (10^{-1} and 10^{-5}) by culturing and subculturing them on both MacConkey Agar and Salmonella-Shigella Agar. These isolates were randomly selected from the large number of organisms present in each sample. Specifically, 5 isolates were selected from MacConkey Agar and 4 isolates were selected from Salmonella-Shigella Agar. These isolates were then characterized, identified and tested for their susceptibility patterns to different classes of antibiotics.

Table 1: Total Plate counts of the three catfish samples

Sample	Dilution	Colony Count	CFU/ml
SFS	10^{-1}	240	2.4 x 10^3
SFS	10^{-3}	190	1.9 x 10^3
SFS	10^{-5}	40	4.0 x 10^2
SFI	10^{-1}	160	1.6 x 10^3
SFI	10^{-3}	120	1.2 x 10^3
SFI	10^{-5}	30	3.0 x 10^2

MFS	10^{-1}	360	3.6×10^3
MFS	10^{-3}	300	3.0×10^3
MFS	10^{-5}	60	6.0×10^2
MFI	10^{-1}	200	2.0×10^3
MFI	10^{-3}	160	1.6×10^3
MFI	10^{-5}	20	2.0×10^2
LFS	10^{-1}	500	5.0×10^3
LFS	10^{-3}	400	4.0×10^3
LFS	10^{-5}	80	8.0×10^2
LFI	10^{-1}	300	3.0×10^3
LFI	10^{-3}	240	2.4×10^3
LFI	10^{-5}	50	5.0×10^2

(**SFS**= Small Catfish Skin, **SFI**= Small Catfish Intestine, **MFS**= Medium Catfish Skin, **MFI**= Medium Catfish Intestine, **LFS**= Large Catfish Skin**, LFI**= Large Catfish Intestine).

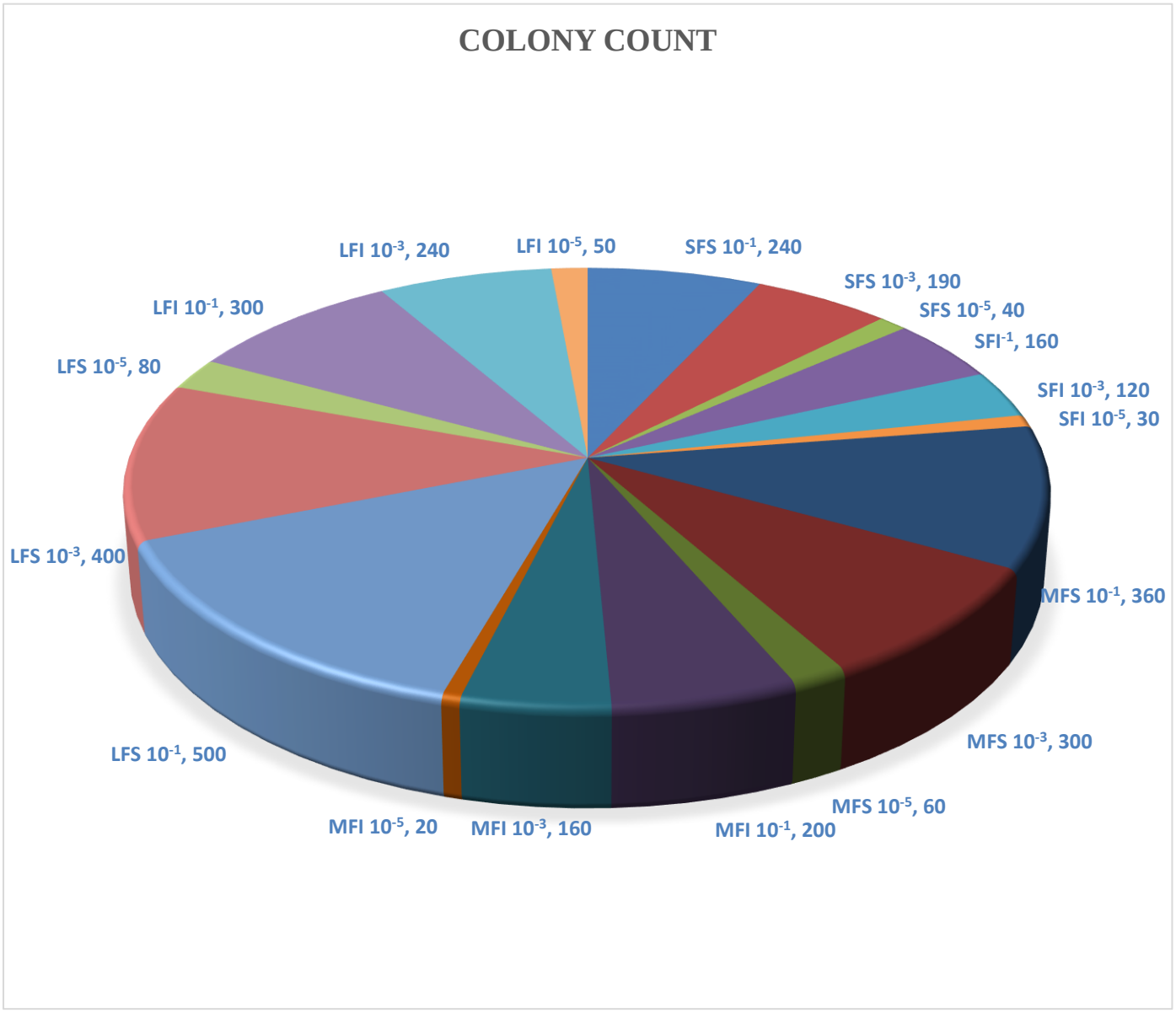

Figure 1: Pie chart showing the Total Plate counts of the three catfish samples

Table 2: Cultural Characteristics of Isolates on Different Media

Isolates	Media Used	Gram Reaction	Cell Shape	Lactose Fermentation
SFS 10^{-5}	MacConkey Agar	-	Bacilli (rod)	Yes
SFI 10^{-5}	MacConkey Agar	-	Bacilli (rod)	No
MFS 10^{-1}	MacConkey Agar	-	Bacilli (rod)	Yes
MFI 10^{-1}	MacConkey Agar	-	Bacilli (rod)	Yes
LFS 10^{-5}	MacConkey Agar	-	Bacilli (rod)	Yes
SFI 10^{-1}	Salmonella-Shigella Agar	-	Bacilli (rod)	No
MFI 10^{-1}	Salmonella-Shigella Agar	-	Bacilli (rod)	No
LFS 10^{-5}	Salmonella-Shigella Agar	-	Bacilli (rod)	No
LFI 10^{-5}	Salmonella-Shigella Agar	-	Bacilli (rod)	No

(SFS= Small Catfish Skin, **SFI=** Small Catfish Intestine, **MFS=** Medium Catfish Skin, **MFI=** Medium Catfish Intestine, **LFS=** Large Catfish Skin**, LFI=** Large Catfish Intestine).

Table 3: Microbiological Characteristics of Isolates

Isolates	Media Used	Gram Reaction	Cell Shape	Lactose Fermentation
SFS 10^{-5}	MacConkey Agar	-	Bacilli (rod)	Yes
SFI 10^{-5}	MacConkey Agar	-	Bacilli (rod)	No
MFS 10^{-1}	MacConkey Agar	-	Bacilli (rod)	Yes
MFI 10^{-1}	MacConkey Agar	-	Bacilli (rod)	Yes
LFS 10^{-5}	MacConkey Agar	-	Bacilli (rod)	Yes
SFI 10^{-1}	Salmonella-Shigella Agar	-	Bacilli (rod)	No
MFI 10^{-1}	Salmonella-Shigella Agar	-	Bacilli (rod)	No
LFS 10^{-5}	Salmonella-Shigella Agar	-	Bacilli (rod)	No
LFI 10^{-5}	Salmonella-Shigella Agar	-	Bacilli (rod)	No

(SFS= Small Catfish Skin, **SFI=** Small Catfish Intestine, **MFS=** Medium Catfish Skin, **MFI=** Medium Catfish Intestine, **LFS=** Large Catfish Skin**, LFI=** Large Catfish Intestine).

Table 4: Biochemical Characteristics of Isolates using API 20E kit

Isolates	API 20E TESTS																						Identification
	ONPG	ADH	LDC	ODC	CIT	H_2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA	OX	NO_2	N_2	
SFS 10^{-5}	+	+	-	+	+	-	-	+	-	-	-	+	+	-	+	+	+	+	+	+	-	+	-	*Enterobacter cloacae*
SFI 10^{-5}	+	+	+	+	+	-	-	-	-	+	+	+	-	-	-	+	-	+	-	+	+	+	-	*Aeromonas hydrophila*
MFS 10^{-1}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	-	*Klebsiella pneumoniae*
MFI 10^{-1}	-	-	+	+	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	-	+	-	*Enterobacter aerogenes*
LFS 10^{-5}	+	-	+	+	+	-	+	-	-	+	-	+	+	-	+	+	+	+	-	-	-	+	-	*Enterobacter aerogenes*
SFI 10^{-1}	+	+	+	+	+	+	+	+	-	-	+	+	+	-	+	+	+	+	+	+	-			*Citrobacter brakii*
MFI 10^{-1}	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	-			*Citrobacter freundii*
LFS 10^{-5}	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	-	+	-	*Enterobacter aerogenes*
LFI 10^{-5}	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+			*Enterobacter cloacae*

(SFS= Small Catfish Skin, **SFI=** Small Catfish Intestine, **MFS=** Medium Catfish Skin, **MFI=** Medium Catfish Intestine, **LFS=** Large Catfish Skin**, LFI=** Large Catfish Intestine**)**.

(ONPG: Ortho-Nitrophenyl-β-galactoside test, ADH: Arginine dihydrolase test, LDC: Lysine decarboxylase test, ODC: Ornithine decarboxylase test, CIT: Citrate utilization test, H_2S: Hydrogen sulfide production test, URE: Urease test, TDA: Tryptophan deaminase test, IND: Indole production test, VP: Voges-Proskauer test, GEL: Gelatin hydrolysis test, GLU: Glucose fermentation test MAN: Mannitol fermentation test INO: Inositol fermentation test SOR: Sorbitol fermentation test RHA: Rhamnose fermentation test SAC: Saccharose (Sucrose) fermentation test MEL: Melibiose fermentation test AMY: Amylase test ARA: Arabinose fermentation test OX: Oxidase test NO_2: Nitrite reduction test N_2: Nitrogen fixation test**).**

Table 5: Antibiotic Susceptibility Pattern of Bacteria Isolates

IDENTIFICATION	TET	COT	GEN	CRX	CHL	CTR	CTX	CIP	AMK	VAN	CAZ	MER	R%	I%	S%	MARI
Enterobacter cloacae	I	R	R	R	R	R	R	R	S	R	R	R	83.3	8.3	8.3	0.8
Aeromonas hydrophila	R	R	R	R	R	R	R	R	S	R	R	R	91.7	0	8.3	0.9
Klebsiella pneumoniae	I	S	S	R	S	R	R	R	S	S	I	R	41.7	16.7	41.7	0.4
Enterobacter aerogenes	R	R	R	R	R	R	R	R	S	R	R	R	91.7	0	8.3	0.9
Enterobacter aerogenes	R	R	R	R	R	R	R	I	I	R	R	R	83.3	16.7	0	0.8
Citrobacter brakii	R	R	R	R	R	R	R	R	S	R	R	R	91.7	0	8.3	0.9
Citrobacter freundii	R	R	R	R	I	R	R	R	S	R	R	R	83.3	8.3	8.3	0.8
Enterobacter aerogenes	R	R	R	R	I	R	R	I	S	R	R	R	75	16.7	8.3	0.8
Enterobacter cloacae	R	R	R	R	R	R	R	R	S	R	R	R	91.7	0	8.3	0.9
Resistance %	**77.8**	**89**	**88.9**	**100**	**67**	**100**	**100**	**78**	**0**	**88.9**	**88.9**	**100**				
Mean ± SD													16.0 ± 1.7	7.7 ± 0.8	11.8 ± 1.3	0.15 ± 0.017

(SFS= Small Catfish Skin, **SFI=** Small Catfish Intestine, **MFS=** Medium Catfish Skin, **MFI=** Medium Catfish Intestine, **LFS=** Large Catfish Skin**, LFI=** Large Catfish Intestine).

Tetracycline= (TET, 10µg), Cotrimoxazole= (COT, 25µg), Gentamicin= (GEN 10µg), Cefuroxime= (CXM, 30µg), Chloramphenicol= (CHL, 10µg), Ceftriaxone= (CRO, 30µg). Cefotaxime= (CTX, 30µg), Ciprofloxacin= (CIP, 5µg), Amikacin= (AMK, 30µg), Vancomycin= (VAN, 30µg), Ceftazidime= (CAZ, 30µg), Meropenem= (MER, 10µg).

R: Resistance - This indicates that the bacteria are not susceptible to the antibiotic and are resistant to its effects.

S: Susceptible - This indicates that the bacteria are susceptible to the antibiotic and are likely to be killed or inhibited by its use.

I: Intermediate - This indicates that the bacteria have some degree of susceptibility to the antibiotic, but the effectiveness may be limited.

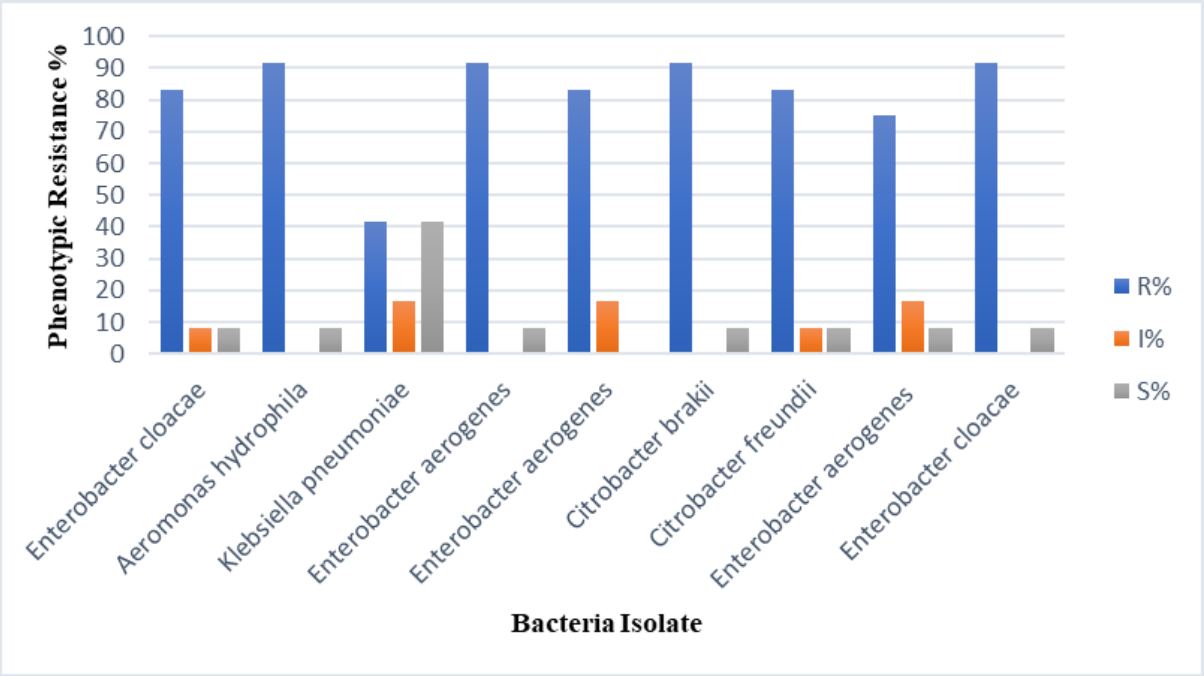

Figure 2: PHENOTYPIC ANTIMICROBIAL RESISTANCE OF ISOLATES

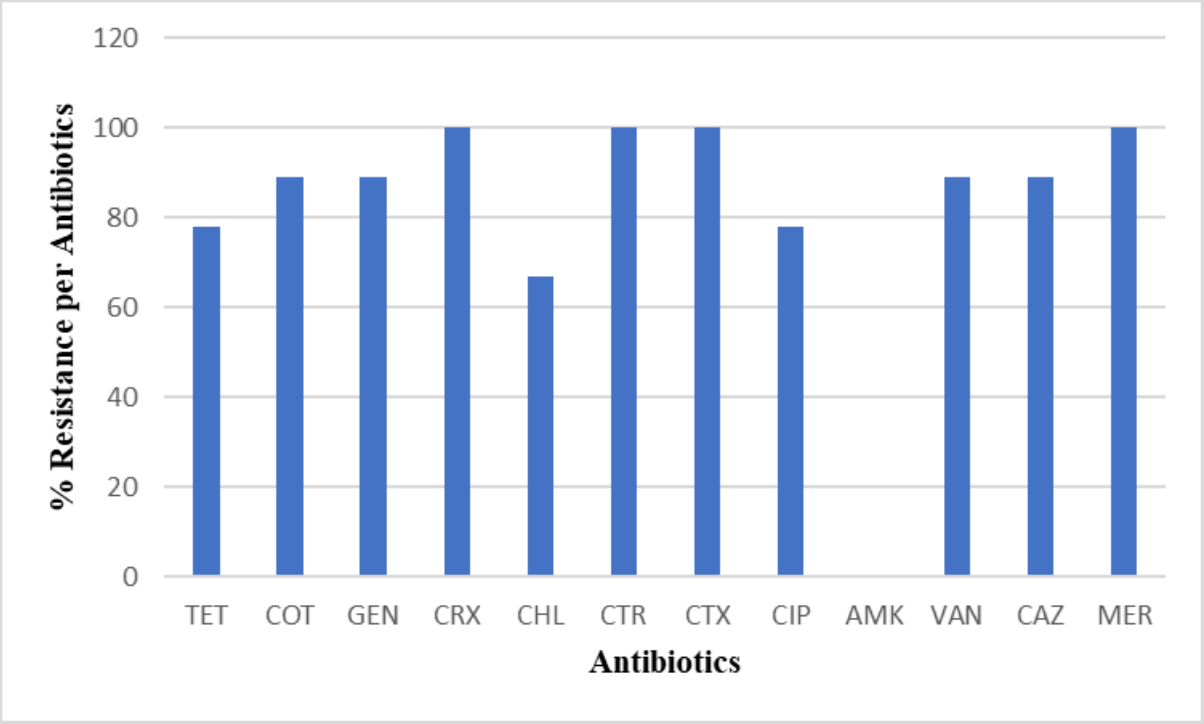

Figure 3: PERCENTAGE RESISTANCE PER ANTIMICROBIAL AGENT

DISCUSSION

Table 1 displays the total plate counts of Gram-negative organisms isolated from different samples of Pond catfish, including Small catfish skin (SFS), Small catfish intestine (SFI), Medium catfish skin (MFS), Medium catfish intestine (MFI), Large catfish skin (LFS), and Large catfish intestine (LFI). The colony counts are reported at different dilutions, along with the corresponding CFU/ml values. The highest colony count was observed in the LFS 10^{-1} sample with 500 colonies and a CFU/ml value of 5.0 x 10^{3}. This suggests a relatively higher bacterial load in the skin of large catfish. The lowest colony count was observed in the MFI 10^{-5} sample with 20 colonies and a CFU/ml value of 2.0 x 10^{2}. This indicates a lower bacterial load in the intestine of medium catfish at the 10^{-5} dilution. Generally, higher dilutions (10^{-3} and 10^{-5}) showed lower colony counts and CFU/ml values compared to the 10^{-1} dilution. This suggests a decrease in the concentration of viable bacteria with increasing dilution. There may be variations in the microbial populations present in the skin and intestine samples from catfish, as shown by the colony counts which varied between various sample types and dilutions. The results of the plate count reveal details on the bacterial load in various catfish samples.

The organisms were cultured on MacConkey Agar and Salmonella-Shigella Agar, their various cultural and morphological characteristics were observed along with the results of the API 20E tests. The result identified the organisms as *Enterobacter cloacae, Aeromonas hydrophila, Klebsiella pneumoniae, Citrobacter brakii, Citrobacter freundii, Enterobacter aerogenes* on the skin and in the intestine of *Clarias gariepinus*.

Furthermore, Antimicrobial Susceptibility Testing was carried out on these isolates to identify appropriate antibiotics to be used against these organisms to ensure successful treatment outcomes and also to identify the antibiotics the isolates are resistant to.

The results of the tests on the Gram-negative organisms isolated from Pond catfish showed a high prevalence of antibiotic resistance, the multiple antimicrobial resistance index (MARI) ranged from 0.4 to 0.9. Most of the isolates had multiple antibiotic resistance patterns, which suggested the presence of multidrug-resistant strains. This is a worrying discovery, as it implies that the organisms have created defenses against the effects of widely used antibiotics.

Among the tested isolates, *Enterobacter cloacae* showed resistance to all tested antibiotics except for amikacin, indicating a high level of resistance. *Aeromonas hydrophila* also exhibited resistance to all antibiotics except for amikacin. These findings suggest that these organisms may pose a significant challenge in terms of treatment options. *Klebsiella pneumoniae*, on the other hand showed a mixed pattern of susceptibility and resistance as it was sensitive to Cotrimoxazole, Gentamicin, Chloramphenicol, Amikacin, and Vancomycin, but resistant to Ciprofloxacin, Ceftriaxone, Cefotaxime, and Meropenem.

Notably, *Enterobacter aerogenes* and *Enterobacter cloacae* exhibited a similar resistance pattern being resistant to all tested antibiotics except for amikacin. This suggests a potential similarity in the resistance mechanisms or a common source of resistance genes in these isolates. Studies have emphasized *Enterobacter cloacae* adaptability and its potential to serve as a repository for antibiotic resistance

genes, stressing the significance of preventing the spread of resistant strains from aquatic environments to human populations **[25]**.

Citrobacter brakii and *Citrobacter freundii* displayed resistance to all tested antibiotics except for amikacin. *Citrobacter freundii* showed intermediate susceptibility to Chloramphenicol. These findings highlight the presence of resistance in *Citrobacter species*, which is a concerning trend **[26]**.

77.8% of the tested isolates exhibited resistance to Tetracycline, Ciprofloxacin. Majority of the isolates around 88.9%, displayed resistance to Co-trimoxazole, Gentamicin, Vancomycin, Ceftazidime. All isolates showed complete resistance (100%) to Cefuroxime, Ceftriaxone, Cefotaxime, Meropenem and approximately 66.7% of the tested bacterial strains demonstrated resistance to Chloramphenicol. None of the isolates showed resistance (0%) to Amikacin.

The observed resistance patterns reflect the alarming prevalence of antibiotic resistance among the tested bacterial strains. The high levels of resistance observed with Tetracycline, Ciprofloxacin, Co-trimoxazole, Gentamicin, Vancomycin, and Ceftazidime indicate a significant challenge in treating infections caused by these strains using these antibiotics. The complete resistance displayed by all strains against Cefuroxime, Ceftriaxone, Cefotaxime and Meropenem highlights the urgent need for alternative treatment options.

The absence of resistance observed with Amikacin suggests its potential as an effective antibiotic against the tested strains. Amikacin may serve as a valuable option for treatment when other antibiotics fail due to resistance. This suggests that Amikacin may still be an effective choice for treating bacterial infections caused by these strains. Among all the Aminoglycoside antibiotics, Amikacin is known to develop resistance very slowly because of its complex structure **[27]**. They are therefore regarded as a backup treatment option when all other antibiotic therapy fails. They are also known for their chemical stability, low incidence of resistance, fast bactericidal activity, and relatively lower cost.

The results of the antibiotic susceptibility testing on Gram-negative organisms isolated from Pond catfish align with existing literature on antibiotic susceptibility patterns in similar organisms. Several studies including **[28]** and **[29]**, have reported high levels of antibiotic resistance in *Enterobacter cloacae*, *Enterobacter aerogenes*, *Klebsiella pneumoniae* and *Citrobacter species*. Consistent with these findings, the current study identified resistance to multiple antibiotics in these organisms, highlighting the persistent challenge of antibiotic resistance in aquaculture settings. The absence of resistance to amikacin among the isolates observed in my study contrasts with the findings reported in the study by **[30]**, the antimicrobial response patterns of the selected bacterial isolates indicated that Ciprofloxacin was the most effective antibiotic, while Penicillin was the least effective antibiotic. The resistance patterns observed in this study align with findings from other studies, a study by **[31]** identified *Aeromonas species* with antibiotic resistance patterns in Lake Erie, including resistance to ciprofloxacin and tetracycline which is consistent with the resistance observed in *Aeromonas hydrophila* in the current study. Additionally, a study by **[32]** reported the identification of carbapenem-resistant *Enterobacter aerogenes* producing DHA-1 AmpC enzyme and KPC-2 carbapenemase, indicating a similarity in resistance mechanisms with *Enterobacter cloacae* as observed in the current study. **[33]** detected carbapenemase-producing *Escherichia coli* and *Klebsiella pneumoniae* implicated in urinary tract infections, highlighting the prevalence of antibiotic resistance in *Klebsiella pneumoniae*, consistent with the resistance pattern observed in this current study.

CONCLUSION

Using total plate counts and the effects of dilution, this study examined the variation in bacterial load in various Catfish samples. The outcomes showed that the various catfish samples and dilutions had different colony counts and CFU/ml values. The skin of Large Catfish had the highest bacterial load, whereas the Medium Catfish's intestine had the lowest bacterial load. With increasing dilution, it revealed a decrease in the concentration of viable bacteria. These results show that the microbial populations in the Catfish samples vary.

Biochemical testing revealed the presence of specific organisms, including *Enterobacter cloacae, Aeromonas hydrophila, Klebsiella pneumoniae, Citrobacter brakii, Citrobacter freundii* and *Enterobacter aerogenes* in the skin and intestine of catfish. These organisms exhibited various cultural and morphological characteristics, aiding in their identification.

Antimicrobial susceptibility testing provided important insights into the resistance patterns of the isolated organisms. Multiple resistance to different antibiotics was observed among the tested isolates, indicating the presence of multidrug-resistant strains. *Enterobacter cloacae and Aeromonas hydrophila* showed resistance to all tested antibiotics except for Amikacin. *Klebsiella pneumoniae* displayed a mixed pattern of susceptibility and resistance, while *Citrobacter species* exhibited resistance to most antibiotics.

The prevalence of resistance among the Gram-negative organisms isolated from Pond catfish indicates the need for careful selection and use of antibiotics in aquaculture and the potential transfer of antibiotic resistance genes to humans. The presence of multidrug-resistant strains raises concerns about treatment options for fish infections and the potential dissemination of resistance to human pathogens. Continuous monitoring of antibiotic susceptibility patterns and preventive

measures are necessary to mitigate the spread of antibiotic resistance.

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