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Characterization and Multiple Antibiotic Resistance of Bacterial Isolates Associated with Fish Aquaculture in Ponds and Rivers in Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The transfer of resistant organisms through consumption of contaminated fish and shell fish poses a substantial risk of environmental contamination because of the practice of using medicated feeds to treat pens or cages. However, antibiotics used for animals either for therapy or prophylaxis can result in transfer of resistant genes from animals to humans and thereby establishing a reservoir of resistant microbes. This study was aimed at identifying bacterial isolates associated with fish pond aquaculture and their multiple resistance pattern to antibiotics. Samples of infected catfishes were collected from a fish pond in Aluu, Rivers state. The fishes were characterized by skin lesions which indicate the infection. Sterile swabs were used to swab the lesions on the skins of the fishes. The antibiotic sensitivity of the isolates was determined using the disc diffusion method. Standardized inoculums of the overnight grown broth cultures were spread on Mueller-Hinton agar plates using

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sterile swabs. The plates were dried at room temperature for 2 h before placing the antibiotic discs at equidistance and incubated for 24 h at 37^0C and the diameter of zone of inhibition was measured. Predominant bacterial isolates from the cultures of swabs from the skins of infected fishes on various media were characterized and identified as *Escherichia coli, Pseudomonas putida, Salmonella* sp, *Shigella* sp, *Staphylococcus aureus, Enterobacter* and *Enterococcus fecalis.* The results obtained for antibiotic sensitivity pattern of isolates show that most of the test isolates were resistant to the activities of Ceftazidine (CAZ), Cetriaxone (CRO), Cefotaxine (CTX), Cephalexin (CL) and Tetracycline (TC) while Gentamycin (CN) and Ciproflaxacin (CIP) were highly susceptible to most test isolates. The fact that transfers of resistant bacteria between aquatic animals and humans through consumption or handling of fish can pose a serious hazard to human health. Therefore the presence of multiple drug resistant bacteria from fish and fish handlers do not only poses risk of disease infection to fishes but also public health hazard to fish handlers and consumers in general.

Keywords: Bacteria; fish pond aquaculture; antibiotic resistance; diseases.

1. INTRODUCTION

Epidemics of bacterial disease are common in dense populations of cultured food or aquarium fish. Predisposition to such outbreaks frequently is associated with poor water quality, organic loading of the aquatic environment, handling and transport of fish, marked temperature changes, hypoxia or other stressful conditions [1]. Generally, ponds and rivers that harbor fish may be the source of the microorganisms due to indiscriminate dumping of human and animal excreta as well as other environmental wastes into natural water bodies or washing of excreta from land into water during the rainy season [2]. These wastes are aesthetically unpleasant, constitute eyesores, produce unpleasant odour especially when their organic compositions are acted upon by putrefying bacteria [3]. Free roaming animals especially dogs and birds and other human activities also contribute to fecal contamination of surface water and ponds [4,5,6]. These microorganisms from faeces or environmental waste contain antibiotic resistant gene that may disseminate and contaminate aquatic environment. On the other hand, aquaculture represents one means that supplement wild fish due to the growing demand for fish protein in the population [7]. The advantage of fish as food is as a result of its easy digestibility and high nutritional value. Fish should be viewed not only as food but also as a ready source of income in the smallholder farming sector [8]. Fish production in earth dams or ponds can revive the once abandoned lands and make them productive. Small scale fish production also improves the livelihoods of the communal people and reduces the number of people who always depend on government for economic assistance.

The transfer of resistant organisms through consumption of contaminated fish and shell fish poses a substantial risk of environmental contamination because of the practice of using medicated feeds to treat pens or cages. Antibiotics used for animals either for therapy, prophylactic or growth promotion purposes at a sub therapeutic dose can result in transfer of resistant genes from animals to humans and thereby establishing a reservoir of resistant microbes [9,10]. Potential biological contamination of aquaculture products can occur from bacteria, viruses, parasites and biotoxins [11,12]. The location of the farm, the species being farmed, water temperature, husbandry systems, postharvest processing, and habits in food preparation and consumption are among the main factors influencing the risk associated with aquatic animals and their products. The use of antibiotics in treatment of fishes also contributes to the pool of multidrug resistant bacteria in water bodies. The effluents from these water bodies are usually discharged into other water bodies which can serve as a source of infection to other aquatic animals. Human infections caused by pathogens transmitted from fish or the aquatic environment are quite common depending on the season, patients' contact with fish and related environment, dietary habits and the immune system status of the exposed individual. The infection source may be fish kept either for food or as a hobby [13]. Several genera like *Salmonella, Shigella dysenteriae, Escherichia coli, Clostridium botulinum, Staphylococcus aureus, Listeria monocytogens Yersinia, Brucella, Pseudomonas aeruginosa* has been reported [14]. This study therefore aims at identifying microorganisms associated with fish pond aquaculture and the risk of transfer of

resistant bacteria to humans from consumption of aquaculture products.

2. MATERIALS AND METHODS

2.1 Sample Collection

Samples of infected catfishes were collected from a fish pond in Aluu, Rivers state. The fishes were characterized by skin lesions which indicates the infection. Sterile swabs were used to swab the lesions on the skins of the fishes. The swabs were labelled or coded S_1-S_{10} and transported immediately in a cooler packed with ice blocks to the laboratory for analysis.

2.2 Sample Preparation

Each sample was reconstituted into test tubes containing 9ml of distilled water and shaken vigorously to a homogenous suspension to form a stock solution. Then 1 ml of the suspension was aseptically transferred using a sterile pipette into 9 ml of sterile distilled water in another test tube to give 10^{-1} dilution. The serial dilution was up to 10^{-3}. From the 10^{-3} dilution, 0.1 ml of the sample was inoculated onto nutrient agar, Eosin Methylene blue agar, *Salmonella-Shigella* agar and Cetrimide agar; and spread by using a bent glass spreader. The inoculated plates were incubated at 37ºC for 24 hours to allow colony formation. After which total microbial count was carried out to estimate the total number of colonies per ml. Plates containing 30-300 colonies were counted and expressed as colony forming units (cfu) per milliliter of samples plated.

2.3 Characterization and Identification of Microbial Isolates

Pure cultures of the isolates, isolated using spread plate technique on nutrient agar as described by Prescott et al. [15] were subjected to determinative schemes of Cowan and Steel [16]. The bacterial isolates were identified in accordance with the schemes of Barond Syrney and the Bergey's manual of Determinative bacteriology [17] based on cultural parameters, microscopic technique and biochemical tests including carbohydrate utilization.

2.3.1 Antimicrobial susceptibility testing

The antibiotic sensitivity of the isolates was determined using the disc diffusion method [18,19]. Standardized inoculum of the overnight grown broth cultures was spread on Mueller-Hinton agar plates using sterile swabs. The plates were dried at room temperature for 2 h before placing the antibiotic discs at equidistance. The plates were incubated for 24 h at 37ºC and the diameter of zone of inhibition was measured. Organisms were classified as sensitive, intermediate or resistant, based on the Clinical and Laboratory Standards Institutes (CLSI).

3. RESULTS AND DISCUSSION

3.1 Microbiological Analyses of Infected Fishes

Predominant bacterial isolates from the cultures of swabs from the skins of infected fishes on various media were characterized and identified as *Escherichia coli, Pseudomonas putida, Salmonella* sp, *Shigella* sp, *Staphylococcus aureus, Enterobacter* and *Enterococcus fecalis* (Tables 1-7). A total of 36 bacterial isolates were obtained from the cultures of swabs from the skins of infected fishes. The results show that the microorganisms isolated from the infected fishes had a percentage rate of 7(19%) for *Escherichia coli* and *Pseudomonas putida*; 4 (11%) for *Salmonella sp* and *Shigella sp*; 8 (22%) for *Staphylococcus aureus* while 2(5%) was recorded for *Enterobacter* sp and 5 (13%) for *Enterococcus fecalis* (Fig. 1).

While the prevalence rate for *Escherichia coli* and *Pseudomonas putida* was 70%), *Salmonella* sp. and *Shigella* sp. had 40%, *Staphylococcus aureus* had 80%, while *Enterobacter* sp. had 20% and *Enterococcus fecalis* 50% (Fig. 2).

3.2 The Antimicrobial Susceptibility Testing of the Isolates Using Disc Diffusion Test

Fig. 3 shows the antimicrobial susceptibility pattern of the isolates using disc diffusion test. The results showed that the isolates were resistant to CAZ, CRO, CTX, CL and TC as demonstrated by their zones of inhibition while CN and CIP were susceptible to the isolates. CIP was highly susceptible to *Pseudomonas putida* and *Escherichia coli with* a diameter of zones of inhibition of 23 mm each while *Staphylococcus aureus* and *Enterococcus sp* showed least resistance activity to CIP with a diameter of zones of inhibition of 19 mm each. CN alone was highly susceptible to *Enterobacter sp* with a

diameter of zone of inhibition of 24 mm while *Enterococcus* sp was resistant to CN with a diameter of zones of inhibition of 9 mm.

Fig. 4 demonstrates the antimicrobial effect of antibiotic combinations on the isolates from infected fishes. The results revealed that combinations of some of the drugs increased their efficacy on the test isolates. For instance, a combination of CIP+LIPO showed increased susceptibility on *Staphylococcus aureus* and *Salmonella typhi* which was demonstrated by an increase in the diameter in the zones of inhibition by 22mm and 27mm respectively. Similar activity was observed with a combination of CN+CIP for same isolates. Also, a combination of CTX+CL, CAZ+CL and CRO+CL showed some degree of susceptibility on the test isolates but these antibiotics when used singly the isolate was resistant to all. However, the test isolates showed resistance to a combination of CAZ+CRO.

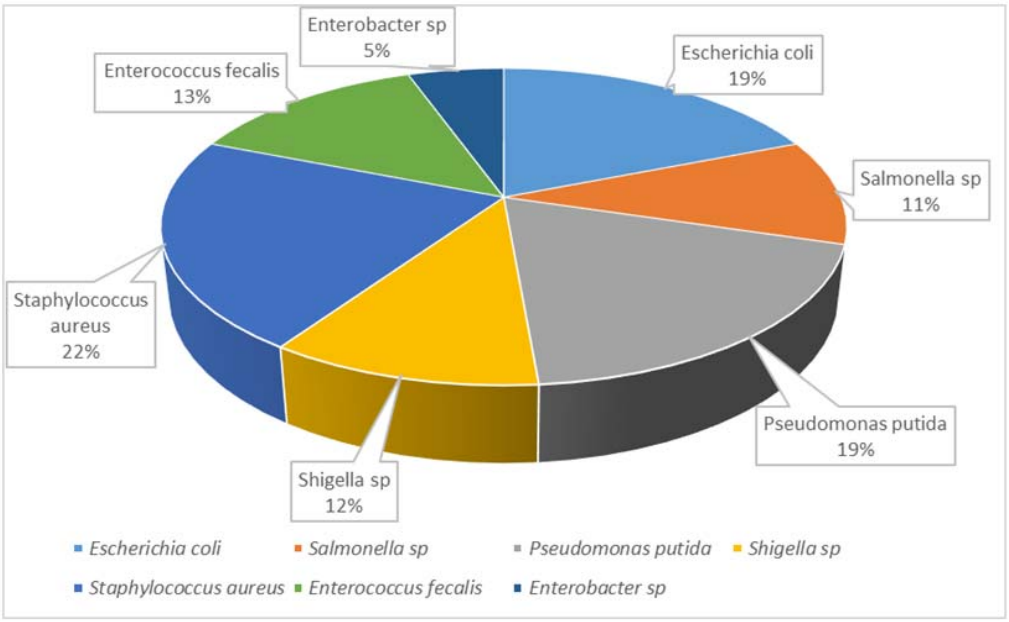


Fig. 1. Percentage rate of occurrence of individual isolates from the infected fishes

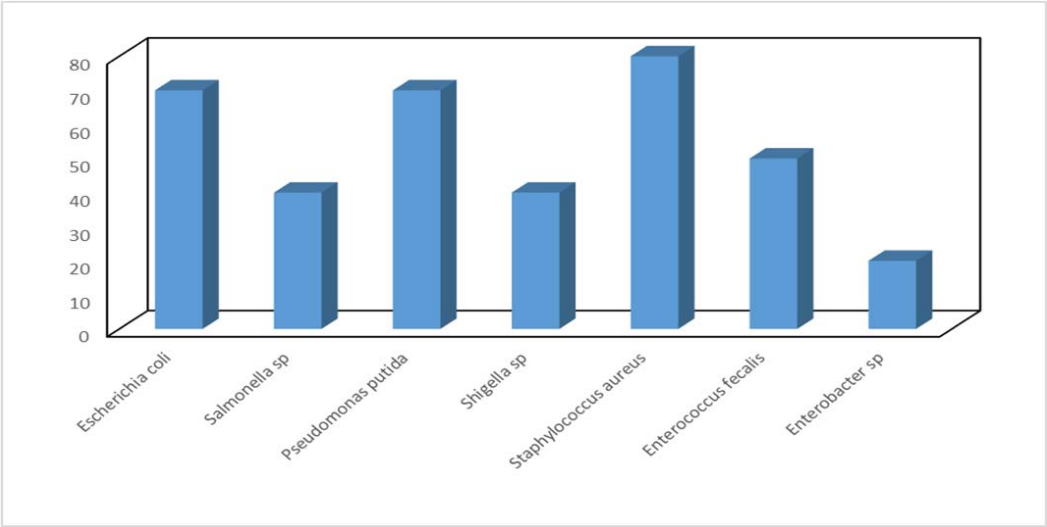


Fig. 2. Prevalence rate of isolates among infected fish

Table 1. Biochemical characteristics of isolates on nutrient agar

Sample code	Colony count (cfu/ml)	Colonial characteristics	Gram reaction	Motility	Spore	Cap	Probable identity
S_1	TNTC	Smooth golden yellow colonies	+	-	-	-	*Staphylococcus sp*
S_2	$2.31x10^6$	Smooth golden yellow colonies	+	-	-	-	*Staphylococcus sp*
		Smooth shiny low convex cream colonies	+	-	-	-	*Enterococcus fecalis*
S_3	$1.76 x10^6$	Smooth shiny low convex cream colonies	+	-	-	-	*Enterococcus fecalis*
S_4	$1.76 x10^6$	Smooth golden yellow colonies	+	-	-	-	*Staphylococcus sp*
S_5	$1.23 x10^6$	Smooth golden yellow colonies	+	-	-	-	*Staphylococcus sp*
		Smooth shiny low convex cream colonies	+	-	-	-	*Enterococcus fecalis*
S_6	$1.44 x10^6$	Smooth golden yellow colonies	+	-	-	-	*Staphylococcus sp*
S_7	$1.16 x10^6$	Smooth golden yellow colonies	+	-	-	-	*Staphylococcus sp*
		Smooth shiny low convex cream colonies	+	-	-	-	*Enterococcus fecalis*
S_8	$1.08 x10^6$	Smooth shiny low convex cream colonies	+	-	-	-	*Enterococcus fecalis*
S_9	$1.08 x10^6$	Smooth golden yellow colonies	+	-	-	-	*Staphylococcus sp*
S_{10}	$9.8 x10^5$	Smooth golden yellow colonies	+	-	-	-	*Staphylococcus sp*

Key. cfu/ml = colony forming units per mililitre; TNTC= Too numerous to count; - Negative; + Positive

Table 2. Biochemical characterization of isolates on EMB agar

Sample code	Colony count (cfu/ml)	Colonial characteristics	Gram reaction	Motility	Spore	Caps	Probable Identity
S_1			NO GROWTH AFTER 24 HRS				
S_2	$1.0x10^4$	Purple metallic sheen	-	+	-	-	*Escherichia coli*
S_3	$3.0 x10^4$	Purple metallic sheen	-	+	-	-	*Escherichia coli*
		Pink mucoid colonies	-	+	-	-	*Enterobacter sp*
S_4			NO GROWTH AFTER 24 HRS				
S_5	$3.0 x10^4$	Purple metallic sheen	-	+	-	-	*Escherichia coli*
		Pink mucoid colonies	-	+	-	-	*Enterobacter sp*
S_6	$3.6 x10^4$	Purple metallic sheen	-	+	-	-	*Escherichia coli*
S_7			NO GROWTH AFTER 24 HRS				
S_8	$4.2 x10^4$	Purple metallic sheen	-R	+	-	-	*Escherichia coli*
S_9	$4.4 x10^4$	Purple metallic sheen	-R	+	-	-	*Escherichia coli*
S_{10}	$1.8 x10^4$	Purple metallic sheen	-R	+	-	-	*Escherichia coli*

Table 3. Biochemical characterization of isolates on *Salmonella- shigella* agar

Sample code	Colony count	Colonial characteristics	Grams reaction	Motility	Spore	Caps	Probable identity
S_1		**NO GROWTH AFTER 24 HOURS**					
S_2	2.1x10^{4}Cfu/ml	Moist, mucoid and shiny light pink colonies	-	-	-	-	*Shigella sp*
		Central black colonies	-	+	-	-	*Salmonella sp*
S_3	1.6 x10^{4}Cfu/ml	Moist, mucoid and shiny light pink colonies	-	-	-	-	*Shigella sp*
S_4	1.3 x10^{4}Cfu/ml	Moist, mucoid and shiny light pink colonies	-	-	-	-	*Shigella sp*
S_5	1.2 x10^{4}Cfu/ml	Central black colonies	-	+	-	-	*Salmonella sp*
S_6		**NO GROWTH AFTER 24 HOURS**					
S_7	1.1 x10^{4}Cfu/ml	Central black colonies	-	+	-	-	*Salmonella sp*
S_8	8 x10^{4}Cfu/ml	Moist, mucoid and shiny light pink colonies	-	-	-	-	*Shigella sp*
S_9	1 x10^{4}Cfu/ml	Central black colonies	-	+	-	-	*Salmonella sp*
S_{10}		**NO GROWTH AFTER 24 HOURS**					

Table 4. Biochemical characterization of bacterial isolates on cetrimide agar

Sample code	Colony count	Colonial characteristics	Motility	Gram stain	Spore	Cap	Probable identity
S_1	1.2x10^4	Small circular creamy white colonies	+	-	-	-	*Pseudomonas sp*
S_2	1.8x10^4	Small circular creamy white colonies	+	-	-	-	*Pseudomonas sp*
S_3	1.6x10^4	Small circular creamy white colonies	+	-	-	-	*Pseudomonas sp*
S_4		**NO GROWTH AFTER 48 HOURS**					
S_5		Small circular creamy white colonies	+	-	-	-	*Pseudomonas sp*
S_6		Small circular creamy white colonies	+	-	-	-	*Pseudomonas sp*
S_7		**NO GROWTH AFTER 48 HOURS**					
S_8		Small circular creamy white colonies	+	-	-	-	*Pseudomonas sp*
S_9		Small circular creamy white colonies	+	-	-	-	*Pseudomonas sp*
S_{10}		**NO GROWTH AFTER 48 HOURS**					

Table 5. Biochemical characteristics of bacterial isolates from nutrient agar

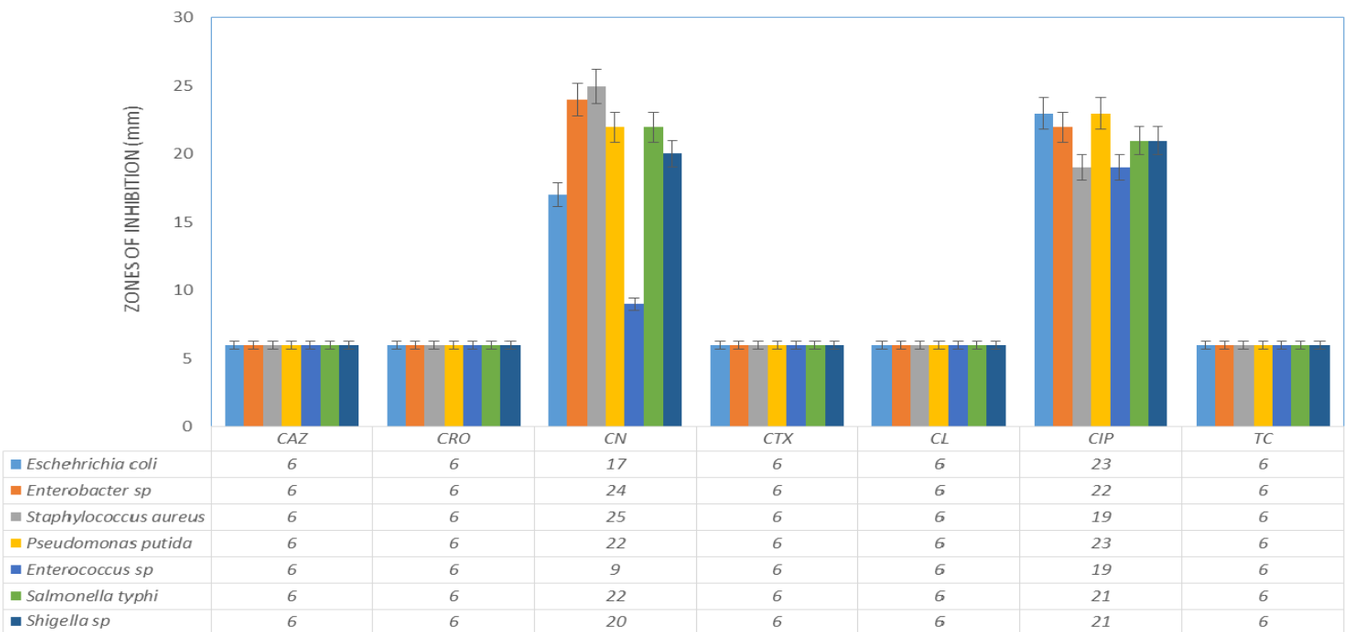
Colony code	Motility	Spore	Catalase	Oxidase	Coagulase	Indole	MR	VP	Citrate	Urea	NO_3	H_2S	Sucr	Lact	Mal	Mann	Gluc	Identity of isolates
S_1X	-	-	+	-	+	-	-	+	-	+	+	-	+	+	+	+	+	*Staphylococcus aureus*
S_2X	-	-	+	-	+	-	-	+	-	+	+	-	+	+	+	+	+	*Staphylococcus aureus*
S_2Y	-	-	+	-	-	-	+	-	+	-	+	-	+	+	+	+	+	*Enterococcus fecalis*
S_3X	-	-	+	-	-	-	+	-	+	-	+	-	+	+	+	+	+	*Enterococcus fecalis*
S_4Y	-	-	+	-	+	-	-	+	-	+	+	-	+	+	+	+	+	*Staphylococcus aureus*
S_5X	-	-	+	-	+	-	-	+	-	+	+	-	+	+	+	+	+	*Staphylococcus aureus*
S_5Y	-	-	+	-	-	-	+	-	+	-	+	-	+	+	+	+	+	*Enterococcus fecalis*
S_6X	-	-	+	-	+	-	-	+	-	+	+	-	+	+	+	+	+	*Staphylococcus aureus*
S_7X	-	-	+	-	+	-	-	+	-	+	+	-	+	+	+	+	+	*Staphylococcus aureus*
S_7Y	-	-	+	-	-	-	+	-	+	-	+	-	+	+	+	+	+	*Enterococcus fecalis*
S_8X	-	-	+	-	-	-	+	-	+	-	+	-	+	+	+	+	+	*Enterococcus fecalis*
S_9X	-	-	+	-	+	-	-	+	-	+	+	-	+	+	+	+	+	*Staphylococcus aureus*
$S_{10}X$	-	-	+	-	+	-	-	+	-	+	+	-	+	+	+	+	+	*Staphylococcus aureus*

Table 6. Biochemical characteristics of bacterial isolates from EMB agar and Cetrimide agar.

Col code	Motility	Spore	Catalase	Oxidase	Coagulase	Indole	MR	VP	Citrate	Urea	NO_3	H_2S	Sucr	Lact	Mal	Mann	Gluc	Identity of isolates
S_2A	+	-	+	-	-	+	+	-	-	+	+	-	+	+	+	+	+	*Escherichia coli*
S_3A	+	-	+	-	-	+	+	-	-	+	+	-	+	+	+	+	+	*Escherichia coli*
S_3B	+	-	+	-	-	-	-	+	+	Nd	nd		+	+	+	+	+	*Enterobacter sp*
S_5A	+	-	+	-	-	+	+	-	-	+	+	-	+	+	+	+	+	*Escherichia coli*
S_5B	+	-	+	-	-	-	-	+	+	Nd	nd		+	+	+	+	+	*Enterobacter sp*
S_6A	+	-	+	-	-	+	+	-	-	+	+	-	+	+	+	+	+	*Escherichia coli*
S_8A	+	-	+	-	-	+	+	-	-	+	+	-	+	+	+	+	+	*Escherichia coli*
S_9A	+	-	+	-	-	+	+	-	-	+	+	-	+	+	+	+	+	*Escherichia coli*
$S_{10}A$	+	-	+	-	-	+	+	-	-	+	+	-	+	+	+	+	+	*Escherichia coli*
S_1D	+	-	+	-	-	-	+	-	+	+	+	-	-	-	-	+	+	*Pseudomonas putida*
S_2D	+	-	+	-	-	-	+	-	+	+	+	-	-	-	-	+	+	*Pseudomonas putida*
S_3D	+	-	+	-	-	-	+	-	+	+	+	-	-	-	-	+	+	*Pseudomonas putida*
S_5D	+	-	+	-	-	-	+	-	+	+	+	-	-	-	-	+	+	*Pseudomonas putida*
S_6D	+	-	+	-	-	-	+	-	+	+	+	-	-	-	-	+	+	*Pseudomonas putida*
S_8D	+	-	+	-	-	-	+	-	+	+	+	-	-	-	-	+	+	*Pseudomonas putida*
S_9D	+	-	+	-	-	-	+	-	+	+	+	-	-	-	-	+	+	*Pseudomonas putida*

Table 7. Characterization of isolates on *Salmonella - Shigella* Agar

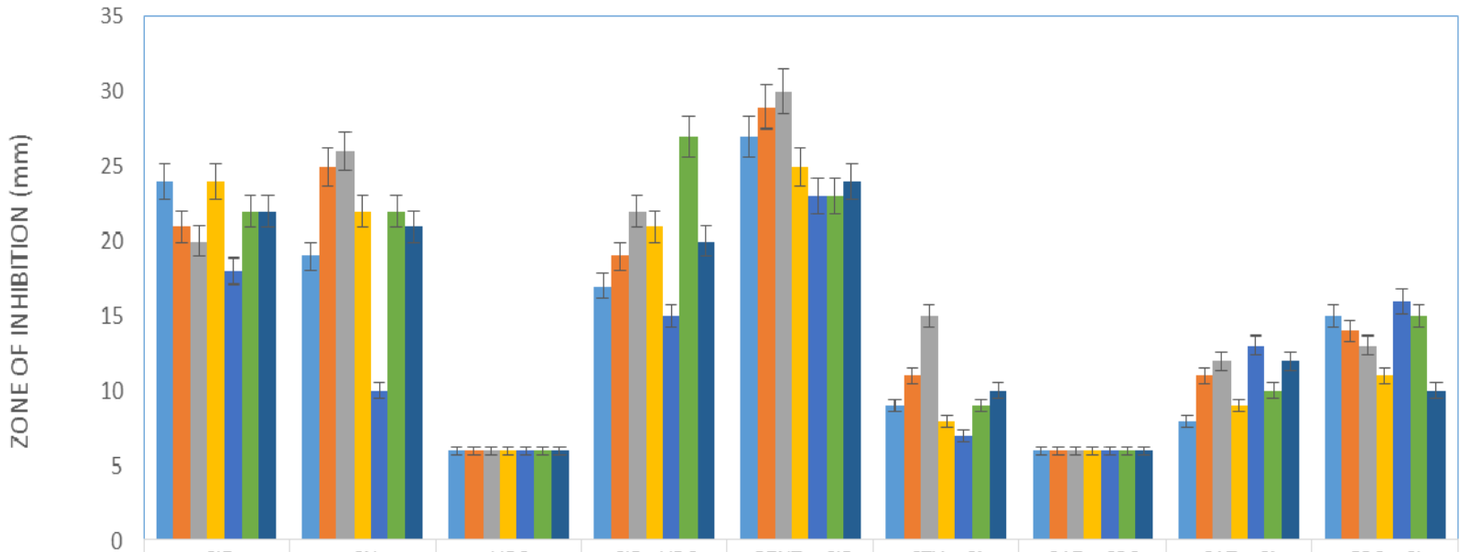
Col code	Motility	Spore	Catalase	Oxidase	Coagulase	Indole	MR	VP	Citrate	Urea	NO_3	H_2S	Sucr	Lact	Mal	Mann	Gluc	Identity of isolates
S_2B	-	-	+	-	-	-	+	-	-	+	-	-	-	-	+	-	-	*Shigella sp*
S_2C	+	-	+	-	-	-	-	+	+	Nd	-	+	-	-	-	+	+	*Salmonella spp*
S_3B	-	-	+	-	-	-	+	-	-	+	-	-	-	-	+	-	-	*Shigella sp*
S_4B	-	-	+	-	-	-	+	-	-	+	-	-	-	-	+	-	-	*Shigella sp*
S_5B	+	-	+	-	-	-	-	+	+	Nd	-	+	-	-	-	+	+	*Salmonella spp*
S_7B	+	-	+	-	-	-	-	+	+	Nd	-	+	-	-	-	+	+	*Salmonella spp*
S_8B	-	-	+	-	-	-	+	-	-	+	-	-	-	-	+	-	-	*Shigella sp*
S_9B	+	-	+	-	-	-	-	+	+	Nd	-	+	-	-	-	+	+	*Salmonella spp*



	CAZ	CRO	CN	CTX	CL	CIP	TC
Eschehrichia coli	6	6	17	6	6	23	6
Enterobacter sp	6	6	24	6	6	22	6
Staphylococcus aureus	6	6	25	6	6	19	6
Pseudomonas putida	6	6	22	6	6	23	6
Enterococcus sp	6	6	9	6	6	19	6
Salmonella typhi	6	6	22	6	6	21	6
Shigella sp	6	6	20	6	6	21	6

Fig. 3. Antibiogram of test isolates from infected fishes

Key: CAZ= Ceftazidine, CRO= Cetriaxone, CN=Gentamycin, CTX=Cefotaxine, CL=Cephalexin, CIP=Ciproflaxacin, TC= Tetracycline



	CIP	CN	LIPO	CIP+ LIPO	GENT + CIP	CTX + CL	CAZ + CRO	CAZ + CL	CRO+ CL
Eschehrichia coli	24	19	6	17	27	9	6	8	15
Enterobacter sp	21	25	6	19	29	11	6	11	14
Staphylococcus aureus	20	26	6	22	30	15	6	12	13
Pseudomonas putida	24	22	6	21	25	8	6	9	11
Enterococcus sp	18	10	6	15	23	7	6	13	16
Salmonella typhi	22	22	6	27	23	9	6	10	15
Shigella sp	22	21	6	20	24	10	6	12	10

Fig. 4. Antimicrobial effect of antibiotic combinations on the isolates from infected fishes

Key: CAZ= Ceftazidine, CRO= Cetriaxone, CN=Gentamycin,CTX=Cefotaxine,CL=Cephalexin,CIP=Ciproflaxacin, TC= Tetracycline, LIPO = Lipocaine

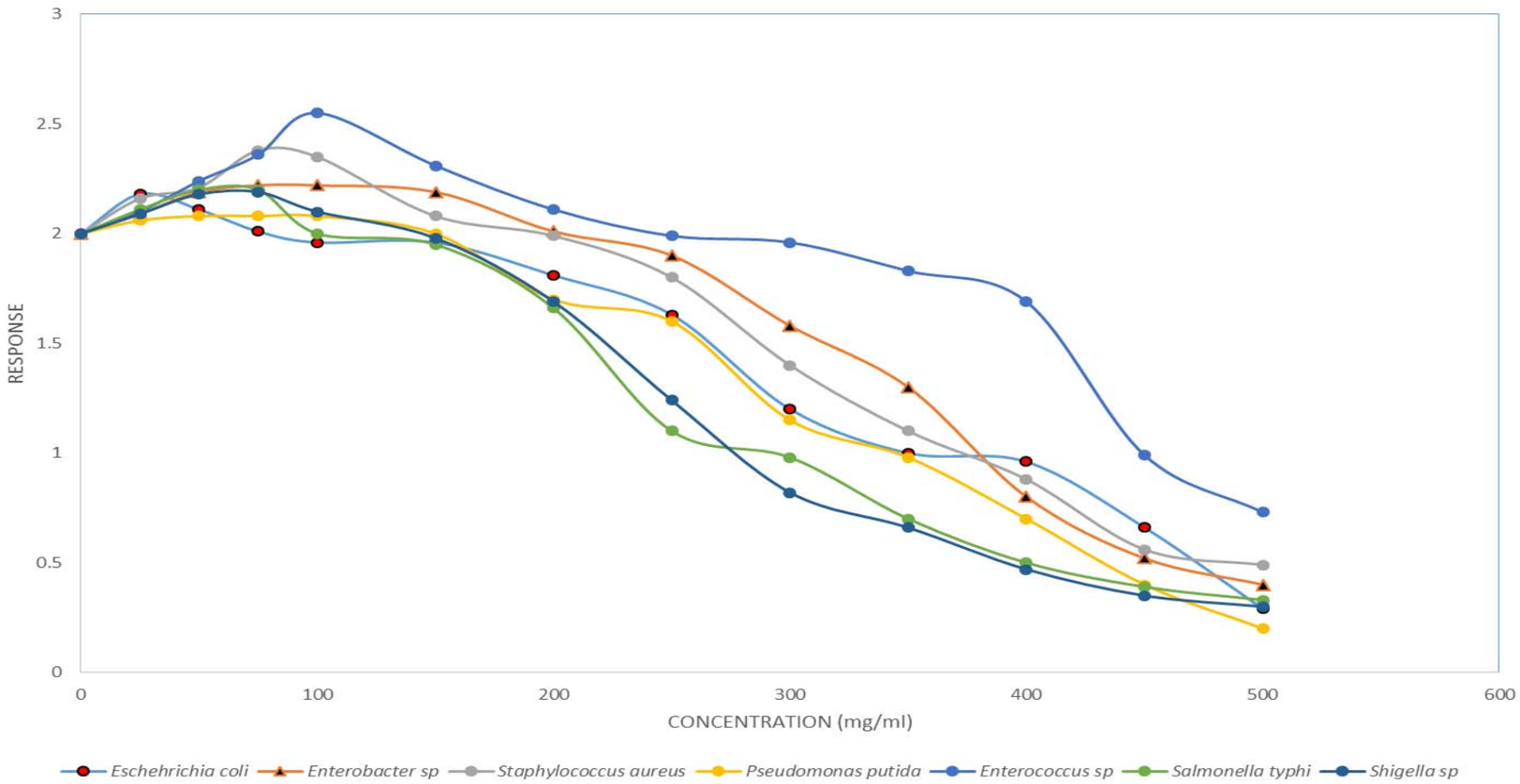


Fig. 5. Response of bacterial isolates to increased dosage of ciprofloxacin

3.3 The Response of Bacterial Isolates to Increased Dosage of Ciprofloxacin

The response of the bacterial isolates to increased dosage of ciprofloxacin was determined and results shown in Fig 5. The results revealed that increased concentration of the antibiotic caused a decrease in the microbial population. This means that there was a decrease in the population of the test isolates with an increase in the concentration of the antibiotic. However, antibiotic concentrations of 0-150 mg/ml, was resistant to most of the bacterial isolates. *Enterococcus* sp showed resistance at this concentration while the concentration was susceptible to *Escherichia coli.*

4. DISCUSSION

Fishes harbour a lot of pathogens which could be as a result of their contact with different streams of their aquatic life. These pathogens are sometimes harmful to the fishes as well as the end consumer [20]. Most of the fish diseases might occur as a result of parasitic infection or environmental pollution [21]. As the consumption of aquaculture products increase, the possibility of contracting zoonotic infections from either handling or ingesting fish products also increases. These microorganisms such as Escherichia *coli*, *Pseudomonas putida, Salmonella sp*, *Shigella sp*, *Staphylococcus aureus*, *Enterobacter sp* and *Enterococcus fecalis* obtained from the study demonstrates that fishes can be potential sources of various infectious diseases. According to Lyhs [22]. Species of *Salmonella, Shigella, Escherichia coli* and *Staphylococcus aureus* are enteric pathogens and are usually found in fishes as a result of fecal contamination. On the other hand, *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium perfringens* and *Salmonella* spp can be found in fish products as a result of bacterial contamination during processing, storage or preparation for consumption. *Pseudomonas* species are predominantly environmental isolates and could be part of the transient microflora in the body of the fishes.

Aquatic environments serves as major reservoirs of *Salmonella* species therefore, fishery products have been reported as carrier of food-borne pathogens [23,24]) *Salmonella* infections of fish products can occur as a result of surface water run-offs, some animals (domestic animals, frogs, rodents, birds, insects, reptiles who act as vectors etc.), contaminated feed and fertilization of ponds Studies indicate that fresh fishes, fish meal, oysters or fishes from ponds as well as imported frozen shrimp and frog legs can be a major source of Salmonella species particularly if they are caught in areas contaminated with faecal pollution prior to harvest and during processing, packaging, storage or distribution under unsanitary conditions and consumed raw or poorly cooked [25,26,27]. According to WHO [28], monitoring and surveillance of antibiotic resistant bacteria in animals intended for human consumption is important for the regulation of antibiotic resistance and to detect trends and changes of their resistance patterns. The results obtained from this study revealed that most of the test isolates were resistant to the activities of CAZ, CRO, CTX, CL and TC while CN and CIP were susceptible to most test isolates. The cases of antibiotic resistance of bacterial isolates are not new and the rate of production of multiple drug resistant isolates is alarming over the past two decades. The antibiotic resistance of test bacterial isolates to about five antibiotics tested supports earlier reports of Overdevest et al. [29], who observed that the Enterobacteriaceae has increased its resistance pattern dramatically during the past decade. Possible influences to the emergence of this multiple drug resistant strains could be due to transfer of resistant bacteria between aquatic animals and humans through food production line especially in fish and fish handlers [30,31,32]. One way of combating antibiotic resistant strains of bacteria is through synergistic drug action. For instance, a combination of CTX+CL, CAZ+CL, CRO+CL in this study, was susceptible to the test isolates despite the fact that these antibiotics were resistant when used singly. Also, the concentration of the drug used played a major role in the action of the antibiotic on the test isolates. Fig. 5 shows that for lower concentrations of 0-150 mg/ml, most of the bacterial isolates were resistant to the treatment of the antibiotic at that concentration range but as the concentration increases, the population of the microorganisms tested decreased. This indicates that there is the need for the right dosage of drug required for the treatment of the diseases to be administered accordingly without abuse against the test isolates. This measure will enhance antibiotic action against the microorganisms responsible for such diseases.

5. CONCLUSION

From the results obtained in this study, antibiotics resistant bacteria are widespread as nearly all the isolated microorganisms were resistant to most of the antibiotics for which they were tested for. This may be due to either the intrinsic resistance of many microorganisms to antibiotics or acquired resistance of the organisms enabled by the transfer of resistance of drug resistance plasmids among members of the isolates. Since antibiotics in animal feed promote animal growth, improved efficiency of feed conversion to body weight, and may also affect disease prophylaxis among the confined microbes in such animals and their subsequent impact on human health, it has increased its indiscriminate use [33].

Some researchers opined for continuous monitoring and surveillance of antimicrobial resistance in microorganisms associated with aquatic animals [34]. This is important due to the fact that transfer of resistant bacteria between aquatic animals and humans through consumption or handling can pose a serious hazard to human health [35]. Therefore the presence of multiple drug resistant bacteria from fish and fish handlers do not only poses the risk of disease infection to the fishes but also public health hazard to fish handlers and consumers in general [36].

Finally, the results of this work show that the use of gentamycin and ciprofloxacin can be efficient in treating diseases resulting from any of the bacterial isolates tested and combination of drugs can also increase the potency and effectiveness of the drug on the test isolates. Treatment with lower concentrations of the drugs may contribute to resistance. Therefore there is the need for the right dosage of drug required for the treatment of the diseases to be administered and avoid abuses or self-medication.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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