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IDENTIFICATION AND ANTIBIOTIC PROFILIING OF GRAM-NEGATIVE BACTERIA ISOLATED FROM CULTURED CATFISH (*Clarias anguillaris* Linnaeus 1758 and *Heterobranchus bidorsalis* Geoffroy 1809)

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ABSTRACT
The present study was conducted to isolate and identify gram-negative bacilli from some African catfish species and to evaluate the antimicrobial susceptibility of the isolates with a view of making suggestions on treatments. One hundred and four apparently healthy catfishes (Clarias anguillaris and Heterobranchus longifilis) were obtained where samples for bacterial cultures from the skin and gastrointestinal tract of the fish. Bacteria species were identified using standard culture method and biochemical tests respectively. This was followed by antimicrobial susceptibility test for the bacteria identified using agar disc diffusion method. A total of 8 genera and 10 species of non-enteric gram-negative rods, and 6 genera and 7 species of Enterobacteriaceae were identified. Biochemical variations were observed within the following species: Klebsiella pneumoniae, Proteus mirablis, Enterobacter agglomerans, Aeromonas hydrophila, Alcaligenes faecalis type II, Flavobacterium menongosepticum, Vibrio alginolyticus, Pseudomonas fluorescens-25 and Pseudomonas fluorescens-35. Multidrug resistance to two or more antibacterial agents was observed for 89% of the isolates tested. Specifically, high susceptibility rates to ciptrofloxacin (93%) and gentamycin (75%) by the isolates were observed. Drug preparations containing ciprofloxacin and gentamycin could be recommended for use in fish culture for bacterial diseases incriminated by these bacteria.

Keywords: Antimicrobials, Bacteria, Characterizations, Susceptibility, Catfish

INTRODUCTION

The ubiquitous nature of bacteria (obligate and opportunistic pathogens) makes epidemic diseases common in densely populated, cultured fish. However, there are non-pathogenic bacteria of fish that are rather beneficial to the fish. The occurrence of these bacterial pathogens could be on and/or inside the host or in the environment surrounding the host (Anja, *et al.*, 2000). The economically significant group of bacteria, especially gram-negative bacteria, constitutes a serious threat to fish and is responsible for high mortalities in both cultured and wild fishes worldwide (Muniruzzaman and Chowdhury, 2004). Bacterial pathogens are among the frequently encountered causes of infectious diseases in stressed condition due to deteriorating water quality, handling or transportation of the fish. The health of the fish and its yield depend on the quality of the water in which the fish lives and absence of sources of stress. According to Adeyemo *et al*. (2009), cultured fish are liable to disease outbreaks that are frequently associated with poor water quality, handling and transport of fish, marked temperature and pH changes, hypoxia, and other stressful conditions. The interaction of fish (host), the pathogen and the environment (water) is unavoidable and thus unfavorable changes in the environment may pose stress to the fish which may compromise the immune system of the fish; and when the immune system is compromised both obligate and opportunistic pathogens become a threat and are capable of causing diseases with low to high mortality.

Most bacterial pathogens of fish are aerobic, gram-negative rods (Taranzo, et al. 2005). The type and number of microorganisms that live in fish vary according to the season, the species and the natural habitat. Blind treatment of fish has been common which has attracted global concern over the development of drug resistance and drug residues in the food producing animal (FAO, 2005). Therefore, proper diagnosis by isolation and identification of the bacterial pathogens, and antimicrobial sensitivity testing of the isolated bacteria is paramount. Antibiotic sensitivity testing before the administration of antibiotics is recommended by CLSI (2014) to avoid the chance of developing drug resistance, most especially multi-drug resistance (MDR). MDR is a common phenomenon in fish and their habitats where different antibiotics are used indiscriminately (Grema, *et al.*, 2015).

Despite the indiscriminate use of antibiotics and the global concern over the development of drug resistance in fish farming, there is paucity of information on the bacteria involved and their antibiotic profiles. Therefore, the objectives of the study were to isolate and identify gram-negative bacilli from catfish and to evaluate their antibiotic sensitivity tests for common antibiotics used in treatment of bacterial diseases of cultured catfish.

MATERIAL AND METHODS

Experimental fish sample

The fish for this study were obtained within Makurdi metropolitan. The fish used were apparently healthy. The fish samples were transported and were stabilized for 7 days. The experiment was conducted in Microbiology laboratory, Department of Veterinary Microbiology University of Agriculture, Makurdi, Benue State, Nigeria. Makurdi town is located between latitude 7°38'N - 7°50'N, and longitude 8°24'E - 8°38'E with elevation is 104m above sea level (Abah, 2013). The city is the capital of Benue state, Nigeria, and the headquarters of Makurdi Local Government Area which is located in the middle belt along the Benue River.

Collection of fish sample

One hundred and four (104) apparently health catfishes (*Clarias anguillaris* and *Heterobranchus longifilis*) were obtained for sample collection for isolation of bacteria from the skin and gastrointestinal tract of the fish as described by Abareethan and Amsath (2015). In the field, the fish species were identified with the aid of schematic diagram according to Olaosebikan and Raji (2004). The fish were transported live to the laboratory inside 10 litre plastic Jery-can. In the laboratory, the fish were euthanized by administering over-dose of trichloromethane ($CHCl_3$) to them. Each fish was placed on a disinfected table inocula were collected from two places on the skin using sterile wire loop. Thereafter the skin was swabbed with 75% ethanol before incising the abdomen with sterile scalpel blade to exteriorize the gastrointestinal tract. The intestine was incised longitudinally to expose the intestinal mucosa, and samples were collected with sterile swab sticks for culture.

Culturing of samples

Samples from fish were directly plated out on brain-heart infusion agar (BHIA) (LAB 048, UK), and MacConkey agar (CM 0007, Toxoid, England). Pure isolates were obtained through repeated sub-culture on Nutrient agar which were incubated at 37°C for 18 - 24 hours aerobically. The cultured plates were examined visually for growth of colonies of bacteria overnight. Presumptive distinct colonies were subsequently streak-purified on nutrient agar (Accumix™ Belgium) and incubated overnight. After incubation, the pure plates were examined microscopically to determine their gram-reactions by subjecting them to Gram-staining technique; and the gram-negative rods were stock-cultured on brain-heart infusion agar slants for further studies.

Identification of bacterial isolates

All the isolated gram-negative rods were identified to species level by standard biochemical test using Microbact™ (Oxoid) gram-negative bacteria identification kit following the instructions of the manufacturer. The kit consist of two separate substrate strips (12A and 12B), each consisting of 12 different biochemical substrates. Bacterial isolates for biochemical tests were prepared from colonies (1 - 3) from fresh cultures of each of the stock-cultures on BHIA. Colonies were suspended in 5mls of sterilized distilled water and thoroughly mixed to obtain a homogeneous suspension before use. Oxidase test was carried out to separate *Enterobacteriaceae* (oxidase-negative) from other gram-negative rods (oxidase-positive) before inoculating the strips. The strips were then incubated at 37°C for 18-24 hours. The octal digits obtained were entered into Microbact™ identification software for interpretation. The highest percentage of identification from the software was regarded the closest species identified.

Antimicrobial susceptibility testing

Thirteen of the isolates were used for this test. The sensitivity test was done by using the agar disk diffusion method (Quinn *et al.*, 1994) on Mueller-Hinton agar. The seeded plates with compliments of the test antimicrobial agents were incubated aerobically at 37°C for 24 hours. The antimicrobial agents tested include ciprofloxacin (5 µg), ampicillin (20 µg), gentamycin (10 µg), tetracycline (30 µg), chloramphenicol (20 µg) and streptomycin (10 µg). The diameters of the zones of inhibition were measured to the nearest mm using a meter rule. An interpretation of the size of the zones of inhibition was made with reference to CLSI (2014) recommendations.

RESULTS

The result of the characterization of the bacteria isolates are presented in Table 1 which shows the biochemical characteristics of species of non-enteric Gram negative rods and enterobacteriaceae identified from the skin and gastrointestinal tract of catfish using Gram negative bacteria identification kit ™ (Toxoid). The Gram negative bacteria isolates were grouped into non-enteric and enterobacteriaceae based on the biochemical characterization.

Table 1: Biochemical Characteristics of Non-enteric Gram negative bacilli and Enterobacteriaceae Isolated from Catfish

Bacterial species	Nitrate	Lysine	Ornithine	H_2S	Glucose	Mannitol	Xylose	ONPG	Indole	Urease	V-P	Citrate	TDA	Gelatin	Malonate	Inositole	Sorbitol	Rhamnose	Sucrose	Lactose	Arabinose	Adonitol	Raffinose	Salicin	Arginine
Non-enteric Gram negative Bacilli																									
Aeromonas hydrophila	+/−	−	−	−/+	+	+/−	−	+/−	+	−/+	+	+	−/+	+/−	+	−	−/+	−/+	+/−	+/−	+/−	−	−/+	−	+/−
Alcaligenes faecalis type II	−	−/+	−	−	−	−	−/+	−	−	−	+	−/+	−/+	−	+	−	−/+	−	−	−	−	−	−	−	−
Burkholderia cepacia	−	+	−	−	−	−	+	−	−	−	+	−	+	−	+	−	−	−	−	−	+	−	−	−	−
Flavobacterium menongosepticum	−/+	−	−	−	−/+	−/+	−	+/−	+	−/+	+	−/+	−	−/+	+	−	−	+/−	−	−	+/−	−	−	−	−
Flavobacterium odoratus	−	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
Xanthomonas maltophilia	−	−	−	−	−	−	−	+	−	−	+	+	−	−	+	−	−	−	−	−	+	−	−	−	−
Morexella species	+	−	−	−	−	−	−	−	−	+	+	+	−	−	+	−	−	−	−	−	−	−	−	−	−
Vibrio alginolyticus	−	−	−	−	+/−	−	−	+/−	+	−	+	+/−	−	+/−	+	−	−	+/−	−	−	+/−	−	−	−	−
Pseudomonas fluorescens-25	−	+/−	−	−	+/−	−	+	−	−/+	−/+	+	+/−	−/+	−	+	−	−	+/−	−	−	+	−	−	−	−
Pseudomonas fluorescens-35	−	−	−	−	−	−	−−	−	−	−	+	−	−	−	+	−	−	+	−	−	+	−	−	−	−
Enterobacteriaceae																									
Klebsiella pneumoniae	+	+	−	+	+	+	+/−	+	−	+	+	+	+/−	−	−/+	+	+	+	+	+	+	+	+	+	−/+
Morganella morganii	−	−	−	−	+	−	+	−	−	+	+	+	−	−	−	−	−	−	−	−	+	−	−	−	−
Proteus vulgaris	+	−	−	−	−	−	−	+	−	+	+	−	−	−	+	−	−	−	−	−	+	−	−	−	−
Proteus mirabilis	+	−	−	−/+	−/+	−	+	−/+	−	+/−	+	−/+	+/−	−/+	−/+	−	−	−/+	−	−	+	−/+	−	−	−
Serretia rubidaea	+	+	−	+	+	+	+	+	−	−	+	+	−	−	−	−	+	+	+	−	+	−	−	−	−
Hafnia alvaei	+	+	−	−	−	−	+	+	−	−	+	−	−	+	−	−	−	+	−	−	+	−	−	−	−
Enterobacter agglomerans complex	+	−	−	−	+/−	−/+	+/−	+/−	+/−	+/−	+/−	+/−	+/−	−/+	+/−	−/+	−/+	+/−	−	−/+	+	−	−/+	−/+	−/+

Notes: +/− = Variable (mostly +), −/+ = Variable (mostly −)

A total 79 species were identified from 104 catfish sampled in this study (Table 2). Thirty one (31) species were identified as non-enteric Gram-negative rods, while forty eight (48) were enterobacteriaceae (table 3). Fifteen species of non-enteric and twenty five (25) enterobacteriaceae were identified from the skin samples. While 16 and 25 species were non-enteric and enterobactericeae respectively identified from intestinal tract of the catfish sampled. The intestinal tract recorded high bacterial species compared to the skin. A total of 8 genera yielding 10 species of non-enteric Gram-negative rods and 6 genera yielding 7 species of enterobacteriecae were identified.

The occurrences of non-enteric Gram negative bacteria in the order of frequency is presented in Table 2. They include: *Pseudomonas fluorescens-25*(9), Aeromonas *hydrophila* (7), *Flavobacterium menongosepticum* (4), *Alcaligenes faecalis* type II (3). *Pseudomonas fluorescens-35* and *Vibrio alginolyticus* occurred only two times each in this study. *Morexella species, Burkholderia cepacia, Xanthomonas maltophilia* and *Flavobacterium odoratus occurred only once in the study. On the other hand the Enterobacteriaceae* in the order of frequency of occurrence are as follows: *Enterobacter agglomeran complex* (7), *Klebsiella pneumonia* (4), *Proteus mirablis* (2), and *Hafnia alvaei, Morganella morganii ssp morganii, Proteus vulgaricis, Serretia rubidaea* appeared only once.

Table 2: Bacteria species isolated from skin and gastrointestinal tract of catfish from cultured facilities

Bacterial Isolates	Isolation sites		Total
	Skin	Intestinal tract	
Non-enteric Gram-negative rods			
Aeromonas hydrophila	4	3	7
Alcaligenes faecalis type II	2	1	3
Pseudomonas fluorescens-25	4	5	9
Pseudomonas fluorescens-35	1	1	2
Vibrio alginolyticus	-	2	2
Morexella sp	-	1	1
Burkholderia cepacia	1	-	1
Xanthomonas maltophilia	-	1	1
Flavobacterium meningosepticum	2	2	4
Flavobacterium odoratus	1	-	1
Sub-total	**15**	**16**	**31**
Enterobacteriaceae			
Hafnia alvaei	-	1	1
Klebsiella pneumonia	1	3	4
Morganella morganii ssp *morganii*	-	1	1
Proteus mirablis	1	1	2
Proteus vulgaricis	1	-	1
Enterobacter agglomeran complex	5	2	7
Serretia rubidaea	-	1	1
Sub-total	**8**	**9**	**17**
Grand Total	**23**	**25**	**48**

There were biochemical variations observed within the species of *K. pneumoniae*, *Proteus mirablis* and *Enterobacter agglomerans* complex (Table 3). Thus 3 biochemically different biovars of *Klebsiella species*, 2 biovars of *P. mirablis* and 7 biovars of *Enterobacter agglomerans* complex were identified in this study. The non-enteric bacilli recorded more biochemical variation compared to those of enterobactericeae. Biovars were observed among *A. hydrophila* (4), *Alcaligenes fecalis type II* (3), *F. meningosepticum* (4), *Vibrio alginolyticum* and *P. fluorescens-35* recorded 2, while the highest variation was observed in P. fluorescens-25 (6).

Table 3: Species/biovars of bacteria isolated from catfish

	Bacterial species	No. of isolates	No. of biovars
Enterobacteriaceae	*Klebsiella pneumoniae*	4	3
	Morganella morganii	1	-
	Proteus vulgaris	1	-
	Proteus mirabilis	2	2
	Serretia rubidaea	1	-
	Hafnia alvaei	1	-
	Enterobacter agglomerans complex	7	7
	Total	**17**	**12**
Non-enteric gram negative bacilli	*Aeromonas hydrophila*	7	4
	Alcaligenes faecalis type II	3	3
	Burkholderia cepacia	1	-
	Flavobacterium menongosepticum	4	4
	Flavobacterium odoratus	1	-
	Morexella species	1	-
	Xanthomonas maltophilia	1	-
	Vibrio alginolyticus	2	2
	Pseudomonas fluorescens-25	9	6
	Pseudomonas fluorescens-35	2	2
	Total	**31**	**21**

The result of antibiotic sensitivity tests for the enterobacteriaceae is presented in Table 4. The isolates were subjected to: Ciprofloxacin (CIP), Ampicillin (AMP), Gentamycin (CN), Tetracycline (TE), Chloramphenicol (C) and Streptomycin (S). *Enterobacter agglomerans* complex, *K. pneumoniae*, *H. alvaei* and *S. rubidaea* were highly sensitive to CIP and CN among the 6 Isolates of enterobacteriaceae tested showing 92% and 62% respectively. Low susceptibility rates were recorded for AMP (31%), TE (46%), C (46%) and S (23%). The susceptibility pattern of the isolates identified showed that *Enterobacter agglomerans* species were susceptible to all the drugs used in this study. *Klebsiella species* were resistant to AMP and S. *Proteus* species were resistant to AMP and C, while the only. *Hafnia species* tested was resistant to TE and S. Serratia species was clearly multi-drug resistant.

Table 4: Frequency of antimicrobial susceptibility of *Enterobacteriaceae* isolated from catfish

Antibiotics (concentration)	Number of susceptible strains (%)					
	***Enterobacter* spp. (n = 4)**	***Klebsiella* spp. (n = 4)**	***Proteus* spp. (n = 3)**	***Hafnia* spp. (n = 1)**	***Serretia* spp. (n = 1)**	**Total (n = 13)**
CIP (5µg)	4 (100)	4 (100)	2 (67)	1 (100)	1 (100)	12 (92)
AMP (20µg)	3 (75)	0	0	1 (100)	0	4 (31)
CN (10µg)	3 (75)	2 (50)	2 (67)	1 (100)	0	8 (62)
TE (30µg)	2 (50)	2 (50)	2 (67)	0	0	6 (46)
C (20µg)	3 (75)	2 (50)	0	1 (100)	0	6 (46)
S (10µg)	2 (50)	0	1 (33)	0	0	3 (23)

Keys: CIP = Ciprofloxacin, AMP = Ampicillin, CN = Gentamycin, TE = Tetracycline, C = Chloramphenicol, S = Streptomycin

The results of antimicrobial susceptibility testing for non-enteric Gram negative bacilli are presented in Table 5. The non-enteric bacteria displayed high rates of susceptibility (60% - 93%) to C, CN and CIP. The susceptibility rates to S (33%), TE (40%) and AMP (40%) were intermediate. The isolates of *Pseudomonas* and *Aeromonas* species recorded susceptibility rates ranging from 17% - 100% and 50% - 100% respectively. With the exception of *Flavobacterium* species, all the other bacteria were susceptible to CIP. Similarly, *Vibrio* and *Xanthomonas* species were the only bacteria that were resistant to CN and C respectively. *Vibrio, Xanthomonas* and *Flavobacterium* species isolated in this study were all resistant to S.

Table 5: Frequency of antimicrobial susceptibility of non-enteric Gram-negative bacilli isolated from catfish

Antibiotics (concentration)	Number of susceptible strains (%)						
	***Alcaligenes* spp. (n = 4)**	***Pseudomona* spp. (n = 6)**	***Aeromonas* spp. (n = 2)**	***Vibrio* spp. (n = 1)**	***Xanthomonas* spp. (n = 1)**	***Flavobacterium* spp (n = 1)**	**Total (n = 15)**
CIP (5µg)	4 (100)	6 (100)	2 (100)	1 (100)	1 (100)	0	14 (93)
AMP (20µg)	0	3 (50)	2 (100)	0	0	1 (100)	6 (40)
CN (10µg)	3 (75)	6 (100)	2 (100)	0	1 (100)	1 (100)	13 (87)
TE (30µg)	3 (75)	1 (17)	1 (50)	0	0	1 (100)	6 (40)
C (20µg)	2 (50)	3 (50)	2 (100)	1 (100)	0	1 (100)	9 (60)
S (10µg)	1 (25)	3 (50)	1 (50)	0	0	0	5 (33)

Keys: CIP = Ciprofloxacin, AMP = Ampicillin, CN = Gentamycin, TE = Tetracycline, C = Chloramphenicol, S = Streptomycin

Multi-drug resistance among these bacteria to 2 or more antimicrobial agents was observed to be 69% of *Enterobacteriaceae* and 20% for non-enteric gram-negative bacteria. The resistance pattern of the enterobacteriaceae and non-enteric Gram negative bacilli are presented on table 6 and 7 respectively. A total of 10 different resistance patterns were recorded for the 13 isolates of enterobacteriaceae, while 9 different resistance patterns were recorded for the 15 non-enteric Gram negative bacilli.

Table 6: Antimicrobial resistance patterns of the *Enterobacteriaceae* isolates

Resistance patterns	Number (%) of strains
AMC + S	1 (8)
TE + S	2 (15)
TE + C	1 (8)
AMP + CN + S	1 (8)
AMP +C + S	2 (15)
AMP + TE + C	1 (8)
AMP + TE + C + S	1 (8)
AMP + CN + TE + S	1 (8)
AMP + CN + TE + C + S	1 (8)
CIP + AMP + CN + TE + C + S	1 (8)

Table 7: Antimicrobial resistance patterns of the non-enteric Gram-negative bacilli isolates

Resistance patterns	Number (%) of strains
AMP	1 (7)
TE	1 (7)
CIP + S	2 (13)
T + S	2 (13)
TE + C	2 (13)
AMP +C + S	2 (13)
AMP +TE + S	2 (13)
AMP + CN + TE + S	2 (13)
AMP + TE + C + S	2 (13)

DISCUSSION

The bacterial isolates identified in this study were similar to those from fish and fish environments identified by Grema, et al. (2005), and those recorded in the reviewed work of Sader and Jones (2005). The fewer number of enterobacteriaceae compared to the non-enteric Gram-negative bacilli recorded in this present study agrees with the work of Sedlacek, *et al.* (2016) who obtained 36% of enteric bacteria and 41% of non-enteric gram-negative bacilli. The study of bacteria associated with fish and its environment is relevant, because bacteria pathogen are among the major cause of infectious diseases and mortality in the wild and fish reared in confined condition. Disease problems constitute the largest single cause of economic losses in aquaculture especially diseases caused by gram negative bacteria that are ubiquitous in their life style and very difficult to deal with. The virulence and host range of existing pathogens have been increasing, posing considerable challenge to fish health; now researchers are looking for more efficient drug to combat bacteria fish disease (Sudheesh, *et al.,* 2012). Although pathogenic species representing majority of existing genera have been implicated in fish diseases and are responsible for important economic loss in cultured fish worldwide. If the management practice towards water quality is maintained to standard, the problems will be reduced to the barest minimum (Sudheesh, *et al.,* 2012).

Bacterial species such as *Vibrio, Flavobacterium, Pseudomonas, Aeromonas*, identified in this study are among the major bacterial pathogens of economic importance which were reported to be threats to fish by Grema, *et al.* (2005) and Sudheesh (2012). Though *Klesiella pneumoniae* is an opportunistic pathogen, when unfavorable condition occurs can be dangerous and can turn in to superbugs almost impossible to fight with common antibiotics (Melan, *et al.*, 2016). *Klebsiella pneumoniae* in this study is of zoonotic importance according to Adesina, *et al.* (2016), who reported that among the 6 species of *Klebsiella. sp* identified in their study, 3 major members of genus that cause human disease are *K. pneumoniae, K. granulomatis* and *K. oxytoca*. The isolation of *Serretia* species in this study is of significance because the organism is associated with a disease outbreak in farmed ornamental fish (Arathi, *et al.,* 2017). So the identification of these bacteria among other organisms is a threat to fish in the environment, although virulent species like *S. marcescens* have not been identified, but there is possibility the bacteria could be present in the environment.

The presence of *Enterobacteriaceae* in this study could be a serious public health risk, although in most cases they are part of normal microbiota in the fish, but when colonizing humans (fish handlers, processors and fishermen) can cause diseases like urinary tract infection (Nagamatsu, *et al.,* 2015; Umar, *et al*., 2016). Renato, *et al*. (2017) observed that humans are exposed to infections with *Enterobacteriaceae* and other microbe from fish during handling and processing or consumption of improperly cooked or raw fish.

The resistance recorded for the *Enterobacteriaceae* against streptomycin and ampicillin could be attributed to the indiscriminate use of these drugs in aquatic environment which cause the development of resistance factors to the bacteria.

Ciprofloxacin was found most effective against most isolates probably because it is commonly used in animal medicine and thus, has not been abused and misused in aquatic environment compared to streptomycin and ampicillin.

Although Akinbowale *et al*. (2006) reported that there were no products registered for use in aquaculture; antimicrobial resistance is present in isolates from aquaculture and aquaculture environments as observed in this study. Antibiotic resistance is a significant human health issues resulting to the transfer of these resistant organisms or their gene to humans via the food chain (Angulo *et al.*, 2004). This study corroborates with the reports of several workers who documented cases of resistance in aquaculture environment, fish and shellfish (Saha and Pal 2002; Michel *et al*., 2003; Hatha *et al*., 2005). Cases of development of resistance and even drug residues in the flesh of food producing animals have become a global issues which necessitated the ban on the use of some antimicrobial agents in developed and some developing countries and recommended the use of biological control like probiotics in food producing animals because of the public health hazard associated with the antibiotics.

Multiple drug resistance was recorded in this study. Multi-drug resistance is defined as resistance of a single bacterium to more than 3 antimicrobials (Oteo, *et al.,* 2005), sometimes even as low as ≥2 antibiotics from 2 different classes, is regarded as multiple drug resistance (Gibbs, *et al.,* 2000, Silpi, et al. 2016). The multi-drug resistance of *Vibrio* spp. isolates from shrimp hatcheries has also been reported by Mirand and Zemelman (2002) in Indonesia; the isolates were resistant to ampicillin, tetracycline, amoxicillin and streptomycin. Mirand and Zemelman (2002) also reported multi-drug resistance in *Hafnia alvei* involving ampicillin, amoxicillin, cephalexin, cephalothin, florfenicol, erythromycin and sulfamethoxazole. This was not in agreement with the antibiogram for *Hafnia* spp. observed in this study in which *Hafnia* spp was susceptible to CIP, AMP, CN, and C, but resistance to TE and S. Multidrug resistance of *Klebsiella pneumoniae* revealed in this present study was in agreement with the report of Ibrahim and Hameed (2015) where

Klebsiella pneumoniae was resistant to 6 antibiotics. Contrary to the work of Kaskchedikar and Chhabra (2010) who recorded multidrug resistance in *Aeromonas hydrophila* to AMP and colistin antibiotics, all the ***Alcaligenes*** **spp.,** ***Pseudomona*** **spp. and** *Aeromonas hydrophila* species isolated in this study were sensitive to all the six drugs tested. This is not strange; it could be due to strain differences and frequent abused of drugs.

The high rate of multidrug resistant bacteria is of global and public health concern. This is because it can lead to increased human and domestic animal healthcare costs and increased morbidity and mortality (Steele *et al.*, 2005) as well as un-responsive cases of zoonotic bacterial disease outbreak among humans. According to Elsaidy *et al.* (2015), the use of organic manure by fish farmers for fertilization may constitute a predisposing factor to antibiotic resistance by transferring of antibiotic residues and resistant bacteria to fish farms. The susceptibility of CIP could be due to the fat that the drug is not in common use in veterinary medicine and thus, yet to be abused compared to other drugs. The highest degree of multidrug resistance was observed with *Serretia* species in this study which was resistant to 5 out of the 6 drugs tested. This finding is in agreement with other workers elsewhere who reported that the pathogen Serretia sp identified from guppy *Poecillia reticulatus* showed multiple drug resistance to 11 out 16 antibiotics tested (Keith and Donald, 2000).

The indiscriminate use of antibiotics to prevent infection in fish and economic loss in aquaculture has resulted to the emergence of resistance strains which is very dangerous for consumers. The study provide evidence that there is an increasing emergence of MDR of bacterial isolates of fish and fish handlers, thus a finding which agrees with the report of Albuquerque, *et al.* (2007) who found increasing emergence of antibiotic resistant isolates from fish and fish handlers. Base on the antibiotics susceptibility test of *Proteus mirablis*, the bacteria was highly sensitive to CIP (67%), but resistant to AMP, which conforms with the work of Umar, *et al.* (2016) who reported the sensitivity of *Proteus* species to CIP (70%), and resistance to AMP. A similar report by Luzzaro, *et al.* (2009) revealed resistance to TE and β-lactam antibiotics like penicillin and amoxicillin by *Proteus mirabilis*.

The resistance patterns observed in this study may be due to the generic and species differences of the isolates (Kathleen, *et al.,* 2016). The detection of antibiotic resistance gene in bacteria that can be transferred to other bacteria as well as to human microbiota has since been reported by Kathleen, *et al.* (2016). The level of antibiotic resistance problem is proportional to the frequency of antibiotic usage in an environment.

In this study high percentage of susceptibility was observed towards CIP, CN and C which is similar to the report of Kathleen, *et al.* (2016) who observed high susceptibility towards CN TE and C. High % of AMP and S resistance was recorded in both *Enterobacteriaceae* and non-enteric gram-negative bacilli in this study which corroborate with the works of Zhang, *et al.* (2013) and Kathleen, *et al.*(2016) indicating that microorganisms generally are developing resistance to most common antibiotics in the aquatic environment. Although, the use of antibiotics in human medicine has increase the emergence of resistant bacteria, the indiscriminate use of antibiotics in animals have contributed to the problems of resistance and complicated the choice of treatment in human disease (Novonty, *et al.,* 2004).

Awareness on antibiotic resistance threat should be instilled in the public and the country (Nigeria) at large to combat further spread or even to call for banning of the use of these common antibiotics that many bacteria have developed resistance to, in aquaculture environment. Similar action has been practiced in Malaysia, Korea, Spain since 1998 when antibiotic resistance problem became rampant and there was relief from that issues (Yoo *et al.,* 2003). The results of this study therefore recommend antimicrobial susceptibility testing before administering antibiotics for treatment of bacterial infection in aquaculture.

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