Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 6 (1): 87-97, 2011
ISSN 1816-4935 / DOI: 10.3923/jm.2011.87.97

Bacterial Species Associated with Anatomical Parts of Fresh and Smoked Bonga Fish (*Ethmalosa fimbriata*): Prevalence and Susceptibility to Cephalosporins

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ABSTRACT

The aim of this study was to determine the prevalence of bacterial species associated with smoked and fresh Bonga (*Ethmalosa fimbriata*) sold at two different markets in Uyo using standard microbiological techniques and their susceptibility to antibiotics (cephalosporins) using Disc Diffusion Technique (DDT). The results of the bacteriological status of both fresh and smoked Bonga fish showed variations in the total bacterial and total coliform counts in different anatomical parts (skins, gills and intestine). The highest total bacterial counts was recorded from gills (9.2×10^5 cfug^{-1}) and lowest in skin (4.3×10^5 cfug^{-1}) in fresh bonga fish, while the highest total bacterial counts was obtained in intestine (7.7×10^4 cfug^{-1}) and lowest in skin (3.1×10^4 cfug^{-1}) in smoked Bonga fish. The total coliform counts of the fresh Bonga fish ranged from 3.3×10^2 to 4.1×10^3, 3.6×10^2 to 3.1×10^3 and 4.3×10^2 to 7.5×10^3 in skins, intestines and gills, respectively. In smoked fish, the skin had the lowest total coliform counts (1.5×10^2), while the highest coliform counts was obtained in gills (3.5×10^3). The prevalence of the *Streptococcus* spp., *Escherichia coli*, *Yersinia* sp., *Enterobacter* sp., *Staphylococcus aureus*, *Enterococcus* sp., *Vibrio cholerae*, *Proteus* sp., *Shigella* sp., *Salmonella* sp. and *Campylobacter* sp. isolated from both fresh and smoked fish samples varied depending on the anatomical parts. The results of antibiotic susceptibility showed that the bacteria isolated from both fresh and smoked fish were more sensitive to ceftazidime, cefoxitin and cefoperazone than cephalothin. However, both fresh and smoked Bonga fish could be carriers of pathogenic bacteria and a vehicle of transferring bacterial food borne infections and intoxication and cephalosporins may be the drugs of choice for the treatment.

Key words: Bonga, infection, prevalence, susceptibility, Cephalosporins, Uyo

INTRODUCTION

The Bonga fish (*Ethmalosa fimbriata)* is pelagic-neritic, catadromous freshwater fish which belongs to the family clupeidae and order clupiformes (Abowei, 2009). Fish is one of the best supplies of proteins, vitamins and minerals and are essential nutrients required for supplementing both infant and adult diets (Abdullahi *et al*., 2001). Fish and fish products play a significant role in the diets of the populations of West African countries and constitute more than 60% of the total protein intake in adults especially in the rural areas. It has a relatively 10% calories content hence

its role in nutrition is recognized. In Nigeria, fish is eaten fresh, preserved or processed (smoked) and form a much-cherished delicacy that cuts across socio- economic, age, religious and educational barriers (Adebayo-Tayo *et al*., 2008).

Fishes are susceptible to a wide variety of potentially pathogenic bacteria (Schmidt *et al*., 2000). Many of these bacteria capable of causing disease are considered to be saprophytic in nature but only become pathogenic when fishes are physiologically unbalanced, nutritionally deficient, or as a result of other stressors such as poor water quality, overstocking, which allow opportunistic bacterial infections to proceed and lead to considerable economic losses in aquaculture as a results of heavy mortalities in both culture and wild fishes throughout the world (Anderson, 1995; Toranzo *et al*., 2005). Among the common fish pathogens are *Staphylococcus* sp., *Aeromonas* sp., *Salmonella* sp., *Shigella* sp., *Enterococcus faecalis*, *E. coli,* Y*ersinia* sp., *V. cholerae* and other vibrios (Kam *et al*., 1995; Lehane and Rawlin, 2000; Isonhood and Drake, 2002). Dietary responses to short term diets of fish and the positive effects on protein calorie malnutrition, asthma, arthritis, auto-immunity, coronary heart diseases and arteriosclerosis have been independently and unanimously reported (Cobiac *et al*., 1991; Gerhard *et al*., 1991; Fafioye *et al*., 2008).

Bacterial infection of fish and fish products may influence human health by inducing disease/infection and cause abdominal pain, acute gastroenteritis, bloody/mucoid diarrhoea, nausea, vomiting and fever upon ingestion of insufficiently heat-treated (smoked) fish or fish products contaminated during the processing and the presence of these bacteria harmful to man generally indicates poor sanitation in handling and processing (Kam *et al*., 1995; Han *et al*., 2001). Most outbreaks of food poisoning associated with fish derived from the consumption of raw or insufficiently heat treated fish, which may be contaminated with bacteria from water environment (*Vibrio* sp., *C. botulinum*) or terrestrial sources (*C. perfringens*, *Salmonella* sp., *Shigella* sp., *Staphylococcus* sp., *V. cholerae*), or fish products recontaminated during heat processing (Khatib *et al*., 1994; Novothy *et al*., 2004). Hot smoking in mild conditions at a temperature in the fish not exceeding 65°C does not inactivate all pathogens or inhibit bacteria during storage. (Novothy *et al*., 2004).

Estimation of bacterial numbers in fish and fish products is frequently used to retrospectively assess microbiological quality and/or to assess the presumptive safety of the product. A number of microbiological methods are used to check that the microbiological status of fish such as Standard Plate Count (SPC) which determines the total number of aerobic bacteria present in fish at mesophylic temperatures (30-37°C). The SPC can be used as general, relative gauge, of the overall load of a product. When SPC is used as a part of a shelf life analysis to determine spoilage, the number generated can be compared from test interval to test to verify if the bacteria initially present are capable of growing. The large-scale settings of aquatic animal husbandry and the continuous use of antimicrobial agents in aquaculture have resulted in an increased antibiotic resistance in bacteria potentially pathogenic to fish (Smith *et al*., 1994; Alderman and Hastings, 1998; Petersen *et al*., 2002; Ibiebele and Sokari, 1989; Cabello, 2006). The most frequently prescribed class of antibiotics are cephalosporins, which are structurally and pharmacologically related to the penicillins. Cephalosporins have a beta-lactam ring structure that interferes with synthesis of the bacterial cell wall by disrupting the synthesis of the peptidoglycan layer of bacterial cell walls (Stephen *et al*., 2007). The apparent occurrence of antibiotic resistance among bacteria

from various areas of animal production during the past years and its possible implications for public health have in many countries lead to an intensified surveillance of bacterial resistance (Aoki, 1992; Hakan *et al*., 2009). In the field of aquaculture, both therapeutic and environmental problems have been addressed, as antimicrobial agents are released into the surrounding water during medical treatment of bacterial fish diseases (Schmidt *et al*., 2000). This research was embarked upon to investigate the bacterial spp associated with anatomical parts of fresh and smoked *Ethanomosal fimbriota* (Bonga fish) species determine the susceptibility of the isolates to cephalosporins.

MATERIALS AND METHODS

Samples collection: Thirty smoked and 30 fresh Bonga fish (*Ethmalosa fimbriata*) were randomly sampled and purchased between August-September, 2009 from three different marketing sites located at two main markets (Itam and Akpan Adem) in Uyo town Akwa Ibom State, Nigeria. The samples were subsequently kept in sterile polyethylene bags prior to bacteriological analysis.

Bacteriological analysis: The fish samples were aseptically removed from the sterile polyethylene bags and a sterile knife was used to bisect the fish samples in order to remove the intestine, gills and skin. Each sample (steaks cut from the gill, intestine and the skin of the fish) was blended separately for homogeneity by the use of mortar and pestle and 1 g was weighed and added to 10 mL of distilled water aseptically. Ten-fold serial dilutions of the homogenates; 0.1 mL of 10^{-2}, 10^{-3} and 10^{-4} dilutions was made and Pour Plate Method (PPM) was used for microbial enumeration. Each dilution was plated by pipetting 1 mL of dilution into sterile Petri dishes. About 15 mL of plate count Agar (Eosin Methylene Blue Agar (Oxoid), MacConkey Agar (Oxoid), Nutrient Agar (Oxoid), Mannitol Salt Agar (Oxoid) and Columbia agar supplemented with Butzler Selective Medium and which have been melted and brought to 45°C were used. The plates were rotated by hand at least 5 times in the clockwise direction and 5 times in anti-clockwise direction for equal distribution of the media. After solidifying, the plates were inverted and placed in incubator at 37°C for 24 h. Plates for isolation of *Campylobacter* sp. were incubated anaerobically using anaerobic jar. The incubated plates were read by observing the cultural and morphological characteristics of the cultures representative colonies. Colonies were selected at random and subcultured to obtain pure isolates on fresh plates containing nutrient agar and then incubated at 37°C for 24 h. The stock cultures were obtained, labeled carefully and were used for conventional identification using Gram's staining, motility, indole production, urease, carbohydrate utilization, catalase, citrate utilization, oxidase, coagulase methyl red tests.

Antibiotic sensitivity testing: *In vitro* susceptibility of the bacterial isolates to four different antibiotics (cephalosporins) was determined using a disk-diffusion technique (Bauer *et al*., 1996). Sterile Petri dishes of Mueller Hinton agar were prepared according to the manufacturer's specification. A 0.1 mL of each bacterial isolates was seeded into each of the Petri dishes containing Mueller-Hinton agar and were allowed to stand for 45 min to enable the inoculated organisms to pre-diffuse. The commercially available discs containing the following antibiotics: Ceftazidime (Caz, 30 μg), cefoxitin (Cft, 30 μg), cefoperazone (Cfp, 30 μg) and cephalothin (Kf, 30 μg) (Oxoid,

UK) were aseptically placed on the surfaces of the sensitivity agar plates and these were incubated for 18-24 h at 37°C. Zones of inhibition after incubation were observed and the diameters of inhibition zones were measured in millimeters. The interpretation of the measurement as sensitive, intermediate and resistant was made according to the manufacturer's standard zone size interpretive manual .The intermediate readings were considered as sensitive for the assessment of the data.

RESULTS AND DISCUSSION

In this study, eleven genera consisting both Gram-positive and Gram negative bacteria were isolated from the 30 smoked and 30 fresh Bonga fish. The isolates were identified as *Streptococcus* sp., *Escherichia coli*, *Yersinia* sp., *Enterobacter* sp., *Staphylococcus aureus*, *Enterococcus* sp., *Vibrio cholerae*, *Proteus* sp., *Shigella* sp., *Salmonella* sp. and *Campylobacter* sp. using their morphological and biochemical characteristics (Gram's reaction, Motility, Catalase, Oxidase, Coagulase, Indole, Urease, Sugar tests) (Table 1). The results of the bacteriological status of both fresh and smoked Bonga fish are summarized in Table 2 with variations found in the total bacterial counts and total coliform counts in the different anatomical parts (intestine, gills and skins). The highest total bacterial counts value was recorded from gills (9.2×10^5 cfug^{-1}) and lowest in skin (4.3×10^5 cfug^{-1}) in fresh bonga fish, while the highest total bacterial counts was observed in intestine (7.7×10^4 cfug^{-1}) and lowest in skin (3.1×10^4 cfug^{-1}) in smoked Bonga fish (Table 2). The

Table 1: Morphological and biochemical characteristics of bacteria isolated from fresh and smoked bonga fish

	Isolates										
Parameters	a	b	c	d	e	f	g	h	i	j	k
Grams reaction	+/cocci	+/cocci	-/rod	-/rod	-/curve	-/rod	-/rod	-/rod	+/cocci	-/curve	-/rod
Catalase test	-	+	-	-	-	-	-	-	-	+	-
Citrate test	-	-	-	-	-	-	-	-	-	-	+
Oxidase test	-	-	-	-	+	-	-	-	-	+	-
Coagulase test	-	+	-	-	-	-	-	-	-	-	-
Indole test	-	-	-	-	+	+	-	-	-	-	-
Urease activity	-	-	-	-	-	-	+	-	-	-	+
Glucose	+	+	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	+	-	+	-	-	-
Sucrose	-	-	-	-	+	-	+	-	-	-	+
Mannitol	+	+	+	-	+	+	+	+	+	+	-
Motility	+	+	+	-	+	+	+	+	+	+	+

a: *Streptococcus* sp.; b: *Staphylococcus aureus*; c: *Salmonella* sp.; d: *Shigella* sp.; e: *Vibrio cholerae*; f: *Escherichia coli*; g: *Yersinia* sp.; h : *Enterobacter* sp.; i: *Enterococcus faecalis*; j: *Campylobacter* sp. and k: *Proteus* sp.

Table 2: Mean bacterial counts of different anatomical parts of fresh and smoked bonga fish (cfug^{-1})

	Fresh Bonga fish (cfug^{-1})		Smoked Bonga fish (cfug^{-1})	
Parts	TBC	TCC	TBC	TCC
Skins	4.3×10^5-6.5×10^5	3.3×10^2-4.1×10^3	3.1×10^4-4.7×10^4	1.5×10^2-2.1×10^3
Intestines	6.2×10^5-7.9×10^5	3.6×10^2-3.1×10^3	5.8×10^4-7.7×10^4	3.0×10^2-2.9×10^3
Gills	8.3×10^5-9.2×10^5	4.3×10^2-7.5×10^3	4.3×10^4-5.5×10^4	2.3×10^2-3.5×10^3

TBC: Total bacterial counts; TCC: Total coliform counts

Table 3: Prevalence of bacteria isolated from fresh bonga fish (n = 30)

Parts/bacterial isolated	No. of occurrence	Percentage of occurrence
Skins		
Staphylococcus aureus	14	24.14
Shigella sp.	08	13.80
Enterococcus faecalis	06	10.34
Salmonella sp.	10	17.24
E. coli	08	13.80
Streptococcus sp.	12	20.68
Total	58	100.00
Intestine		
Enterobacter sp.	06	11.54
Salmonella sp.	08	15.38
Shigella sp.	05	09.62
Enterococcus faecalis	09	17.30
E. coli	11	21.15
Campylobacter sp.	02	03.85
Yersinia sp.	05	09.62
Proteus sp.	05	09.62
V. cholerae	01	01.92
Total	52	100.00
Gills		
Proteus sp.	06	13.64
Salmonella sp.	06	13.64
Shigella sp.	07	15.91
Enterococcus faecalis	10	22.72
Campylobacter sp.	01	02.72
E. coli	09	20.45
Yersinia sp.	05	11.36
Total	44	100.00

total coliform counts ($cfug^{-1}$) of the fresh Bonga fish ranged from 3.3×10^2 to 4.1×10^2, 3.6×10^2 to 3.1×10^3 and 4.3×10^2 to 7.5×10^3 in skins, Intestines and gills, respectively. In smoked fish, the skin had the lowest total coliform counts (1.5×10^2), while the highest coliform counts was obtained in gills (3.5×10^3) (Table 2). A total of 154 bacterial species were obtained from the three anatomical parts of fresh bonga fish while in smoked bonga fish, 103 bacterial species were isolated. In fresh bonga fish, *Staphylococcus aureus*, *E. coli* and *Enterococcus faecalis* had the highest prevalent rates of 24.14, 21.15 and 22.72% in skins, intestine and gills, respectively (Table 3). The bacterial species with the highest percentages of occurrence in smoked Bonga fish were *Staphylococcus aureus* (29.27%), *Enterococcus faecalis* (21.87%) and *E. coli* (26.67%) (Table 4). The prevalence of both Gram positive and Gram negative bacteria isolated from different anatomical parts of both fresh and smoked fish are shown in Table 3 and 4. The results of antimicrobial susceptibility tests of the Gram-positive and Gram-negative bacteria species isolated from fresh Bonga fish showed that (75-84%) *Streptococcus* sp., (71-85.7%) *Staphylococcus aureus*, (72-85.5%) *Enterococcus* sp., (81.8%) *Proteus* sp., (75-85.5%) *Shigella* sp., (62.7-71%) *Salmonella* sp., (64-71.4%) *Escherichia coli*, (70%) *Yersinia* sp., (67-81%) *Enterobacter* sp. (33.3-67%) *Campylobacter* sp. were susceptible to ceftazidime and cefoperazone, while (100%) *Vibrio cholerae* were susceptible to ceftazidime and (100%) *Vibrio cholerae* resistant to cefoperazone (Fig. 1, 2).

Table 4: Prevalence of bacteria isolated from smoked bonga fish (n = 30)

Parts used bacteria isolated	No of occurrence	Percentage of occurrence
Skins		
Staphylococcus aureus	12	29.27
Salmonella sp.	07	17.07
Shigella sp.	04	09.76
Enterococcus fæcalis	04	09.76
E. coli	06	14.63
Streptococcus sp.	08	19.51
Total	41	100.00
Intestines		
Salmonella sp.	03	09.38
Shigella sp.	04	12.50
Enterococcus fæcalis	07	21.87
E. coli	07	21.87
Enterobacter sp.	05	15.62
Campylobacter sp.	01	03.13
Yersinia sp.	03	09.38
V. cholerae	02	06.25
Total	32	100.00
Gills		
Proteus sp.	04	13.30
Salmonella sp.	06	20.00
Shigella sp.	05	16.67
Campylobacter sp.	01	03.33
Enterococcus fæcalis	06	20.00
E. coli	08	26.67
Total	30	100.00

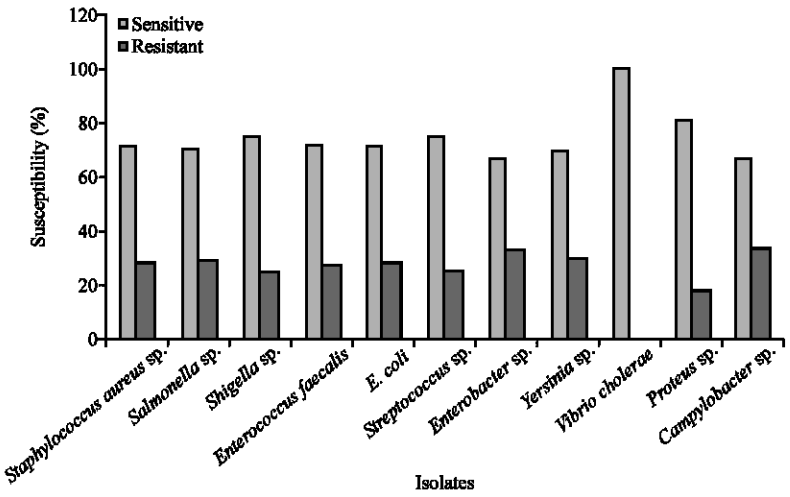

Fig. 1: Susceptibility profile of bacterial species isolated from fresh Bonga fish to Ceftazidime

The results also showed the bacterial spp isolated from the fresh Bonga fish were more susceptible to cefoxitin than cephalothin (Fig. 3, 4). The incidence of antimicrobial drug sensitivity of Bacteria species isolated from smoked Bonga fish to these four antibiotics also varied during the study period

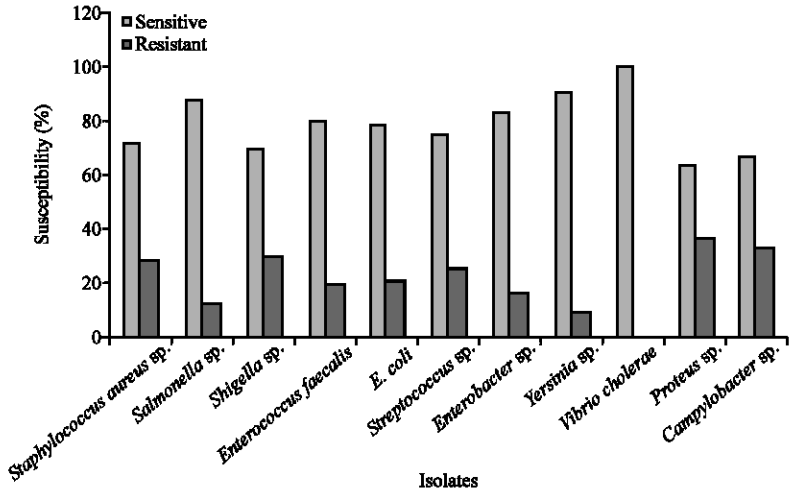

Fig. 2: Susceptibility profile of bacterial species isolated from fresh Bonga fish to Cefoxitin

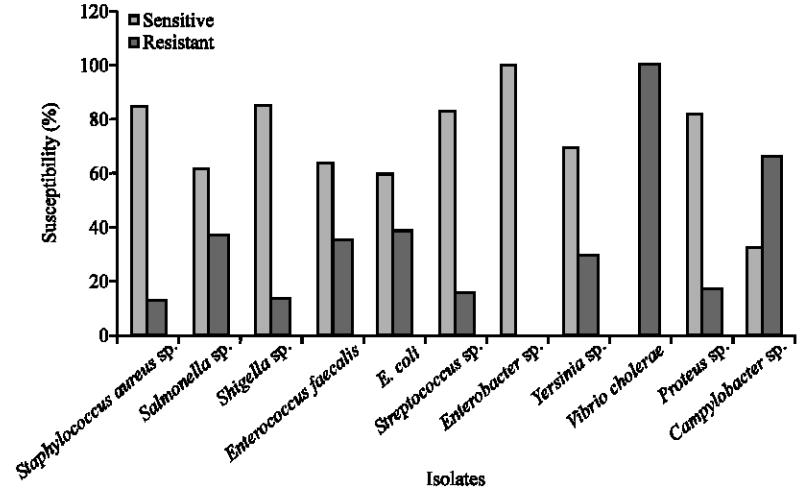

Fig. 3: Susceptibility profile of bacterial species isolated from fresh Bonga fish to Cefoperazone

with *Streptococcus* sp., *Yersinia* sp., *Vibrio cholerae* and *Proteus* sp. having the highest susceptibility valued of (100%) to ceftazidime (Fig. 5). (92.3%) *Shigella* sp. and (87.5%) *Streptococcus* sp. were sensitive to cefoperazone (Fig. 6). *Enterobacter* sp. and *Vibrio cholerae* were more susceptible to cefoxitin than other bacterial sp. isolated from smoked Bonga fish (Fig. 7), while 23.5-100% of the bacteria were resistant to cephalothin (Fig. 8).

The bacteriological study of bacterial sp. associated with different anatomical parts of both smoked and fresh Bonga fish (*Ethmalosa fimbriata*) showed that these parts contained at least eleven genera of bacteria. The total bacterial counts and total coliform counts obtained in this study don't concur with the results of Yagoub (2009). The occurrence of some Gram-negative bacteria (*Yersinia* sp., *Shigella* sp., *Proteus* sp.) and Gram positive bacteria (*Streptococcus* sp., *Enterococcus faecalis* is in concurrence with Turker and Usta (2008). In this study, isolation of highly pathogenic Enterobacteriacaea such as Salmonella sp., *Shigella* sp. and the pathogenic *E. coli* from the collected samples indicated public health hazards and concern. The presence of *Salmonella* sp. in

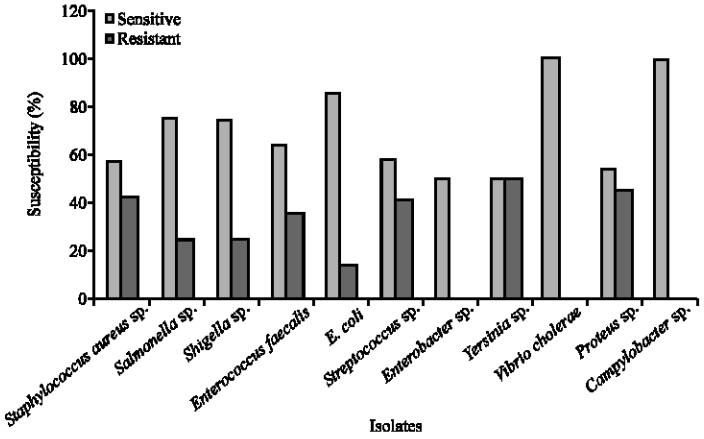

Fig. 4: Susceptibility profile of bacterial species isolated from fresh Bonga fish to Cephalothin

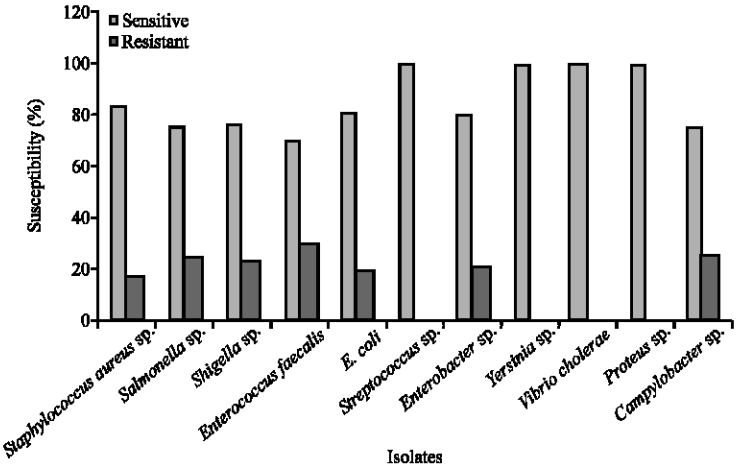

Fig. 5: Susceptibility profile of bacterial species isolated from fresh Bonga fish to Ceftazidime

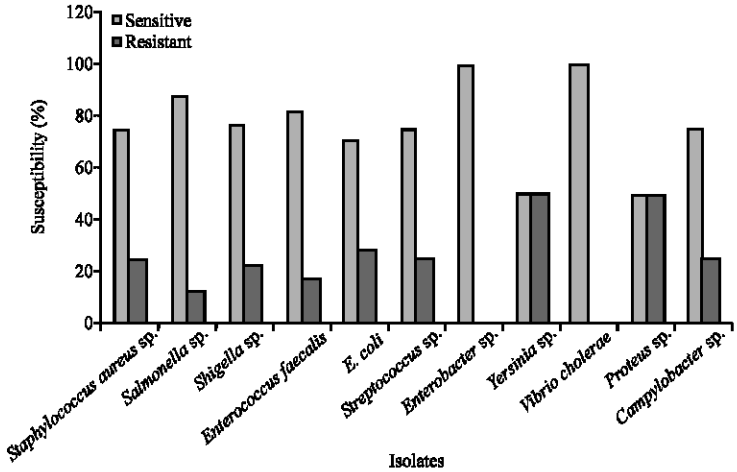

Fig. 6: Susceptibility profile of bacterial species isolated from fresh Bonga fish to cefoxitin

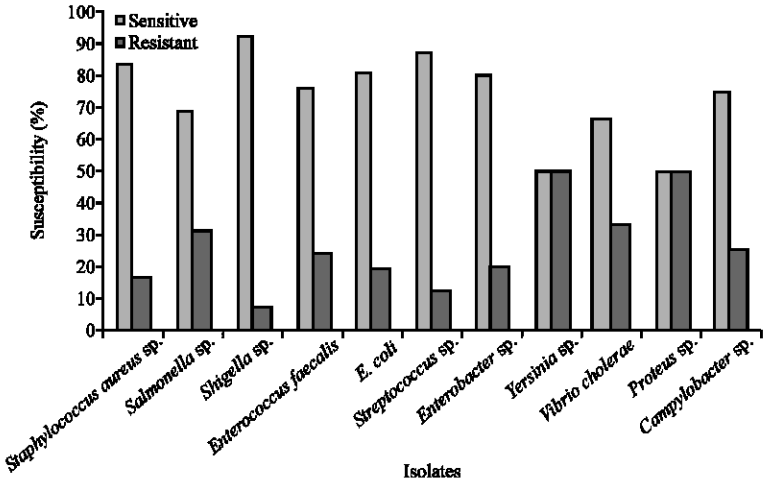

Fig. 7: Susceptibility profile of bacterial species isolated from fresh Bonga fish to Cefoperazone

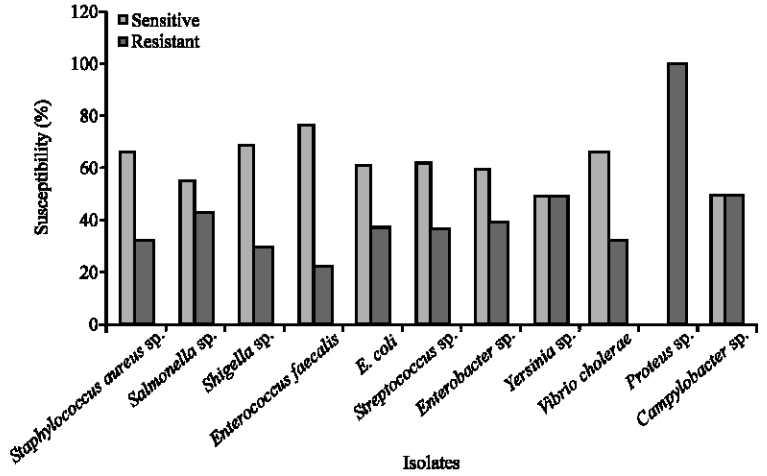

Fig. 8: Susceptibility profile of bacterial species isolated from fresh Bonga fish to Cephalohin

the fish sampled from the three different marketing sites in this research is in conformity with the result obtained by Hatha and Lakshmanaperumalsamy (1995). The isolation of *Klebsiella* sp. and *Proteus* sp., *Shigella* sp. from this fish indicated faecal and environmental pollution and this support the findings of Yagoub and Ahmed (2004) and Najiah *et al.* (2008). The occurrence of these pathogenic bacteria especially *E. coli* in food (fish and fish products) may influence human health by inducing diseases/infections and cause abdominal pain, acute gastroenteritis, bloody/ mucoid diarrhoea, nausea, vomiting and fever (Akinjogunla *et al.*, 2009).

Pettibone *et al.* (1996) has also reported antibiotic resistance of the fish pathogens obtained from fish and this is in conformity with our results and the resistance may be as a result of the continuous use of antimicrobial agents in aquaculture (Schmidt *et al.*, 2000; Sorum and L'Abée-Lund, 2002; Cabello, 2006; Turker and Usta, 2008). Antibiotics resistance profile of some Gram negative bacteria isolated from fish have been recorded by McPhearson *et al.* (1991) and this agrees with the results obtained in this research.

In conclusion, this study revealed that fresh and smoked fish sold in Uyo could be a source of food- borne bacterial pathogens and this could lead to outbreaks of food poisoning associated with fish derived from the consumption of raw or insufficiently heat treated fish.

Consequently, the use of heavy antibiotic in aquaculture, improvements in handling and processing of fish are needed to minimize the prevalence of the pathogenic bacteria and reduce the emergence of antibiotic resistant pathogenic and environmental bacteria.

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