Journal of Applied & Environmental Microbiology, 2014, Vol. 2, No. 3, 81-85
Available online at http://pubs.sciepub.com/jaem/2/3/4

DOI:10.12691/jaem-2-3-4

Isolation and Identification of Bacteria Associated with Fresh and Smoked Fish (*Clarias gariepinus*) In Minna Metropolis, Niger State. Nigeria

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Received January 18, 2014; Revised March 29, 2014; Accepted March 31, 2014

Abstract An in-vitro assay was conducted to ascertain and identify major bacterial contaminants of fish, which hitherto had constituted an imported dietary intake of the people of Minna Metropolis, Nigeria. Fresh and smoked fish samples were collected from three different markets, the bacterial load of the samples was determined using the pour plate method. Identification and characterization of various isolates were based on gram-staining technique and biochemical tests. *Clarias gariepinus* was used for the study. In-vitro assay result revealed that the samples were contaminated by six bacteria species, which include *Staphilococcus aureus, Bacillus subtilis, Staphilococcus epidermis, Salmonella epidermis, Salmonella typhii, Streptococcus* spp. and *Shigella* sp. The mean bacterial load for the fresh fish was 1.84 x 10^6 cfu/ ml. and for the smoked fish 2.06 x 10^6 cfu/ ml.

Keywords: *Clarias gariepinus, bacterial load, fresh fish, smoked fish, Minna metropolis*

Cite This Article: Ibrahim B. U., BABA J., and Sheshi M. S., "Isolation and Identification of Bacteria Associated with Fresh and Smoked Fish (*Clarias gariepinus*) In Minna Metropolis, Niger State. Nigeria." *Journal of Applied & Environmental Microbiology*, vol. 2, no. 3 (2014): 81-85. doi: 10.12691/jaem-2-3-4.

1. Introduction

The importance of fish cannot be overemphasized. Fish is a low fat food, a great source of protein, vitamins and minerals. Over the years, agriculture has gained a rapid interest due to the importance of fish as a cheap source of protein, since beef meat and even goat meat are beyond the reach of an average Nigerian citizen (Fang *et. al.,* 2010). Fish constitute about 45% of the total amount of protein (FDF, 2007), even for a long time to come, this trend will continue. In Nigeria, the demand for fish greatly exceeds supply. This problem is aggravated by the low level of domestic fish production against the increase human production (Adeleke, 1999). As a result of the crucial role played by fish production in meeting the protein demand, information on the parasites of fish becomes particularly important, as they are known to significantly affect yield in fisheries (Hundson *et. al.,* 2005).

Fish parasites may be grouped broadly as bacteria, fungi, virus e.t.c. These parasites put together are responsible for about 45% loses in fish farms (Kabata, 2008).

One of the earliest reports of fish parasites in Nigeria, was that of Awachie (1996) on the parasite of fish in the area of Kainji Reservoir, with 30% of *Sarotherodon niloticus* infected by acanthocephalan, and 9% of *Clarias gariepinus* infected by cestodes. In Eastern Nigeria, Jonah (1990), carried out a survey of Helminthes parasites of fish in Imo River, and recorded a low 7.7% level of infection. Robert (1999), reported that intensification of pond fish culture favour increase of disease agents and disease outbreak. Kabata (2008), also reported that pathological conditions arising from parasitic infection posed a serious consequences especially under a crowded conditions.

In various ways, fish could be contaminated by micro organisms. The aim of the present study is to determined and identify the bacterial pathogens contaminating fresh and smoked fish in Minna metropolis, Niger State.

2. Materials and Methods

2.1. Study Area

The study was conducted in Minna metropolis, Niger State, located within longitude 6°33'E and latitude 9°3'N, with a land mass of 88 $Km^{2.}$ The vegetation of the area reflect that of Savannah zone, dominated by grass with scattered trees species. The climate presents two distinct seasons, a rainy season between April and October, and, a dry season (November-March) completely devoid of rain.

2.2. Collection of Fish Samples

Fresh and dried fish samples were obtained from three different markets within Minna metropolis (Kure Ultra modern market, Tunga Goro market and Chanchaga

market), between 7.00 a.m. and 8.00 a.m. local time. They were packed in a leather bag and transferred to the laboratory for identification and biological assays.

3. Sample Preparation

3.1. Preparation of Serial Dilution

Sample preparation was made using the method described by Obi and Krakowiaka (1983). The part of the fresh fish body were scraped and swab stick was used to swab the fish body and inserted into the first test tube containing 9 ml of distilled water as a stock, and five other test tubes also containing 9 ml of distilled water were arranged serially in the test tube rack. 1 ml. of the stock was collected using a pipette to the first test tube and from the first test tube to the second test tube up to the fifth test tube respectively i.e. 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} respectively. 10^{-4} and 10^{-5} were used as the dilution factor and 1 ml. was taken from each factor into a sterilized petri dish in duplicate. All plates were incubated at a temperature of 37°C for 24 hrs, before colony counting and isolation procedures.

3.2. Media Preparation

Nutrient agar was prepared by weighing 28 g and dissolved in 1 litre of distilled water. The dissolved nutrient agar was then autoclaved at a temperature of 121°C for 15 minutes. The media was allowed to cool down and pour into each of the 8 petri dishes containing 1 ml. of the diluents. It was then allowed to solidify and incubate at temperature of 37°C for 24 hrs.

3.3. Bacteria Colony Count

Bacteria colonies were counted using colony machine. The number of colonies on the plate was multiplied by the reciprocal of the dilution factor and calculation was done for 1 ml of original sample, and plating was done in duplicate foe each dilution. An average count was taken to obtain the total count.

3.4. Identification and Characterization of the Isolates

All isolates were sub-cultured to obtain a pure culture and a gram-staining carried out. Identification of the isolates was carried out based on the method described by Sakazaki and Shimad (1986), Collins *et. al.*,(1989) and Cheesebrough (2002).

4. Results

Table 1. Total Bacteria count of fresh fish samples

SAMPLING AREA	PATHOGENS IDENTIFIED FROM FISH SAMPLES COLLECTED	DILUTION FACTOR	No. OF COLONY	POPULATION IN cfu/ml.
KURE ULTRA MODERN	*Staphylococcus aureus, Bacillus subtilis, Shigella spp.*	10^{-4}	102	1.02 X 10^{6}
TUNGA GORO	*Staphylococcus epidermis, Shigella spp., Bacillus subtilis*	10^{-4}	219	2.19 X 10^{6}
CHANCHAGA	*Staphylococcus aureus, Bacillus subtilis, Shigella spp., Staphylococcus epidermis, Salmonnella typhii*	10^{-4}	232	2.32 X 10^{6}

Table 2. Total Bacteria count for smoked fish samples

SAMPLING AREA	PATHOGENS IDENTIFIED FROM FISH SAMPLES COLLECTED	DILUTION FACTOR	No. OF COLONY	POPULATION IN cfu/ml.
KURE ULTRA MODERN	*Staphylococcus aureus, Bacillus subtilis, Shigella spp., Staphylococcus epidermis*	10^{-4}	193	1.93 X 10^{6}
TUNGA GORO	*Staphylococcus aureus, Bacillus subtilis, Shigella spp.*	10^{-4}	185	1.85 X 10^{6}
CHANCHAGA	*Staphylococcus aureus, Bacillus subtilis, Shigella spp., Staphylococcus epidermis*	10^{-4}	241	2.41 X 10^{6}

Bacterial colony count of fresh fish (*Clarias gariepinus)* from the three markets above revealed that samples from Chanchaga market, Minna metropolis has the highest number of Bacteria load, and the following species of bacteria were identified from the samples from Chanchaga market *S. aureus, B. subtilis, Shigella sp., S. epidermis* and *S. typhii.* It also has the highest number of species identified, while Kure ultra modern market and Tunga Goro market has three species identified from each of them as follows respectively *S. aureus, B. subtilis, Shigella sp.*, and, *S. epidermis, Shigella sp., B. subtilis.*

Bacterial colony count of smoked fish (*Clarias gariepinus)* from the three markets sampled above in Minna metropolis, Niger State, revealed that samples from Chanchaga has the highest number of bacterial load, similar to what was obtained for fresh fish in Table 1. Chanchaga market and Kure ultra modern market recorded the following four bacteria species identified from the smoked sampled fishes collected *S. aureus, B. subtilis, Shigella sp. and S. epidermis.* While Tunga Goro market has three bacteria species identified i.e. *S. aureus, B. subtilis* and *Shigella sp.* from the smoked sample fishes collected.

The results of the gram-stain and the biochemical tests the isolates were subjected to are presented in Table 3 and Table 4.

Table 3. The result of the biochemical and identification of bacterial isolate of the smoked fish specimens (*Clarias gariepinus)*

Sample code	Gram Rxn	Shape	Cat	Coa	s.u.	*S.sa*	m.sa	E.mb	Glu	Fruct	Suc	Lact	Man	Ind	Cit	Mr	Vp	TSIA H_2S	Slope Butt	Suspected organism
S1	+	COCCI	-	-	-	-	-	-	+	+	+	+	+	-	+	+	-	-	R	*S. aureus*
S2	+	COCCI	+	+	-	-	-	-	+	+	+	-	-	-	+	-	+	-	Y	*B.subtilis*
S3	+	ROD	-	-	+	-	-	-	-	+	+	+	-	-	+	+	-	-	Y	*B.subtilis*
S4	-	COCCI	+	+	-	-	-	-	+	+	+	-	+	-	-	-	+	-	Y	*S. aureus*
S5	+	ROD	-	-	+	-	-	-	+	+	+	+	-	+	-	-	+	-	Y	*Shigella* sp.
S6	-	COCCI	-	-	-	-	+	+	+	+	+	+	-	-	+	+	-	+	R	*S. epidermis*
S7	+	ROD	-	-	-	-	-	-	+	+	+	+	-	+	+	-	+	-	R	*Shigella* sp.
S8	+	COCCI CHAIN	-	-	+	+	-	+	-	+	+	+	-	-	+	+	-	-	Y	*B.subtilis*
S9	+	COCCI	-	-	-	-	+	-	+	-	-	+	-	+	+	-	+	-	Y	*S. aureus*
S10	-	COCCI	+	+	-	-	-	-	+	-	+	+	+	-	+	-	+	-	Y	*S. aureus*
S11	+	ROD	-	-	+	-	-	-	-	+	+	+	-	-	+	+	-	-	Y	*B.subtilis*
S12	-	ROD	-	-	+	-	-	-	-	+	+	+	-	-	+	+	-	-	Y	*B.subtilis*

+ = Positive, - = negative, cat = catalase, coa =coagulase, s.u. = starch utilization, *S. sa= Salmonella shigella* agar, ind = indole test, cit = citrate, suc = sucrose, mr = methyl red, vp = voges proskauer, man = mannitol

Table 4. The result of the biochemical and identification of bacterial isolate of the fresh fish specimens (*Clarias gariepinus)*

Sample code	Gram Rxn	Shape	Cat	Coa	s.u.	*S.sa*	m.sa	E.mb	Glu	Fruct	Suc	Lact	Man	Ind	Cit	Mr	Vp	TSIA H_2S	Slope Butt	Suspected organism
S1	+	COCCI	+	+	-	-	+	-	+	-	+	+	+	-	+	-	+	-	Y	*S. aureus*
S2	+	ROD	+	-	+	-	-	-	-	+	+	+	-	-	+	+	-	-	Y	*B.subtilis*
S3	+	COCCI	+	+	-	-	+	-	+	-	+	+	+	-	+	-	-	-	Y	*S. aureus*
S4	-	ROD	-	-	-	+	-	+	+	+	+	+	-	-	-	-	-	-	R	*Shigella* sp.
S5	+	COCCI	+	-	-	-	+	+	+	+	+	+	-	+	+	+	+	-	R	*S. epidermis*
S6	-	ROD	-	-	-	+	-	-	+	-	+	+	-	-	+	+	+	+	Y	*S. typhii*
S7	+	COCCI CHAIN	+	-	-	-	-	-	+	-	-	+	-	+	+	-	+	-	Y	*S. aureus*
S8	+	ROD	+	-	+	+	+	+	-	+	+	+	-	-	+	+	+	-	Y	*B.subtilis*
S9	+	COCCI	+	-	-	-	-	+	+	+	-	+	-	-	+	+	+	-	R	*S. epidermis*
S10	-	ROD	-	-	-	+	+	-	+	-	+	+	-	-	+	+	+	-	R	*S. typhii*
S11	+	COCCI	+	-	-	-	-	+	+	+	+	+	-	-	+	+	+	-	R	*S. epidermis*
S12	-	ROD	-	-	-	+	-	-	+	-	+	+	-	-	+	+	+	-	R	*S. typhii*

+ = Positive, - = negative, cat = catalase, coa =coagulase, s.u. = starch utilization, *S. sa= Salmonella shigella* agar, ind = indole test, cit = citrate, suc = sucrose, mr = methyl red, vp = voges proskauer, man = mannitol

5. Discussion

The study revealed a mean bacteria count value of 1.84 x 10^6 cfu/ml. for the fresh fish and 2.06 x 10^6 cfu/ml. for the smoked fish. The bacteria count for the fresh fish ranged from 1.02 x 106-2.32 x 106 cfu/ml. which is less than that of smoked fish that ranged from 1.85 x 106-2.41 x 106 cfu/ml. The mean and the range of fresh samples is less than those of smoked fish. This could be due to sanitary conditions under which the smoked fish samples are handled and kept (Tiamiyu *et. al.,* 2011).

The result of this study revealed that *Staphylococcus aureus, Shigella* spp., *Staphylococcus epidermis, Bacillus subtilis* were the common pathogenic bacteria found associated with fresh and smoked fish in Minna metropolis. The presence of *S. aureus* was attributed to the contamination of the fish samples by man through handling and processing. Clucas and Ward (1996), recorded *S. aureus*, but stated that if ever, it seldom occurs as natural microflora of fish and shellfish. Its main habitat is humans and animals, and found mostly in the nose, throat and skin of healthy individuals (Clucas and Ward, 1996). This indicates that fresh and smoked fish with this bacteria pathogens, must have been contaminated through handling during post harvest. In a similar study carried out by Moshood and TengkuHaziyamin *(2012), Bacillus aureus, Staphylococcus aureus, protens mirabilis, Klebsiella* sp., *Salmonella typhii* and *Streptococcus* sp. were all found to be associated with smoked fish. It was suspected that these organisms may have contaminated the smoked fish through human handlers, air and soil. The findings of Moshood *et. al.,* 2012 corroborates the findings in this study, since common bacteria such as *Staphylococcus aureu, Salmonella typhii* and *Bacillus subtilis* were also isolated. The presence of these organisms in the smoked fish samples of C*larias gariepinus* might be due to increase in moisture content of the product during storage, and also increase in temperature that favours the growth of these organisms. During handling of fish, the natural flora of fish environment will be contaminated with organisms associated with man, such as *Salmonella typhii* and *Staphylococcus aureu*, both isolated in this investigation, can grow well at 30°C - 37°C (Brown, 2004). In a related development, Tiamiyu *et. al.,* 2011, isolated and identified *Staphylococcus aureu, Bacillus sp., Salmonella sp.* and *Streptococcus sp.* from the skin of *Clarias gariepinus* also supports the outcome of this study. *Salmonella sp.* may be present naturally in tropical aquatic environments (Tiamiyu *et. al.,* 2011). It is well established that aquatic birds spread *Salmonella Sp*. and other pathogen in the environment (Fenion, 1983; Beveridge, 1989). *Bacillus sp., Escherichia coli, Salmonella sp., Streptococcus sp.* and *S. aureus* have been implicated in fish-borne diseases of humans (Babu, 2000). Ikpi and Offem (2011) in a study carried out on bacterial infection of mudfish *Clarias gariepinus* isolated and identified *Staphylococcus aureu, E. coli* and *Pseudomonas fluorescens* however dominating. *Staphylococcus epidermis*, one of the microorganism isolated in this study was among the predominant microorganisms isolated from both gills and skin of *Clarias gariepinus* in a study conducted by Hassan *et. al.,* (2010). In support of *Samonella typhii* that was isolated in this study, *Salmonella* sp. have been recovered from gills, intestine and whole body of catfish *Clarias gariepinus* and sea food in Malaysia (Bundiati *et. al.,* 2011; Bremer *et. al.,* 2003; Kumar *et. al.,* 2009; Heinitz *et. al.,* 2000; and Ponce *et. al.,* 2008). Also, in a study conducted by Efuntoye *et. al.,* (2012), *Salmonella typhii* and *Salmonella entridis* were isolated among other organisms. This constitutes a food safety problem, because catfish could be a potential agent of transfer of these species to unsuspecting customers.

The pathogenic state of species of streptococcus is alarming. For instance, Streptococcus parauberis has become important disease agent in the aqua culture industries of North East Asia (Korea, Japan and China), most especially among olive flounder aqua culture farms (Seong *et. al.,* 2013). Only recently, Nho *et. al.,* (2009), reported that S. parauberis is the dominant etiologicalagent of Streptococcus characterized y clinical symptoms, such as chronic wasting syndrome, heamorrhagic septicaemia, exophtaimia and meningitis with abnormal swimming. Streptococcal diseases have been reported worldwide in wild and farmed populations of diverse fresh water and marine fish (Austin and Austin, 1993, Kusuda and Salati, 1993). In 1993, there was an important epizootic outbreak of Streptococcosis in turbot Scophthalmus maximum cultured in Galacia (NW Spain), that was initially thought to be caused by an Enterococcus species-like bacterium (Toranzo *et. al.,* 1994). The disease has been the main limiting factor of the turbot culture in spain (Toranzo *et. al.,* 1995, Romalde et. al., 1996). Other *Streptococcus sp.* that have been found to be associated with aquatic contamination include Streptococcus pyogenes, Streptococcus pneumonia etc.

The public health importance of bacterial flora of Nigeria fish species have not been adequately defined due mainly to mode of food preparation in the tropics, which involved cooking for considerable length of time. The heat would have eliminated most, if not all the bacterial flora (Sowunmi *et. al.,* 2008).

It is noteworthy that sanitary condition under which fishes are handled, processed and stored be improved upon to reflect standard or good practices.

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