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BACTERIOLOGICAL EXAMINATION OF BARBECUE FISH

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Abstract:
This study was carried out for Bacteriological examination of barbecue fish in Ozoro, Isoko-North, Delta State, Nigeria. Samples of barbecue fish were obtained from two (2) different locations in Ozoro. Five bacteria species were isolated from the barbecue fish; Clostridium species, Escherichia coli, Staphylococcus aureus, Proteus mirabilis and Enterococcus species. It was observed that the isolated organism with highest percentage of occurrence was Clostridium species (30%) and the lowest occurrence was Enterococcus Species (10%). Also the results showed that the range of total heterotrophic plate count from bacterial isolates in sample (A) was from 4.4 x 10^3 to 7.6 x 10^3 cfu/ml, while for sample (B) ranges from 3.2 x 10^3 to 6.4 x 10^3 cfu/ml. The bacterial isolate from the barbecue fish were most likely to be as a result of cross contamination from improper handling, storage facilities and display, as well as their survival mechanisms during the preparation process. These bacterial are involved in a number of diseases of public health concerned.

Key Words: ***Bacteriological, Examination, Barbecue, Fish***

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INTRODUCTION:

Seafood has traditionally been a popular part of the diet and main supply of animal protein in many parts of the world. Fish and fishery products constitute an important food components for large section of world population, more so in developing countries, where fish forms a cheap source of protein (Amusan *et al*., 2010).

In the last two decades there has been an increase in awareness about the nutritional and health benefits of fish consumption. The low fat content of some fish and the presence of polyunsaturated fatty acids in red meat fishes which are known to reduce the risks of coronary heart diseases, have increased the dietary and health significance of seafood consumption. The food and Agriculture organization in 1994 asserted that fish contributes about 60% of the developing world, derives more than 30% of their annual protein from fish (Amusan *et al*., 2010).

However, in Nigeria, fish constitute 40% of the animal protein feeding (Olatunde, 1998). They can be easily being contaminated at various stages of handling and processing and moreover the quality is the major concern to food processors and public health authorities (Oramadike *et al.,* 2010 and Amusan *et al.,* 2010). Cat Fish (Silurformes) is locally called barbecued when prepared in a ready-to-eat manner and done in most bar/beer parlours. The nutritional value of fish has led to its worldwide acceptance and consumption by humans. Raw fish has become the most susceptible of all food to microbial spoilage as microbe's e.g, fungi, bacteria and virus are commonly associated with fresh fish (Donnenberg *et al*., 2005).

Street-food are foods and beverage which are sold by street vendors or hawkers, and the foods and beverages could be raw or cooked or barbecued (Ameko *et al., 2012*). Some of the various varieties of street-foods evolved are, Legumes like Cowpea and Groundnuts, vegetables like tomatoes, Onions etc and types of fishes (Ameko *et al,* 2012).

Pathogens, just as it is as a microorganism is readily available on air, in view of this proven fact on exposure of these barbecue fish, there is every tendency that this food (barbecue fish) must be associated or infected by pathogenic microorganisms (bacteria). In other to identify these pathogens, the significances of this research work are no more farfetched.

Sea foods (Barbecue fish) when it is not adequately protected from flies and dust will definitely lead to contamination with micro-organisms (Bryan et al., 1992, 1997). This present study evaluates the bacteriological quality of barbeque fish.

MATERIALS AND METHOD:

STUDY AREA

Ozoro is the administrative headquarter of Isoko North Local Government Area, Delta State. The people are Isoko and hospitable and they are mainly farming, and trading of most agricultural products.

Sterilization of Glasswares

The glass-wares that were used for this project were washed with detergent rinsed thoroughly and sterilized using autoclave at 121°C for 15 minutes.

Methods

Sample Collection

The barbecue fish samples were purchased from two different locations in Ozoro, Isoko North Local Government Area, Delta State. The samples were wrapped with foil paper at point of purchase and were labeled A1-A5 and B1-B5. They were transferred to the laboratory where analysis was carried out.

Analysis

Isolation of test organisms

The samples were blended and serially diluted according to the method of Cheesbrough (2002). Media prepared was according to the manufacturer's instruction and then used for enumeration of isolated bacteria.

The plates were incubated at 37°C for 24 hours. Purified isolates where identified according to their morphological characteristics and reactions to biochemical test.

Morphological Characteristics

Gram Staining: Smear of each bacterial isolate was made on a grease free clean glass slide with a drop of normal saline, air dried, and heat fixed by quickly passing the slides over flame. The smear was flooded with crystal violet for one minute (1mm) then washed the crystal and adds lugol's iodine solution for 1 minute and then washed with water which it was decorourized with 95% alcohol for 15 sees and rinsed off with water again. The slide was then flooded with safranine red for one minute to counter stains, washed off with water, dried, and examined under the microscope using oil immersion and x l00 objective.

Biochemical Test

The biochemical analysis carried out was in accordance with procedures reported by Cheesbrough (2002).

Citrate Test

The bacterial isolate were tested for their ability to utilize citrate as the sole carbon source. Simmons citrate medium was used. Bacterial isolates were inoculated into Simmons citrate medium in test tubes and incubated at 37°C for 24- 48 hours. The culture media was observed for a colour change from green to blue. Positive showed no growth with intense blue colour, while negative test showed no growth and the colour of the medium remained green (Bello, 2002):

Triple Sugar Iron Agar Test (TSI)

Bacterial isolates were stabbed into TSI slant media and also streaked on the surface of the slant after which the medium was incubated at optimal temperature of 37°C for 24 hours. The TSI slant medium was used to check for the present of the following:

Gas: If bubble is present in the media (gas positive)
H2S: If black is present in the media (H2S positive)
Lactose: If the top of the media turn from pink to yellow (lactose positive)
Glucose: If the bottom of the media turn from pink to yellow (glucose positive)

Catalase Test

This test detects the presence of catalase enzyme when present in a bacterium, it catalyse the breaking down of hydrogen peroxide with the release of oxygen as bubble.

$2H_2O_2 \quad 2HO + O_2$

With a wire loop, a colony was picked from the pure culture and was transferred to the centre of a glass slide. 1 -2 drops of 3% hydrogen peroxide was added to the bacterial isolates. Immediate production of bubbles indicated positive result and if no bubble indicated negative.

Indole Test

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole, which then accumulates in the medium for indole production.

Bacterial isolates were inoculated into peptone water medium contained in sterile test tubes then incubated at 37°C for 48 hours. After the incubation period about 3 drops of Kovac's indole reagent was added to the peptone water culture. The bottles were shaken thoroughly and allowed to stand and observed for colour development. A red colour ring at the interface of the medium denotes a positive result. And if the isolate is negative, the reagent layer will remain yellow or slightly cloudy (Bello, 2002).

RESULT AND DISCUSSION:

Result

The bacteria isolated from the samples were *Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Clostridium species, and Enterococcus species*. The highest occurrence Organisms of all the samples is *Clostridium species* and the lowest occurrence was *Enterococcus species*.

Some bacterial isolates have shown the ability to utilize sugar as their substrate which was shown in Table 1. Shows the Cultural, Morphological and Biochemical Characteristics of Bacteria Isolates. Table 2. Shows the occurrence of bacteria isolates identified and their heterotrophic count in different barbecue fish sample, while Table 3 shows the total heterotrophic count from bacteria isolates from barbecues fish samples.

Table 4.1: Cultural, Morphological and Biochemical Characteristics of Bacteria Isolates

Isolates			Gram stain	Morphological characteristic					Citrate	Catalase	Idole	Glucose	lacttose	H_2S	Gas
Staphylococcus aureus	GPC	Cocci	-	+	+	+	+	-	+						
Escherichia coli	GNB	Rods	-	+	+	+	+	-	+						
Clostridlum species	GPB	Rods	-	+	-	+	-	+	-						
Proteus mirabilis	GBN	Rods	+	+	+	+	-	-	+						
Enrerococcus species	GPB	Rods	+	+	-	+	+	-	-						

Key: + = positive, - = Negative, GPB = Gram Positive Bacillus, GNB = Gram Negative Bacillus, GPC Gram Positive Cocci.

Table 2 Percentage of Occurrence of Bacteria Isolates identified in Different Barbecue Fish samples

Bacterial Isolates	% Occurence
Clostridium species,	30
Staphylococcus aureus,	20
Proteus mirabilis	20
Enterococcus species	10
Escherichie coli	20

Table 3. The total heterotrophic count of bacteria isolates.

Simple	Cfu/ml
A 1	**$4.4x\ 10^3$**
A2	**$4.8\ x\ 10^3$**
A3	**$7.6\ x\ 10^3$**
A4	**$6.7\ x\ 10^3$**
A5	**$7.2\ x\ 10^3$**
B1	**$5.9\ x\ 10^3$**
B2	**$6.4\ x\ 10^3$**
B3	**$3.2\ x\ 10^3$**
B4	**$3.9\ x\ 10^3$**
B5	**$5.4\ x\ 10^3$**

Discussion

Based on the result obtained in this study on Bacteriological examination of barbecue fish, it clearly emphasize the cultural, morphological, chemica1 characteristics in accordance with the procedures reported by Cheesbrough (2002), the bacteria isolated from barbecue fish and their ability to utilize sugar as their substrate were shown in (Table 1) and other test carried out. The isolation of pathogenic and spoilage organisms such as *Staphylococcus aureus, Escherichia coli, and Proteus mirabilis* raises public health concerns about safety in consuming barbeque fish products and cause a high rate of spoilage leading to shorter life of the product (Ramos, 1999). Contamination of fish with these organisms is attributed mainly to poor handling by processors and traders who expose fish to unsanitary conditions.

E. coli is often implicated in the gastroenteritis associated with poor handling of food in addition to chemicals found in smoke like sodium chloride *Staphylococcus aureus* is known to cause enterotoxigenicity due to the production of enterotoxin and also known to cause Staphylococcus food poisoning which is a major type of food intoxication. *Clostridium botulinum* the bacteria that may cause botulism is the most harmful of these bacteria (Long, 2009)

The bacterial isolated were *Clostridium species, Staphylococcus aureus, Proteus mirabilis, Enterococcus species, and E. coli* (Table 1).
It should be noted however, that the organisms isolated from the samples were pathogenic organisms that could become harmful and cause food borne intoxication. *Staphylococcus aureus* in Barbeque fish samples can be attributed to post-processing contamination.

This result from this study is in agreement with Nwachukwu and Madubuko (2013) who Isolated *E coli, Staphylococcus aureus, Listeria monocytogenes* from smoked white cat fish (*Chrysichthys nigrodigitatus*).

CONCLUSION AND RECOMMENDATION:

Conclusion

Ready to eat foods contains the indigenous microflora of the raw materials from which they are prepared. Pathogens may form part of the microflora,

causing a public health problem. Pathogens such as *Staphylococcus auerus, E. coli, Clostridium species, Enterococcus species*, were the main identified organisms in barbecue fish which are the most common diseases reported from the health sector of most regions in Africa.

Recommendation

Based on the study and research carried out on the street vended ready eat barbecue fish, it has been observed that many people purchase the barbecue fish and keep it for a while before consumption, thus, encouraging the growth of bacteria which may cause food poisoning on consumption. It is therefore recommended that the fish be consumed immediately after purchased in order to reduce the risk of food poisoning to a minimum rate.

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