Isolation of Bacterial Flora from Beans Cake Sold at the Commercial Area of Federal Polytechnic Damaturu, Yobe State

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ABSTRACT

Ten (10) samples of beans cake (Kosai or Akara) were collected from the Federal Polytechnic Damaturu environment to determine the bacteriological quality, level of concentration and to isolate and identify some of the organisms present in Kosai. 0.1mls of the Akara sample which is prepared by dissolving 5.0g of the sample in 10mls of sterile distilled water. The samples were spread plated on nutrient and macConkey agar and then incubated at 37^{o}C for 24 hours. The bacterial colonies were counted using a colony counter. The result revealed that the total microbial load range from 0.7 – 18.9X10^{3} cfu/g and the preliminary gram staining of the colonies revealed the presences of both gram positive and negative organisms in most of the colonies. Four organisms were identified from the colonies which include; Shigella spp (17.9%), Escherichia coli (28.50/0), Salmonella spp (17.90/0) and Staphylococcus aureus (35.7%) respectively. These isolates were commonly found to be the contaminants in food and water. Sources might be through handling, packaging and production process. Staphylococcus aureus which occurs in about 35.7% of the samples that were studied calls for more proper hygiene and handling as these isolate were known to cause infection even at a very low concentration. Base on this study one can deduce that the handling process is fairly good and is evident in the low amount of bacterial count and the absence of fastidious isolate such as Pseudomonas spp and also the limited number of isolated organisms. It's therefore recommended that proper hygiene and handling process be encouraged among the sellers and buyers of beans cake in oder to limit the chances of infections caused by these microorganisms.

1.0 INTRODUCTION

Food elaborated with satisfactory hygienic standards is one of the essential conditions for promoting and preserving health, and inadequate control is one of the factors responsible for the occurrence of food borne disease outbreaks (Oliveira *et al.*, 2016). Illness resulting from food borne disease has become one of the most widespread public health problems in the world today. The surveillance of food-borne disease outbreaks is fairly established in developed countries but in spite of that only less than 10% are recorded in official statistics. In case of developing countries, it could be even less than 1% (WHO, 2006). International studies have shown that a significant proportion of food borne diseases arise from practices in the kitchen of a home (Scoff, 2019; Bryan, 2017, Redmond and Griffith, 2004). Several studies assessing different kinds of consumer groups identified food prepared in the family home as a major source of food poisoning (Jay *et al.*, 2018; Anderson *et al*., 2004).

There is an increasing tendency for the populace to patronize out-door foods also called snacks or "Take-away-foods" in the Polytechnic Campuses in particular and in the society at large. The term "Snack" is used to describe "High-Energy" foods such as crisps of all types, fried fish or meat, and even African delicacies such as "Akara" (fried beans cakes), "Moin-moin" (steamed bean pastry food), oil-fried ripe plantain (or "Dodo") etc. Snack foods are very popular worldwide especially among children and the working class. Furthermore, snack foods are increasingly becoming choice foods as a result of general food-shortages and poverty in the third world countries, (as a result of low income, which encourages expenditure of limited money on the often cheaper snacks); and urbanization influence which encourages more hours of work away from home.

Foods are usually contaminated with microorganisms as a result of inadequate preparation, unsanitary handling, ineffective storage, improper packaging and unsanitary exposure. According to Isara *et al.*, (2010) the prevalence of food contamination in the fast food restaurants in Benin in Nigeria, was found to be 37.5%, in which *Bacillus cereus* and *Staphylococcus aureus* were the most commonly isolated bacteria, while salad, meat pie and fried rice were the most commonly contaminated foods. Foods are easily contaminated as they serve as rich substrates for most microorganisms including different pathogens which could cause gastroenteritis and food poisoning. (Jay, 2005).

2.0 FOOD POISONING

Food poisoning syndrome results from ingestion of water and wide variety of food contaminated with pathogenic microorganisms (such as bacteria, viruses, protozoa, and fungi), their toxins and chemicals. Food poisoning must be

suspected when an acute illness with gastrointestinal or neurological manifestation affect two or more persons, who have shared a meal during the previous 72 hours. The term as generally used encompasses both food-related infection and food- related intoxication. Some microbiologists consider microbial food poisoning to be different from food-borne infections. In microbial food poisoning, the microbes multiply readily in the food prior to consumption, whereas in food-borne infection, food is merely the vector for microbes that do not grow on their transient substrate. Others consider food poisoning as intoxication of food by chemicals or toxins from bacteria or fungi (Sridhar, 2006).

2.1 Bacterial Etiology of Food Poisoning:

Food infections by bacteria can be divided into two types:

1. Those in which the food does not ordinarily support the growth of pathogens but merely carries them. E.g. Salmonella, Shigella, Vibrio etc.

2. Those in which the food can serve as a culture medium for growth of pathogens to numbers that can infect the person.

Food borne infections by bacteria can also be classified as toxication and food-infections. Intoxication, the toxins are released by bacteria such as *Clostridia*, *Bacillus* and *Staphylococcus species*. In food infections, the bacteria are ingested, which later initiate the infection.

Staphylococcus aureus

S. aureus is gram positive cocci that occurs in singles, pairs, short chains, tetrads and irregular grape like clusters. It is present ubiquitously in the environment, but only those strains that produce enterotoxin can cause food poisoning. Food is usually contaminated from infected food handler. The food handler with an active lesion or carriage can contaminate food. The incubation period is usually 1 -6 hours since the ingested food contains preformed toxin. The clinical features are sudden and characterized by vomiting and diarrhea, but no fever. The illness lasts less than 12 hours. There are no complications and treatment is usually not necessary.

Laboratory diagnosis: The presence of a large number of *S. aureus* organisms in a food may indicate poor handling or sanitation; however, it is not sufficient evidence to incriminate a food as the cause of food poisoning.

Staphylococcal food poisoning can be diagnosed if they are isolated in large numbers from the food and their toxins demonstrated in the food or the isolated *S. aureus* must be shown to produce enterotoxins. Dilutions of food may be plated on Baird-Parker agar or Mannitol Salt agar. Enterotoxin may be detected and identified by gel diffusion.

Escherichia coli

Escherichia coli are bacterium that is common, but certainly not the most abundant among bacterial, inhabitant of human intestine. It was also lives in the intestine of many other animals, wild as well

as domestic animals (USA FDA, 2002). Normally *Escherichia coli* do not cause disease; rather it is to serve a useful function in the body suppressing the growth of harmful bacteria species and by synthesizing appreciable amount of vitamins. Although some strains, causes diarrhea and it is the most common causes of urinary tract infections. Currently there are four recognized classes of entero virulent *Escherichia coli* that cause gastroenteritis in humans. Among these is the enterohemorrhagic (EHEC) strain designated as *E. coli* 0157:H7 is particularly virulent and has been responsible for several dangerous out breaks in people eating contaminated foods. According to US food and drug administration (2007), said that drinking water is tested for the presence of *E. coli* and related bacteria not because the bacteria are dangerous but because they are an indication of contamination by sewage, and m contain organisms like *Salmonella* that are dangerous. E. *coli* is the one of the most thoroughly studied living things (USA, 2007).

Diseases Caused By *Escherichia coli*

The disease caused by E. *coli* is characterized by severe cramping (abdominal pain) and diarrhea which is initially watery, but becomes grossly bloody, occasionally vomiting occurs. Fever either low grade or absent. Because E. *coli* lives in the intestine of humans, this has raised fear that genetically engineered version might escape from the laboratory (or factory) and takes up residence in human's producing a product that might be harmful, example it produce large quantities of one or more related, potent toxins that cause severe damage to the living of the intestine. These toxins (Vero toxin shiga-like toxin) are closely related or identical to the toxin produced by *Shigella dysentaniae* (Douglas, 2016).

Salmonella typhi

Salmonella typhi is a gram negative organism also, it is a rod-shaped, predominantly motile entero bacteria with diameters around 0.7-1.5ktm, length from 2-5ktm and flagella which grade in all direction (i.e peritrichous). They are chemoorganotrophs, obtaining their energy from oxidation and reduction reactions using organic sources, and are facultative anaerobes.

Most species produce hydrogen sulfate, which can readily be detected by growing them on media containing ferrous sulphate. Most isolates exist in two phases that is a motile phase I and a non-motile phase Il. Cultures that are non-motile upon primary culture may be switched to the motile phase using a cragic tube. *Salmonella* is closely related to the *Escherichia* genus and are found worldwide in cold and warm-blooded animals (i.e. including humans), and in the environment (Geidam, 2009).

Diseases Caused By *Salmonella typhi*

These diseases are caused by *Salmonella typhi*, they can cause illness like typhoid fever, pxch

I aratyphoid fever and food borne illness. The key to avoiding infection of

Salmonella typhi is prevention of fecal contamination in drinking water and food supplies. Since the only sources of thus agent are infected humans, it is possible to control transmission by proper hygiene, waste management, water purification and treatment of the sink. These measures are attained in developed societies attributing to the low incidence (Hensel, 2009).

3.0 METHODOLOGY

Sample Collection

Ten (10) samples each were collected from five different locations; Federal Polytechnic Damaturu and environs in well labeled plastic containers. The locations are Staff quarters (Q), Staff School (P), Main commercial area (A), Male Hostel (D) and Female Hostel (C).

Determination of Total Bacterial Count

0.1ml of each of the Akara sample were spread plated on nutrients and macConkey agar an incubated at 37ºC for 24 hours for determination of the total bacterial count all colonies were counted using the colony counter while the organisms with different morphologies on agar were isolated and sub-cultured for further identification.

Characterization and Identification of Organisms

Characterization was made by noting the morphological characteristics of the different isolates the agar plates. Physiological examination such gram's on as staining reaction and determination of motilities were all carried out to aid identification of the organisms. Biochemical tests such as indole, methyl red, Vosges Proskauer, catalase and coagulase tests were also carried out.

Motility

The medium (agar with concentration of 0.2-0.5) was inoculated with the test organism. A stab of each inoculate was made at the center of each tube. The tubes were incubated at 37ºC for 24 hours. The temperature was reduced for Pseudomonas. A diffused growth at the place of inoculation is considered as positive and restricted growth is considered as negative.

Indole Production

This was done by colorimetric reaction with P—Dimethylamino-benzaldehyde (Kovac reagent) to determine the ability of isolate to decompose the amino acid, tryptophan to indole. Peptone broth was prepared and dispensed in test tube and were inoculated with the isolate and incubated at 37ºC for 4 days. 0.5m1 of Kovac reagent was added to each tube shaking gently. Positive results were characterized with red alcohol layer.

Methyl Red

This was used to detect the production of sufficient acid during fermentation of glucose which is indicated by change in the color of the methyl red indicator. Isolates were inoculated into tube of previously prepared glucose peptone water and incubated at 37ºCfor 2 days. 5 drops of methyl red solution was added to each tube and color change was observed. Positive

results give red color while negative results give yellow with the indicator.

Voges-Proskauer

Tubes of glucose phosphate peptone water were inoculated and incubated at 37ºC for 2 days. 1ml of 40% KOH and 3m! of 5% solution of cz-naphtol in absolute ethanol was added to each tube. A positive result gives crimson color in 30 minutes.

Catalase Test

This test was performed in a test tube. 2m1 of 3% hydrogen peroxide was placed in a clean test tube. A sterile wire loop was used to pick a colony of the test organism and mixed with 2ml of 3% hydrogen peroxide in the test tube and observed for the production of gas bubbles which indicates a positive reaction.

Coagulase Test

The ability to liberate coagulase enzymes was tested by using human plasma. A loopful of human plasma was added to culture isolate on slide. Positive isolate give precipitation reaction with plasma, Test was also carried out at 37^0C for 24 hours. Positive tubes showed coagulation of the plasma in the tube.

4.0 RESULTS AND DISCUSSION

The study conducted on the microbial contamination of beans cake (kosai) revealed that the total microbial load range from 0.7-18.9 x 103cfu/g (Table l) and the preliminary Gram staining of the colonies revealed the presence of 'both Gram positive and negative organisms in most of the colonies. The organism with the highest frequency of occurrence taken as the baseline. Four (4) isolates were obtained which include; *Shigella spp* (17.9%), *Escherichia coli* (28.5%), *Salmonella spp*. (17.9%) and *Staphylococcus aureus* (35.7%) as indicated in Tables 2 and 3.

Table 1: Bacterial cell count and Gram staining of colonies

Sample	Bacterial Count (cfu/g)	Gram Stain Reaction
C1	1.6 X 10^3	Positive
C2	1.9 X 10^3	Positive
Q1	18.9 X 10^3	Negative
Q2	14.5 X 10^3	Positive
A1	9.2 X 10^3	Negative
A2	8.4 X 10^3	Negative
D1	0.7 X 10^3	Positive
D2	0.8 X 10^3	Positive

P1	2.0 X 10^3	Negative
P2	5.4 X 10^3	Positive

Table 2: Summary of the suspected microorganisms

Sample	Suspected Microorganisms
C1	*Staphylococcus aureus*
	Shigella spp
C2	*Escherichia coli*
	Shigella spp
	Staphylococcus aureus
Q1	*Escherichia coli*
	Staphylococcus aureus
	Shigella spp
Q2	*Escherichia coli*
	Shigella spp
	Staphylococcus aureus
A1	*Escherichia coli*
	Staphylococcus aureus
	Shigella spp
A2	*Escherichia coli*
	Shigella spp
	Staphylococcus aureus

Table 3: Summary of isolates frequency of occurrence

Organism	**Frequency**	**Percentage (%)**
Escherichia coli	8	28.5
Salmonella spp	5	17.9
Shigella spp	5	17.9
Staphylococcus aureus	10	35.7

5.0 CONCLUSION

In summary, microbial contamination of Kosai in the Federal Polytechnic Damaturu environs is low. The commonly isolated organisms determined are *Staphylococcus aureus* (35.7%) *Escherichia coli* (28.5%), while Shigella *spp* and *Salmonella spp* has (17.9%) each. These organisms can cause various and serious health problems. Proper hygiene and handling of the baked beans cake can help to limit the number of these organisms in baked beans.

6.0 RECOMMENDATIONS

From the work performed, the following recommendations are suggested:

1. Proper hygiene and handling process should be encouraged among the sellers and buyers of beans cake in order to limit the chances of infections caused by Microorganisms.

2. Further studies are to be conducted in oder to expand the coverage and also to identify the organism to their various strains and identify the toxins produced by them if at all any exist.

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