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Bacteriological Assessment of Meat Pie Sold at Ochanja Market Onitsha, Anambra State

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Abstract— *Ten meat pie samples were purchased from different eatery points in Ochanja Main Market, Onitsha and analyzed for the presence of pathogenic bacteria using standard microbiology and biochemical techniques. The following bacteria genera were isolated and identified from the meat pie; Escherichia coli (39%), Staphylococcus aureus (35%), and Bacillus cereus (26%). The percentage distribution showed that Escherichia coli were the most prevalent in the meat pie samples while Bacillus cereus was the least. The meat pie samples sold within Ochanja Main Market were considered fit for human consumption since the distributions of the bacteria isolates were below standard threshold limit.*

Keywords— Meat Pie, Bacteria and Food borne diseases.

I. INTRODUCTION

There has been a notable and remarkable increase in the consumption of convenience and ready to eat foods by the people in recent times. Ready to eat foods can be described as foods that were meant for immediate consumption at the point of sale. It could be raw or cooked, hot or chilled and can be consumed without further heat treatment [Tsang, 2002]. Different terms have been used to describe such ready to eat foods include, convenient, ready, instant or fast foods. Examples of such ready to eat foods include pastries, meat pie, sausage rolls, burger, moi-moi, salad, fried meat, fried chicken, milk and milk products [Alexander and Tittiger, 1971]. Meat and meat products have been a constant food for man as far back as there has been any evidence of civilization on the face of earth.

Meat pie is a food product that comprises of savory pie with a filling of meat and other savory ingredients [Clarkson, 2009]. It is made up of meat enclosed in a biscuit dough or pastry. Meat pie contains at least 25% meat, protein boosters such as soya protein thus giving it a high protein content. A locally made meat pie usually comprises of meat, salt, water, nutmeg, egg thyme, olive oil, onions, garlic and some other savory ingredients [Adesiyun, 1995; Oluwafemi and Simifaye, 2005]. Meat pie contributes about 20-40% of daily recommended intakes of sodium for adults. It is a good source of carbohydrates. The flour content of meat pie provides high percentage of carbohydrates to the consumers [Bickert, 2010].

The susceptibility of meat pie to spoilage by micro-organisms gives it a shelf-life of 72 – 96 hours (3-4days) [Adesiyun, 1995].

Micro-organisms play an important role in the quality of meat products before, during and after processing by limiting many undesirable biological changes in it [Ukut *et al.*, 2010]. Meats and meat products undergo spoilage as a result of microbial action on the fats and proteins [Adesiyun, 1995]. Food contamination is the introduction or occurrence of a contaminant (any biological or chemical agent, foreign matter or other substance not internationally added to food which may compromise food safety or suitability) in food or food environment [Omoloya and Adeleke, 2013]. Food is prone to contamination at every stage in the food chain. The consumption of food contaminated by micro-organism will result in food borne illnesses. These are usually either infectious or toxic in nature, caused by agents entry into the body through ingestion of food [WHO, 2002]. A food borne infection involves the ingestion of pathogen, followed by growth in the host, including invasion and/or release of toxin [Brener, 2005]. Food supply issues of processed meat are usually as a result of contamination (bacterial) introduced exogenously during activities such as harvesting, processing and preparation [WHO, 2002]. Food borne illnesses have continued to form a significant part of morbidity and mortality of Nigerians and have been in the increase in recent times. The international impact of food borne illnesses is difficult to estimate. Bacteria are the causative agents of food borne illnesses in 60% of cases requiring hospitalization [Mead *et al.*, 1999]. Enterotoxigenic

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Staphylococcus, *Escherichia coli*, *Clostridium perfrigens*, *toxoplasma gontu* and *salmonella pp* have been isolated from foods implicated in illnesses [Bello *et al*., 2013; Cencil *et al*., 2003]. Research has shown that food and water is the vehicle for many illnesses [WHO, 2002].

Street foods are frequently associated with diarrhea diseases, which occur due to improper use of additives, the presence of pathogenic bacteria, environmental contaminants, disregard of good manufacturing practices and food hygiene. [WHO, 2002] reported that vendors are often poorly educated, unlicensed, untrained in food hygiene and they work under crude unsanitary conditions with little or no knowledge about the causes of food borne diseases .Data on issues of food borne are well documented worldwide[Thomas *et al*.,2006].Food borne illnesses is a major international health problem with consequent economic reduction. In Nigeria, a number of foods have been reported to have high incidence of bacteria [Adestan, *et al*., 2013]. Constant bacteriological surveillance is required to ensure wholeness and quality of ready to eat foods consumed by the people.

The need by the vendors to focus more on food hygiene as well as regulatory agencies to ensure compliance with approved standards underscored the reason behind this experiment.

II. MATERIALS AND METHODS

Sample Collection

Ten meat pie samples were obtained from different fast food retail and vending centers in Ochanja market, Onitsha. The samples were immediately wrapped in sterile aluminium foil to prevent contamination and then transported to microbiology section in spring board laboratory, Awka for microbial analysis.

Preparation and Inoculation of Samples

10g of each food sample was weighed out and homogenized into 10ml of sterile distilled deionized water using a sterile warming blender. Tenfold dilutions of the homogenates were prepared and up to 10^{-7} dilution factors of the homogenate were plated in triplicates on the Mueller Hinton agar, Mac-Conkey agar and Manniton salt agar using the spread plate techniques. The plates were then incubated at 37^{o}C for 24 – 48hrs. Mac-Conkey agar was used for coliform enumeration while mannitol salt agar was used for isolation of *S. areus*. Total viable bacteria count was performed in Mueller Hinton agar. At the end of the incubation periods, colonies were counted using illuminated colony counter. The count for each plate were expressed as colony forming unit per gram of the sample (cfu/g).

Identification of Isolates

Colonies identifiable on the Hinton agar were carefully examined macroscopically for control characteristics such as color, size e.t.c. Gram staining as well as appropriate biochemical tests according to (Mead *et al*., 1999) were carried out.

III. RESULTS AND DISCUSSION

Table.1: Plate count of viable bacterial isolates from the meat pie samples sold at Ochanja market, Onitsha.

Sample	Bacteria counter per gram
1	86
2	65
3	81
4	67
5	80
6	57
7	76
8	58
9	65
10	68
Mean	**69.5**

The mean bacteria isolates from the samples was 69.5cfu/g. The mean viable bacteria isolates was found to be within 10^{2}cfu/g threshold limits of foods fit for human consumption.

Table.2: Plate count for different colonial forms from the meat pie samples

Sample	Designated of colonies (cfu/g)		
+	A	B	C
1	34	35	17
2	21	23	21
3	26	32	23
4	25	26	16
5	30	30	21

6	13	19	19
7	27	29	20
8	17	23	16
9	24	26	16
10	27	25	16
Mean	**24.4**	**26.8**	**18.4**

Where A = White coloured colonies
B = Red coloured colonies
C = Pink coloured colonies

The mean bacteria isolates were macroscopically counted for cultural characteristics in colour. The colour count of bacteria isolates decreased as follows:
B > A > C.

Table.3: Cultural characteristics and gram reaction for bacterial identification

Sample	Colonial characteristics	Gram reaction	Probable identification
A	Opaque, smooth, white coloured, colonies measuring 0.1 – 0.3mm on Mac Conkey Agar.	Gram positive cocci, occurring in sample.	*Staphylococcus aureus*
B	Smooth red coloured colonies on Mac Conkey Agar measuring 0.2, 0.5mm.	Gram negative straight rods occur in sample.	*Escherichia Spp.*
C	Pink coloured colonies, irregular and flat, measuring 1 – 2mm on the Mac Conkey Agar.	In chains	*Bacillus cereus*

Table.4: Biochemical tests for identification of bacterial isolates

Identified bacteria	Biochemical Tests									
	Catalase	Coagulase	Citrate	Oxidase	Urease	Indole	Glucose	Lactose	Sucrose	Maltose
Staphylococcus aureus	+	+	-	-	+	-	+	+	+	+
Escherichia Spp.	+	-	+	-	-	+	+	+	+	+
Bacillus cereus	+	N/A	+	N/A	N/A	-	N/A	-	N/A	N/A

Table.5: Percentage distribution of the bacteria isolate

Identified bacteria	Mean conc. per gram	Percentage distribution
Staphylococcus aureus	9	35
Escherichia Spp.	9.1	39
Bacillus cereus	6	26
TOTAL	**23**	**100**

Table 5 showed the percentage distribution of the identified bacterial isolates in the studied sample. *Escherichia coli* was the most prevalent bacteria isolated from the meat pie samples with highest percentage of 39% while the least isolated bacteria was *Bacillus cereus* with percentage distribution of 26%. The result of this finding is in accordance with the reports of [Adesiyun, 1995; Bickert 2010] were they isolated similar organisms from sausages and sea food processors respectively. The presence of these organisms in meat pies depicts a deplorable state of poor hygienic and sanitary practices employed in the processing and packaging of these food products. From the results obtained, the meat pie samples were contaminated with *Escherichia coli, Staphylococcus aureus*and *Bacilus cereus* however the bacterial distribution were within the permissible threshold limits of foods fit for human

consumption. *Escherichia coli* and *Staphylococcus aureus* are named flora in human and animals. Their presence in food products is an indication of excessive human handling [WHO, 2002]. The result obtained in this research agrees with [Adesiyun, 1995; Okonko *et al*., 2009] that foods of animal origin either cooked or uncooked were predominantly contaminated with *Escherichia coli* and *Staphylococcus aureus*. They further stated that the presence of *Escherichia coli* in food products is an indication of fecal contamination of the water sources that were utilized during the processing of the food products. The presence of *Bacillus cereus* in the meat pie samples could be due to improper handling of raw materials from harvesting to processing points [WHO, 2002]. All the three isolated bacteria in the meat pie samples have been incriminated to contribute to life threatening food borne illnesses.

IV. CONCLUSION

The study shows that the meat pie samples sold within Ochanja market,Onitsha,were fit for human consumption since the bacterial load of the three isolates were below the permissible level in sausage foods (10^2cfu/g). This could be attributed to adherence to proper personal and environmental hygiene, use of portable water, proper cooking of the products and use of efficient storage techniques. Since the three bacteria isolates have seen implicated in many life threatening food borne illness, it is good news that their distribution in the meat pie consumed by the people were below recommended threshold limits.

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