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ISOLATION AND CHARACTERIZATION OF SOME MICRO-ORGANISMS ASSOCIATED WITH SLAUGHTERED COW MEAT AT EKE-OKO, ANAMBRA STATE.

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KEYWORDS:
Micro-organisms, Isolation, Cow Meat, Slaughtered, Characterization.

Abstract

The study of isolation and characterization of bacteria associated with slaughtered low meat sold in Eke Oko, Orumba North L.G.A Anambra State was under taken from cow meat samples were obtained from different locations within oko. Each samples were slended, serially diluted up to 10^{-6} folds then cultured and observed for cultural, morphological and biochemical characteristic. Bacteria recovered were salmonella spp, Escherichia coli, Staphylococus Spp. Websiella Spp and streplo coccus spp, which might be duej to poor hygiene and sanitation from the meat sellers and due to unsterilized equipment and utensils used in preparing the cow meat. Hence, cow meant should be carefully prepared under proper sanitation, hygienic environment with sterilized utensils and tools. Relevant government agencies such as NAFDAC should organize seminars and workshops for cow meat sellers as these sellers may be counterproductive while unfit cow meat consumption can serve as vehicles for transmission of diseases.

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INTRODUCTION

Generally, meat is excellent in supplying high quality protein, vitamins and mineral salts. Similarly, it has been reported as ideal for the growth of wide range of spoilage bacteria (May *et al,* 2003), accounting to a great extent why it is perishable. In the world today, traditionally processed meat products are consumed in different countries, amongst which is the meat delicacy called cow meat (Vilar *et al.,* 2000). Cow meat is a traditional meat product gotten from boneless meat hung on stick and spiced with peanut cake, salt, vegetable oil and other flavors followed by roasting around a glowing charcoal fire (Alonge and Hiko,1981). It has become very popular as a street delicacy in several countries, particularly those in West Africa (Inyang *et al,* 2005).

The high ultimate pH of meat generally makes it very susceptible to microbial growth even under the best handling or manufacturing condition and practice (Hedrick *et al.,* 1994).

Sequel of these developments, some researchers elsewhere had noticed sporadic cases of gastroenteritis and symptoms of infection after consumption of cow meat which indicated that the product indeed constitute a food safety risk (Odusole *et al.,* 2003; Inyang *et al.,*2005).

Literature has it that microbial organisms isolated from cow meat are of public health significance, as study conducted on cow meat sold in Ado and Akure, south west Nigeria revealed bacteria, molds, yeast and fungi (Egbebi and Seidu, 201 1).

The main factors which determine food hygiene include handling, preparation techniques and storage practices (Ifediora, *et al.,* 2006). In Nigeria, not much work has been done to try and investigate the occurrence of pathogenic microorganisms in roasted cow meat taking into consideration the method of preparation and storage practices. However, the conditions of preparation and selling are sources of contamination

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especially germs involved in poisoning of cow meat (Bukar *et al.*, 2010). Diseases resulting are a major problem of food safety and a reason of mortality in developing countries (Elmahmood and Doughari, 2007). Osho (2004) also evaluated the bacteria contamination of cow meat processed in Abeokuta, South Western Nigeria and found up to 103cfu gentero-bacteriaceae in 40% of the 622 samples collected; more than 104cfu/g aerobic mesophiles including *Staphylococcus aureus* in all collected samples. Inyang *et al.,* (2005) also evaluated the bacterial quality of cow meat sold in Markurdi, Northern Nigeria and concluded that faecal coliforms were the main bacterial contaminants although they occurred within acceptable limit.

Meanwhile, Edema *et al.,* (2008) who evaluated the microbial hazards associated with processing of cow meat reported that processing water, meat processing slabs, utensils, spices and raw meat revealed contamination with potential pathogens such as *Bacillus cereus, Staphylococcus aureus, Salmonella species* and aflatoxigenic molds with aerobic mesophilic counts in the order of 105 cfu with the highest value (7.17)observed in the packaging material and the lowest value (1 .47) observed in the processing water. It is imperative to note that the tremendous growth in the production and consumption of cow meat in the South-South zone of Nigeria has made it a great concern to study and to know its microbial quality.

Hence, this study is embarked upon to investigate the safety and quality of traditional ready-to-eat meat collected from typical vendors surrounding a major point in Eke-Oko, Orumba North L.G.A of Anambra state.

Preparation of Cow Meat

Usually in Nigeria, skinless, boneless flesh of cattle used for commercial preparation of meatr. Finely grounded roasted peanut cake, red pepper, salt, grounded ginger, grounded garlic, chuncked fresh tomatoes and

minced fresh onions are required on suitable conditions depending on the quality of meat required to be prepared.

The process of preparation involves a few steps, First is the grounding of peanut. The shell and the skin are removed from the peanut before grinding into fine powder using mortar and pestle or crushed with rolling pin. If the powder is oily, it is wrapped with an absorbent paper and squeezed for a minute or two. Next, the grinded pepper, garlic, ginger are stirred into the peanut powder and mixed properly.

The meat is then cut into small sizes or thin sliced, dipped and rolled in a bowl containing the mixed peanut spice and allowed to coat completely. The minced meat are then kept for thirty minutes or more for the peanut cake to stick to it after which the meat slices are threaded unto skewer and brushed with vegetable oil and roasted on the glowing charcoal fire for fifteen to twenty minutes. It is finally removed from the skewer and served hot in a newspaper with sliced onions, tomatoes and cabbage (Judge et al., 2002).

Nutritional Values of Meats

Meat plays an important role in human diet contributing both macro and micro nutrients that are required for growth and good health maintenance. The rate of increase in per capita consumption of meat was found to be very high in developed countries when compared with developed nations (Anjaneyulu *et al.,* 2007). Meat is high in nutrients, but very prone to spoilage and to prevent this from occurring value addition to meat is essential (Anna *et al.,* 2005). This involves processing and preservation of meat so as to prolong its shelf life and improve its acceptability (Eyas Aimed *et al.,* 2006). Processing aids in producing varieties and convenient meat products in order to meet various lifestyle requirements while preservation aided by processing extend to shelf-life of meat and meat products (Sharma and Kondaiah, 2005). The need for effective, cheap and simple preservative

technique cannot be ignored and one of such is intermediate moisture food processing such as suya (tsire) (Omojola, 2008). It is a mass consumer fast food which is processed and sold along streets under unhygienic conditions (Uzeh *et al.,* 2006).

Meat Spoilage

Meat is spoilt when it loses its nutritive value, texture and brings out offensive odour thus rendering it unfit for human consumption. The most important bacterial meat spoilage is caused by lactic acid bacteria; these include many species such as Lactobacillus, Leuconostoc, Pediococcus and Streptococcus which are physiologically related to a group of fastidious and ubiquitous gram-positive organisms.

Meat spoilage is usually associated with gram negative proteolitic bacteria which literally decompose the protein with production of offensive odour (Frazier & Westhoff, 2004). A number of factors could cause meat spoilage (Nester *et* a/.,2001). Meat deterioration or spoilage of meat could be caused by bacteria, yeast and mould. When meat is not properly handled, it leads to microbial spoilage which makes it unfit for human consumption (Sokari *et al.,* 1999).

Factors that Affect the Growth of Microorganisms Meat

A lot of factors affect the growth of microorganisms on meat. The factors include temperature, pH, water availability, presence of nutrients, moisture, acidity (intrinsic factors), gaseous requirement, atmosphere of storage (extrinsic factors) (Nester *et al.,* 2001).

Temperature

Microorganisms have optimum, minimum and maximum temperature that they can grow. *Listeria monocytogenes* have been found to grow at 1°C and even survive freezing. This ability of growing at low temperature provides

opportunity for proliferation in contaminated meat products. *Pseudomonas species* grow at temperature less than 20°C (Dineen *et al.,* 1999). Psychrophiles have optimum temperature between 2°C and 7°C; mesophiles between 10°C and 40 C and thermophiles from 43°C or 66°C (Favaretti, 2000). Examples of psychrophiles are Achromobacter, Streptococcus, Salmonella, most yeast, mould. Examples of mesophiles are *Bacillus stearothemophiles.* Alcaligens, Pseudomonas, 2. 8.2 PH Most bacteria grow optimally at above pH 7 and not well below pH 4 or above pH 9 (Abdul Raouf *et al.,* 1993). But the pH of maximal growth is determined by the simultaneous operation of variables other than the degree of acidity or alkalinity. Proteolitic changes operate best near pH

Water Availability

Water is required by microorganism so reducing water below the optimum level will prolong shelf life of meat. Since most spoilage bacteria can grow only on the surface, drying the meat. Surface will not favour their growth but *Staphylococcus aureus* can grow in meat with 0.86% which is lower than that of other spoilage bacteria (Nester *et al,* 2001).

Nutrients

Microorganisms depend on nutrients from meat product or meat for survival. Meat contains protein, fat, vitamins and phosphorus which support the growth of microorganisms. *Pseudomonas aeruginosa* synthesizes its vitamins and so cause spoilage even in a medium without vitamin. *Staphylococcus aureus* require about 6.5% of sodium chloride for growth and is usually found in salty meet products. (Boles *et al,* 2000).

Contamination of Meat

Animals have a normal or natural microflora that is established very early in life. Besides this microflora, animals tend to harbour the types of organisms found in their environment since they are contaminated by soil, water, feed and excreta (Jay, 2003).

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The contamination of meat by equipment begins with slaughtering the animal. Eva (1984) reported that during the slaughtering operation, the handling of the carcass as well as its contact with various piece of equipment can lead to the spread of salmonella throughout the slaughtering operation. Contamination can also be through slaughtering of sick animals, washing the meat with dirty water by butchers, contamination by flies through processing done close to sewage or refuse dumps site, transportation by rickety vehicles, use of contaminated equipment such as knife and other utensils and addition of unclean spices. The microorganisms grow on meat causing visual, textural and organoleptic changes when they release metabolite.

Material and Method

Sample Collection

Slaugutered caw meat were purchased from Eke oko market and mashed in a sterile laboratory type mortar and pestle into a paste.

Sterilization of Equipment

ml equipment and culture median that were used for this project work were sterilized by autarchy at 12°c at. 1. fVnsi for ISminutes and was kept until needed. This wireloop were sterilized using the business burner flame.

Serial Dilution

Ten-fold serial dilution of the sample were made, These were done with (1ml) syringe and nine rriilliliter sterile water as diluents, it was done until-$10\sim^{3}$ dilution was achieved. O.lml was then pipette from. 10^{-3} dilution on the surface of the petri-dish containing; the solidified media, and then spread evenly with sterile glass spreader. These were done in all the prepared agar plates according to the number of the samples labeled and they were incubated for 24hours at 37oc of the bacterial colonies was done using the

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stunt digital colony counter and the number of cells per ml of each sample were determined with the formular below:-

VD
Wherens n - number of cells per ml .(GFU/ML)
N = are rage number of colonies
V - Volume plated out
D = dilution.

Inoculation of the organism

The samples were serially diluted using Irnl sterile pipette and 9ml sterile water in test tubes as diluents. Aliquots of O.lml of 10^2 dilution' were used to inoculaie on solidified prepared macconkey agar plate using a spread plate method with aid of a sterile glass spreader. The plates were incubated at 37°c for 24hours.
After incubation the pink colonies were counted using colony counter and the number of cells per nil of the sample were determined.

Identification of organisms

Based on the result obtained in the biochemical tests arid the colony morphology- on. the selective media, the pathogen were identified. Pure cultures of the particular pathogen were then maintained in nutrients agar slants and nutrients agar plates for antimicrobial testing.

Results

The isolation and characterization of bacteria associated
With slaughtered cow meat from Eke Oko market within the campus of the Federal Polytechnic Oko, Orumba North Local Government Area, Anambra State are presented in Tables 1-3

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Table 1: Morphological characterisation of bacteria isolates
From slaughtered cow meat Eke Oko market

Test	Cultural and Morphological Appearance	Suspected Micro Organisms
Isolate + NA	Formation of numerous spherical shape colonies of uniform size occurring in clusters	*Staphylococcus spp suspected*
Isolate + MCA	Cocci shaped typhically arrange in chains	Streptococcus Spp suspected
Isolate +NA	Rod like mucoid pink colonies	Klebsiella spp
Isolate + MCA	Short rod-like pale coloured colonies with black center	Salmonella spp

Table 2
Biochemical tests of bacteria isolated from the suya samples.

Biochemical Test	Suspected bacteria obtained
Gram staining	The cocci appeared with dark-blue background that, suspected *Staphylococcus spp* and *Streptococcus spp* are gram positive
Motility test	Thesuspected *Staphylococcus spp* and *Streptococcus spp* showed Brownian motion and does riot form spore which is motility test negative.
Catalase test	Effervescence or bubble was observed as the suspected *Staplylococcus spp* was introduced into hydrogen

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	peroxide solution (H.'O,:) which confirmed the presence of *Staphycococcus spp*
Coagulase test	*Staphylococcus spp* which was suspected showed clumping of the plasma in Macconkey test wiLhin iu seconds which is eoagulase positive
Citrate test	The simmon citrate agar changed from green to blue colour after 24 hours of incubation which is positive, confirming the presence of *Klebsiella spp*
Oxidase test	On the introduction of the suspected *micrococcus spp* into the oxidase reagent. strip,. th ere was n o form ati o n of blue purple colour within 20 seconds and this confirmed the presence of *Streptococcus spp* showing that it is oxidase positive
Urease test	The' absence of pink colour after inoculating the organism in the bijour bottle and introduced to warm water for 30 minutes and incubated for 24hours, showing • no colour change which- indicates the presence of *Escherichia spp,* which is urease negative
Sugar fermentation	There was no'-fermentation of galatose by the suspected *Staphylococcus spp* confirming their presence as non galatose fermenting organisms and their ability to ferment other suger.
Indole test	On the introduction of the suspected *Escherichia spp* isolated into Kovacs reagent and after 10 mintes there was .formation -of-, red lager (redring) this confirmed the presence o *Escherichia coli* which is indole positive

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Table 3: score for biochemical test on the bacterial isolates

Scores of Biochemical Test					Biochemical test		
C		r	r	r	c		shape
+		+	—	—	+		
		gramstaining					
—		—	—	+	+		motility test
—		+	—	+	+		catalase
	test						
—		+	—	—	—		coagulase
	test						
—		—	+	—	+		indole test
+		+	—	—	—		citrate - ultilization
						test	
+		+	—	—	—		urease test
—		—	—	—	—		oxidase test
—		+	+	+	+		mannitol
—		+	—	+	+		sucrole
—		+	+	+	+		glucose
—		—	—	—	+		fructose
—		—	—	+	+		galatose
—		+	+	—	—		lactose

Strep tococ cus spp	Dis Klebs iella spp	Esche richia spp	Salm onella spp	Staph yloco ccus spp

The study on the isolation and characterization of bacteria associated with slaughtered cow meat in Oko Orumba North L.G.A Anambra state was

carried out between march April 2022. Slaughtered cow meat from Eke Oko market within Oko Market , Orumba North L.G.A Anambra state. Each sample was blended, serially diluted, cultured and observed for cultural, morphological and biochemical characteristics. The bacteria recovered are *Escherichia coli*} *Staphylococcus spp, Streptococcus spp, Salmonella spp* and *kiebsiella*

Streptococcus spp were isolated from cow meat different samples. Streptococcus spp can be contaminated in areas of poor sanitation arid where water supplies are polluted. It was reported earlier .by Adeleye (2005) to cause some human diseases like nausea, vomiting and diarrhea, due to poor sanitation.

Salmonella spp was isolated from cow meat as a tracellula pathogen causing illness. It can be transferred from animal to human and from human to human. Food and water can also be contaminated with *Salmonella spp* when in contact with the faeces of an infected person or animal David (2009).

Escherichia coli was isolated from all the slaughtered cow meat samples as earlier reported by Jackson (1997) as a coliform bacteria.

E. coli is one of the strains and produces a toxin known as shiga. It is one of the most powerful toxin and can cause intestinal infection. *E. coli* is commonly found in the lower intestine of warm blooded organism . The presence of *E. coli* in cow meat is due to poor hygienic and sanitary conditions, exposure to dints, dust and contaminated water used in and washing the meat.

Staphylococcus spp were isolated from all the cow meat samples. This findings is similiar, to earlier report presented Alwell (2011). *Staphylococcuss* spp as earlier reported is the most dangerous of all many *staphylococcal* bacteria. It has been reported to cause skin infectous disease, pneumonia, heart value infectations and borne infections and attack the heart of an individual due to improper sanitation and exposure to dust, air and flies to

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pech on the suya meat *Klebsiella spp* were isolated and characterized from samples as earlier, reported by Dickson (2012). This bacteria. is mostly found in the gut of man, animal and moist environment. The recovery of *Klebsiella spp* in suya meat could be due to contamination of food, air, dust, meat and environment. And it can be decontaminated by washing and sterilizing of utensils, hands before and after, use, and the environment where cow is prepared.

Recommendations

From the findings of this project, it is therefore recommended that;

- Perssonels that slaughtered cow meat should make sure that the environment and vendors are well cleansed and sanitized to avoid contamination
- The method of slaughtered cow meat should be enhanced.
- The utensils and equipment' should be highly sterilized before and after use.
- National agency for food drugs administration and control (NAFDAC) should set up inspections on the. establishment of methodology of suya preparation and hawking.

Conclusion

It may be concluded from this study that mostly slaughtered meat in Oko Orumba North L.G.A. Anarnbra state are contaminated, with a wide variety of potentially bacteria. The quality assurance should be thoroughly enforced and monitored in the production, preparation and distribution of cow meat from the beginning to the final products, as well as conveyance from the vendors to the final consumers of cow meat

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