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Pathogenic Microbial Contaminants from Roasted Pork Sold in Uyo Metropolis, Nigeria and Public Health Implications

By Dora Imefon Udoh, Emem Nsima Ekpe & Emem Ibanga Akpan
University of Uyo

Abstract- Isolation of pathogenic microbial contaminants from roasted pork sold in Uyo metropolis, Nigeria was conducted using standard microbiological techniques. Pathogenic microorganisms isolated were Escherichia coli, Staphylococcus aureus, Salmonella spp, Enterobacter spp, Vibrio spp, Penicillium spp and Aspergillus spp. Total heterotrophic counts (THBC) for freshly prepared and exposed roasted pork (FPERP) samples ranged from 2.0x104 CFU/g - 4.2x104CFU/g while for dried and exposed roasted pork (DERP) samples ranged from 5.3x104(CFU/g) to too numerous to count (TNTC). The total enterobacteriaceae count (TEC) and total coliform counts (TCC) values were higher in DERP. Total Vibrio count (TVC) and total mycological count (TMC) were recorded only in DERP. The high microbial counts and diversity of these contaminants from these pork samples is an indication of its low microbiological quality. Thus, proper hygienic condition is recommended before and after preparation of the pork to prevent it from being a potential source of infections to the public.

Keywords: *roasted pork, pathogenic contaminants, infections.*

GJMR-C Classification: *NLMC Code: QW 25.5.M6*

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Dora Imefon Udoh [α], Emem Nsima Ekpe [σ] & Emem Ibanga Akpan [ρ]

Abstract- Isolation of pathogenic microbial contaminants from roasted pork sold in Uyo metropolis, Nigeria was conducted using standard microbiological techniques. Pathogenic microorganisms isolated were *Escherichia coli, Staphylococcus aureus, Salmonella spp, Enterobacter spp, Vibrio spp, Penicillium spp*, and *Aspergillus* spp. Total heterotrophic counts (THBC) for freshly prepared and exposed roasted pork (FPTP) samples ranged from $2.0x10^4$ CFU/g – $4.2x10^4$CFU/g while for dried and exposed roasted pork (DERP) samples ranged from $5.3x10^4$(CFU/g) to too numerous to count (TNTC). The total Enterobacteriaceae count (TEC) and total coliform counts (TCC) values were higher in DERP. Total *Vibrio* count (TVC) and total mycological count (TMC) were recorded only in DERP. The high microbial loads and diversity of these contaminants from these pork samples is an indication of its low microbiological quality. Thus, the proper hygienic condition is recommended before and after preparation of the pork to prevent it from being a potential source of infections to the public.

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I. Introduction

Pork is known as "pig meat" serves as food and is an important source of protein, vitamin and also fats for most people in many parts of the world (Yannick *et al.,* 2013). Pork is one of the most perishable of all food since it contains sufficient nutrient needed to support the growth of microorganisms (Magnus, 1981). The proportion of fat in pork usually ranges from 10 – 16%, but can be much higher, depending on the level of trimming and various other factors. According to Murphy (2011), some health benefits derive from pork includes muscle mass maintenance and adequate intake of pork helps in the high - quality nutrient that may help preserve muscle mass and enhanced exercise performance. Vitamins like thiamin, selenium, vitamin B, niacin, phosphorus are found in pork as well as some other compounds like creatine, taurine, and cholesterol (Murphy, 2011). Many people in Nigeria especially in the South-Eastern part of the country like to consume pig meat that is why pig keeping and consumption are rapidly increasing, and pork joints are located on some busy streets and roads. Pork joints are a mix of pork butchering and a snack bar where ready- to - eat or take away food are sold. Apart from their popularity among Nigerian, the joints are centers that attract flies and other pests. Flies are carriers of parasites and bacteria and are well known for cross - contamination of infections in farms, hospitals, and public places. In the study by Heilmann *et al*., (2015a) in Uganda, they asserted that the feeding habit and breeding of the flies in a filthy environment make flies vectors for various infectious diseases, and a specific reference was made to synanthropic flies which live close to humans, use foodstuff, feces, and other organic materials as protein source. Houseflies have siphoning mouthparts which allowed them to suck up food and whenever they do this, they vomit a mixture of enzymes and previously absorbed food particles with their potential contaminants to liquefy their feed for easy sucking. Thus food contaminants can occur through contaminated feces and mechanical contamination through the flies' body parts as well as pathogenic microbes harbored by flies in their crops. Roesel *et al..,* (2013) and Heilmann *et al*., (2015b) reported that flies, together with other pests such as rats, cockroaches and birds in the pork' joints are the potential source of contamination of the products and responsible for food - borne infection. According to Okonko *et al*., (2013), food-borne microbiologic hazards may be responsible for frequent cases of illness, and thus pose a food safety challenge. Food - borne illnesses are infections are caused by food that contain harmful or pathogenic bacteria, parasites, viruses or chemicals which results in the manifestation of many clinical signs such as vomiting, abnormal cramps and irritations of the gastrointestinal tracts (Scallan *et al.,* 2011). Food - borne diseases encompass a wide spectrum of illness and are growing public health problem worldwide. They arise as a result of ingestion of food stuffs contaminated with microorganisms or chemicals contamination of food production which the contaminants may come from environment and may include polluted water, soil or air (WHO, 2015). Moreover, according to Rao *et al*., (2009) some meat products that have the water activity approximately 0.99 which is suitable for microbial growth. The serious aspect of public concern is that

Author α ρ: Department of Microbiology, Faculty of Science, University of Uyo, Uyo, P. M. B. 1017, Uyo. Akwa Ibom State, Nigeria.
e-mail: dorly4udoh@yahoo.com
Author σ: Department of Microbiology, Faculty of Biological Science, Akwa Ibom State University, Ikot Akpaden, Akwa Ibom State, Nigeria.

which linked to numerous food scandals associated with animals such as those surrounding bovine spongiform encephalopathy and food and mouth disease epidemic (Okonko *et al*., (2013). Given the danger as well as the complications arising from food-borne infections, this research study focused on the isolation of pathogenic microbial contaminants from roasted pork sold in Uyo metropolis, Nigeria, with views to highlight the public health risk and medical implications of consuming contaminated pork.

II. Materials and Methods

a) Collection of Samples

The samples used for this research work were collected from four (4) different selling points in some of the major roads in Uyo metropolis, Nigeria. These selling points are located on the busiest roads where many customers patronize them. These samples points were Ikpa Road, Ikot Ekpene Road, Abak Road and Nwaniba - Use offot Road respectively all in Uyo metropolis, Akwa Ibom State, Nigeria. At each selling point, two (2) types of pork are sold; freshly prepared and exposed roasted pork (FPTP) and dried and exposed roasted pork (DERP). The FPTP is the meat produced and kept within a day while the DERP are that leftover of the freshly prepared that are subjected for heat treatment until they become dry**.** The two samples were kept opened and exposed without any covering materials even in the busiest environments for consumers to see and buy. The samples were collected aseptically, wrapped in a sterile aluminum foil and put in sterile containers. The samples were immediately transported to the Microbiology Laboratory, Department of Microbiology, University of Uyo, Akwa Ibom State, Nigeria for analysis using the standard technique.

b) Proximate Analysis

The sample containers were opened aseptically, and samples were cut using sterile forceps and knife into sterile containers. The proximate analysis was carried out to determine on the pork samples to determine the moisture content, ash content, crude lipid, crude fibre, protein and carbohydrate. The methods of AOAC (2000) were adopted in which the moisture content was determined as the loss in weight that results from drying a known weight of the pork sample at 100^{0}C. The ash content was determined by the ignition of a known weight of the pork sample at 550^{0}C until all carbon has been successfully removed. The crude lipid was derived by hydrolytic methods and the resultant residue was subjected to successive treatments with boiling acid and alkali respectively and at defined concentration; the organic residue was the crude fiber. The crude protein content was determined by the Kjeldahl method and was calculated from the nitrogen content of the pork sample obtained from stepwise digestion of the food substance using chemical reagents (sulphuric acid, sodium hydroxide). Ammonia was the end product obtained and it was measured using standard colorimetric method. The carbohydrate content was determined as nitrogen - free extract (NFE). The percentage carbohydrate was calculated as the difference between 100 and the total of all the proximate composition of each sample.

c) Microbiological Analysis

i. *Processing and Culturing of Samples*

The pork samples collected for this study were processed aseptically in the Laboratory. Serial dilution method for pour plate technique described by Fawole and Oso, (2001) was adopted. Each roasted pork sample was ground using a blender (Lab Blender 400 series, UK). Ten (10) grams of each sample was weighed out, and homogenized into 90ml of sterile distilled deionized water and vigorously shaken to dislodge adhered bacteria. Tenfold dilution of the homogenates was made using sterile pipettes and one (1) ml from the aliquot was transferred serially to other test tubes containing 9ml of distilled water up to 10^{-6}. One (1) ml of the diluents of 10^{-4} was aseptically dispensed into sterile Petri dishes containing 15ml of the already prepared molten agar. The media used were Nutrient Agar (Oxoid, USA), MacConkey Agar (Oxoid, USA), Eosin Methylene Blue (Oxoid, USA), Cysteine Lactose Electrolyte Deficient agar (Difco Laboratories, Detroit, Mich), Mannitol salt agar ((Difco Laboratories, Detroit, Mich), Thiosulphate citrate bile-salt agar (Oxoid, USA), and Sabourad Dextrose Agar (Difco Laboratories, Detroit, Mich) plates. A culture of each sample was done in triplicates. All plates were incubated at 37^{o}C for 24 hours in an incubator. Sabourad Dextrose Agar (SDA) plates were kept for 1week at room temperature for isolation of fungi. The plates were observed and the colonies were counted using colony counter to obtain the total heterotrophic bacteria counts (THBC), total Enterobacteriaceae Count (TEC), total coliform count (TCC), total *Vibrio* count (TVC) and total mycological count (TMC). The average numbers of colonies were taken since the culture was in triplicate. The number of colonies counted was multiplied by the reciprocal of the dilution factor to determine the microbial load in colony forming unit per gram (CFU/g). The colonies were sub-cultured to obtain pure colonies. Pure isolates of bacterial colonies were Gram differentiated and biochemically characterized and identified using the standard taxonomic schemes of Holt *et al*., (1994) and Cheesbrough, (2003) The isolates were maintained in Nutrient agar slants in McCartney bottles and preserved in a refrigerator at 4^{o}C and for further analysis.

III. Results

a) Proximate Analysis of Roasted Pork

Proximate analysis result showed that pork sample had values of moisture content (52.10%), Ash

content (3.42%), Crude lipid (6.603%), Crude fiber (1.226%), Protein (32.60%,) Carbohydrate (4.051) and Caloric value (336.016 Kcal) Table 1.

Table 1: Proximate Analysis of Roasted Pork

Parameters	Values (%)
Moisture Content	52.10
Ash Content	3.42
Crude Lipid	6.603
Crude Fiber	1.226
Protein	32.60
Carbohydrate	4.051
Caloric Value	336.016 Kcal

A total of 12 isolates were obtained from the freshly prepared and exposed roasted pork (FPTP) with percentage of occurrence (33.3%) of *Staphylococcus aureus* as the predominant pathogenic bacteria species, *Escherichia coli* and *Salmonella* spp had 25% respectively and *Enterobacter* spp had 16.7%. Diverse number of isolates were obtained from dried and exposed roasted pork (DERP) with a total number of 15 isolates with *Staphylococcus aureus* being frequently isolated bacterial species with percentage of occurrence of 26.7%, *Escherichia coli had* 13.3%, *Vibrio* spp, *Penicillium* spp and *Aspergillus* spp were also obtained from DERP had 6.67%, 13.3% and 20.0% respectively (Table 2).

Table 2: Percentages of Occurrence (%) of each Isolates from Roasted Pork Studied

Pathogenic Isolate from Pork Samples Studied	Number and Percentages of Occurrence (%) of each Isolate from Freshly Prepared and Exposed Roasted Pork (FPTP)	Number and Percentages of Occurrence (%) of each Isolate from Dried and Exposed Roasted Pork (DERP)
E Coli	3 (25.0)	2 (13.3)
Salmomella Spp	3 (25.0)	1 (6.67)
S Aureus	4 (33.3)	4 (26.7)
Vibrio Spp,	-	1 (6.67)
Enterobacter Spp	2 (16.7)	2 (13.3)
Penicilium Spp	-	2 (13.3)
Aspergillus Spp	-	3 (20.0)
Total	12 (100)	15 (100)

Microbial counts for freshly prepared and exposed roasted pork (FPTP) samples screened showed the total heterotrophic counts (THBC) ranged from $2.0x10^4$ CFU/g – 4.2x104CFU/g. The highest THBC recorded from samples obtained from Abak road, Uyo. Total Enterobacteriaceae count (TEC) ranged from 2.1 $x10^4$ CFU/g - $4.4x10^4$CFU/g and the highest TEC obtained from Ikpa road. Total coliform counts (TCC) ranged from $1.6x10^4$CFU/g to $2.3x10^4$CFU/g. There was no total *Vibrio* count (TVC) and no total mycological count (TMC) from these samples (Table 3).

Table 3: Microbial Counts for Freshly Prepared and Exposed Roasted Pork (FPTP)

Samples	THBC (CFU/g)	TEC (CFU/g)	TCC (CFU/g)	TVC (CFU/g)	TMC (CFU/g)
Abak Road	4.0x104	3.0x104	1.3x104	-	-
Ikot Ekpene Road	3.0x104	2.1x104	1.6x104	-	-
Ikpa Road	2.0x104	4.4x104	1.7x104	-	-
Nwaniba -Use Offot	2.4x104	2.3x104	1.6x104	-	-

Keys: THBC = Total Heterotrophic Counts, TEC = Total Enterobacteriaceae Count, TCC= Total Coliform Counts (TCC), TVC = Total Vibrio counts, TMC = Total mycological count, - = No microbial colony

Microbial counts results carried out on dried and exposed roasted pork (DERP) showed that THBC for samples from Abak road was 6.4 $x10^4$ CFU/g, samples from Ikot Ekpene road was $5.3x10^4$ (CFU/g). Samples from Ikpa road and Use offot road respectively had colonies on the plates that were too numerous to count (TNTC). The TEC ranged from$1.1x10^4$ CFU/g - $9.2x10^4$ CFU/g with the highest TEC of microbial loads recorded from samples obtained from Nwaniba-Use offot road, Uyo. The TCC ranged from $3.9x10^4$ (CFU/g) - $9.7x10^4$ (CFU/g), with the highest TCC loads from Abak road. Moreover, samples from Ikot Ekpene road yielded a TVC of $3.0x10^4$ (CFU/g) while there was no *Vibrio* count in others. The TMC was also recorded from dried and exposed roasted pork from Abak road, Ikot Ekpene road, and Nwaniba-Use offot road respectively, with the range of $1.3x10^4$ (CFU/g) - $2.4x10^4$ (CFU/g). (Table 4).

Table 4: Microbial Counts in Dried and Exposed Roasted Pork (DERP)

Samples Locations	THBC (CFU/g)	TEC (CFU/g)	TCC (CFU/g)	TVC (CFU/g)	TMC (CFU/g)
Abak Road	$6.4x10^4$	$5.9x10^4$	$9.7x10^4$	-	$1.3x10^4$
Ikot Ekpene Road	$5.3x10^4$	$1.1x10^4$	$5.5x10^4$	3.0x103	$2.3x10^4$
Ikpa Road	TNTC	$1.6x10^4$	$6.4x10^4$	-	-
Nwaniba-Use Offot	TNTC	$9.2x10^4$	$3.9x10^4$	-	$1.3x10^4$

Keys: THBC = Total Heterotrophic Bacteria Count, TEC=Total Enterobacteriaceae Count, TCC = Total Coliform Count, TVC = Total Vibiro Count, TMC =Total Mycological Count, - = No microbial colony

IV. Discussion

Pork contains nutrients such as protein, lipid, fiber, carbohydrate, as well as moisture. These constituents make the meat product susceptible to microbial growth. According to Jay, (2000) most organisms utilize protein, a carbohydrate in the presence of moisture to multiply and thrive very well. All pork samples analyzed contained pathogenic microbial contaminants and were *Eschrichia coli, Salmonella* spp*, S aureus, Vibrio* spp, *Enterobacter* spp*, Penicillium* spp, and *Aspergillus* spp. *Staphylococcus aureus* was found with the highest percentage of frequency of occurrence. Yannick *et al*., (2013) in their work also confirmed the presence of bacterial pathogens in pork with *Staphylococcus aureus* as the predominant organisms found with the highest percentage of frequency of occurrence. Tinega *et al*., (2016) reported the presence of *Salmonella* in the pork screened in their work. Whyte *et al.,* (2004) in their work stated that .the wide spread distribution of the meat product makes the consequence of contamination with food poisoning microorganisms more serious. The isolation of these organisms from roasted pork is public health importance because of they are pathogenic organisms and is worrisome on the fact that in the study area, many people like to consume this food product.

Salmonella species are important food - borne pathogens.. They are known to cause typhoid and non-typhoid illnesses (Ikumapayi *et al.,* 2009), and tends to be more severe with people in immunocompromised condition (Afessa *et al.,* 2001; Udoh *et al., 2009*). *Salmonella* causes an acute life - threatening illness (CDC, 2008), and is mainly transmitted through urine or feces of infected people or a chronic carrier. Some serotypes of *Salmonella* species are known cause non-typhi salmonellosis of which results in gastroenteritis in humans. The symptoms include acute watery diarrhea accompanied by nausea, cramps and fever. Blood in the stool may occur. Animals are the main reservoir, and transmission occurs by ingestion of contaminated food products (CDC, 2008).

Staphylococcus aureus is a normal flora of some body parts of man. According to Tauxe (2002), it can be transmitted from person to product through unhygienic practices. Therefore, presence of *Staphylococcus aureus* in the roasted pork studied is an indication of possible contamination from human sources to the meat from the skin, mouth or nose of the handler which can be introduces directly into the food by contact or other aerial-droplet mechanisms such as coughing or sneezing (Yannick *et al*., 2013). However, according to Evenson *et al.,* (1988), and Nema *et al., (*2007), enterotoxin producing strains of *S. aureus* is a leading cause of food intoxication as it can produce extremely potent gastrointestinal toxin *Escherichia coli* and *Enterobacter* species isolated in the study are enteric organisms. Their presence in the pork is an indication generally traceable to fecal contamination either direct or indirect means. They are normal flora of the intestine in human and animals and are widely distributed in the environment contaminating food and water. Moreover, their presences in foods are usually as a result of excessive human handling and possible contamination of pork itself during sales (Clarence et *al*., 2009). The pork that has been processed and kept for some days to be sold stand a chance to been contaminated especially when exposing such meat for consumers to see. *Escherichia coli* and *Enterobacter* species have been implicated in the ability to initiate the pathogenic cascade of sepsis leading to septic shock (Prescott *et al.,* 2002). Notably is the fact that *Enterobacter* species are bacteria commonly known to further cause gastroenteritis, meningitis, and infection in the bladder (Nester *et al.,* 1995). More so, an enterotoxigenic strain of *E. coli* is the most common cause of traveler's diarrhea and some strains of this pathogen can cause a wide variety of infections such as other forms of diarrhea and other gastrointestinal problems especially in a community setting (Donnenberg *et al.,* 2005). Pork or other food products that contain E. coli in its infective dose can be a continuous source of infections leading to complications and death especially among children and immunocompromised individuals (Ternhag et al., 2008).

The presence of *Vibrio* species is one of the potential sources of diarrhoeal diseases. These organisms are normally found in marine and estuarine environments throughout the world (McLaughln, 1995). The major mode of transmission is through contaminated water and food, or person-to-person spread in the overcrowded and unhygienic environment.

Vibrio species especially *Vibrio cholerae* causes severe watery diarrhea, which can reach up to 20 liters per day (McLaughln, 1995; Udoh and Itah, 2012). *Vibrio cholerae* produces a potent enterotoxin called choleragen that is responsible for the symptoms of cholera which could cause dehydration and many more diseases (Nester *et al.,* 1995; Sack *et al*., 2004). According to WHO (1995), diarrheal diseases have been known and recognized throughout history as one of the prevailing cause of childhood death and more potential life loss than all other causes combined. In developing country, food-borne infection such as diarrheal diseases can have long- term effects especially on children's growth as well as their physical and cognitive development and can lead to many complications and death of both children and adults (Adak et al., 2005).

The fungi isolated from this study were mainly Aspergillus niger and Penicillium spp. They have been known to produce mycotoxin which causes food intoxication to consumers (Udoh et al., 2018).The Aspergillus spp is of medical significance because of the production of their aflatoxin. Their presence in food could be due to poor handling of the meat, unhygienic environment, improper storage facility and condition as well as lack of proper personal hygiene. (Licorish et al., (1985) and WHO, (2015) reported that the presence of Penicillium spp in food must be avoided since it can lead to allergic reactions. and arising of penicillin resistance in human pathogenic bacteria.

The microbiological counts in this study showed the microbial density in both freshly prepared and exposed roasted pork (FPTP) and dried and exposed roasted pork (DERP). Freshly prepared and exposed roasted pork (FPTP) had lesser microbial count as compared to dried and exposed roasted pork (DERP). The level of microbial contamination of the pork samples was further observed as some samples had microbial loads that exceeded the recommended as limit of bacterial counts (10^5 CFU/g) of the international standards for micro-organisms in foods (ICMSF 2011). Most outstanding were especially observed from DERP samples, notably those from Ikpa road and Nuaniba - Use offot road, in which their enumeration of THBC were too numerous to count (TNTC) exceeding the international standards of (10^5 CFU/g). To further showed that the DERP samples were highly contaminated pathogenic organisms, *Vibrio* and fungal microbes were also isolated obtained from DERP.

Their presence of these microbial contaminants in the pork samples may be due to the unhygienic status of the slaughter houses, which portrays that the pork was poorly prepared and even the prolonged exposure to the surroundings. Other pre-disposing factors of contamination of the meat that could warrant the presence of these organisms could also be processing points, handling and selling (Yannick *et al*., 2013). According to Ellis, and Goodacre, (2001), and Tauxe, (2006), the health status of animals prior to slaughtering, and prevailing circumstances in the slaughter contributes to the quality of meat from such animals. It was also noticed that in the study area, there is none of the station that cover this meat product but rather, they are placed on the net for passerby to see and patronize. Hence, there is every tendency for atmospheric organisms to settle on these products thereby contaminating them. The customers' effect of touching and selecting the ones to buy, talking and interacting the sellers before the net where the products are kept, even coughing, and sneezing at the sell points can bring the isolates to settle on the products. Moreover, the condition of handlers packing the left-over that has not been sold into the containers to be exposed the next day, and the method of preservation of the meat equally is the source of microbial contamination. Other predisposing factors could account for the growth of these organisms in pork could be the feeding habit of the pig. Mossel *et al*., (1995) made a point that pig mostly feed on corn and soybean with a mixture of vitamins, and minerals added to the diet, the feed could serve as medium for the growth of these organisms. Moreover, the isolation of these organisms in roasted pork indicates a state of poor hygiene and environmental sanitation in some places where the meat is being processed to where it is being sold (Daniyan, 2011). The roasting, exposure as well as handling could also affect the meat quality (Mossel et al., 1995).

V. Conclusion

Roasted pork sold in Uyo metropolis harbor microorganisms. It is very necessary that pork should be in good quality, and this comes as a result of good rearing condition, handling during slaughter, preparation method and transportation. Therefore, pork processors, handlers, and sellers should observe strict hygiene measures so that they may not serve as a source of inoculation of the microorganisms into the meat product. Meat handlers should be educated on the adverse effect of lack of proper personal, and environmental hygiene, and sanitation. Veterinary doctors should inspect the animal before it is slaughtered to establish the fitness of the meat for consumption. Government should set up local regulatory bodies to monitor and regulate the sale of pork. Emphasizing the need of clean environments and placing of the pork in well covered show- case. Consumers should insist on adequate reheating of the pork to destroy vegetative cells. Public heath programme is of good necessity to enlighten and educate the general public on the health implications of consuming contaminated meat products, highlighting the fact that the presence of these pathogenic microbial contaminants with high counts in the pork consumed could lead to an outbreak of disease in the study area and beyond.

Conflicts of Interest

We state that the work has no potential conflicts of interest.

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