

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

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6

7 **Abstract**

8 - Isolation of pathogenic microbial contaminants from roasted pork sold in Uyo metropolis,
9 Nigeria was conducted using standard microbiological techniques. Pathogenic microorganisms
10 isolated were Escherichia coli, Staphylococcus aureus, Salmonella spp, Enterobacter spp,
11 Vibrio spp, Penicillium spp and Aspergillus spp. Total heterotrophic counts (THBC) for
12 freshly prepared and exposed roasted pork (FPERP) samples ranged from 2.0×10^4 CFU/g
13 â?? 4.2x104CFU/g while for dried and exposed roasted pork (DERP) samples ranged from
14 5.3x104(CFU/g) to too numerous to count (TNOC). The total enterobacteriaceae count
15 (TEC) and total coliform counts (TCC) values were higher in DERP. Total Vibrio count
16 (TVC) and total mycological count (TMC) were recorded only in DERP. The high microbial
17 counts and diversity of these contaminants from these pork samples is an indication of its low
18 microbiological quality. Thus, proper hygienic condition is recommended before and after
19 preparation of the pork to prevent it from being a potential source of infections to the public.

20

21 **Index terms**— roasted pork, pathogenic contaminants, infections.

22 rapidly increasing, and pork joints are located on some busy streets and roads. Pork joints are a mix of pork
23 butchering and a snack bar where ready-to-eat or take away food are sold. Apart from their popularity among
24 Nigerian, the joints are centers that attract flies and other pests. Flies are carriers of parasites and bacteria
25 and are well known for cross-contamination of infections in farms, hospitals, and public places. In the study
26 by Heilmann et al., (2015a) in Uganda, they asserted that the feeding habit and breeding of the flies in a filthy
27 environment make flies vectors for various infectious diseases, and a specific reference was made to synanthropic
28 flies which live close to humans, use foodstuff, feces, and other organic materials as protein source. Houseflies
29 have siphoning mouthparts which allowed them to suck up food and whenever they do this, they vomit a mixture
30 of enzymes and previously absorbed food particles with their potential contaminants to liquefy their feed for easy
31 sucking. Thus food contaminants can occur through contaminated feces and mechanical contamination through
32 the flies' body parts as well as pathogenic microbes harbored by flies in their crops. Roesel et al., (2013) and
33 Heilmann et al., (2015b) reported that flies, together with other pests such as rats, cockroaches and birds in the
34 pork joints are the potential source of contamination of the products and responsible for food-borne infection.
35 According to ??konko et al., (2013), food-borne microbiologic hazards may be responsible for frequent cases of
36 illness, and thus pose a food safety challenge. Food-borne illnesses are infections caused by food that contain
37 harmful or pathogenic bacteria, parasites, viruses or chemicals which results in the manifestation of many clinical
38 signs such as vomiting, abnormal cramps and irritations of the gastrointestinal tracts ??Scallan et al., 2011).
39 Food-borne diseases encompass a wide spectrum of illness and are growing public health problem worldwide.
40 They arise as a result of ingestion of food stuffs contaminated with microorganisms or chemicals contamination
41 of food production which the contaminants may come from environment and may include polluted water, soil
42 or air ??WHO, 2015). Moreover, according to Rao et al., (2009) some meat products that have the water
43 activity approximately 0.99 which is suitable for microbial growth. The serious aspect of public concern is that
44 P Abstract-Isolation of pathogenic microbial contaminants from roasted pork sold in Uyo metropolis, Nigeria

5 C) MICROBIOLOGICAL ANALYSIS I. PROCESSING AND CULTURING OF SAMPLES

45 was conducted using standard microbiological techniques. Pathogenic microorganisms isolated were Escherichia
46 coli, Staphylococcus aureus, Salmonella spp, Enterobacter spp, Vibrio spp, Penicillium spp, and Aspergillus spp.
47 Total heterotrophic counts (THBC) for freshly prepared and exposed roasted pork (FPTP) samples ranged from
48 2.0×10^4 CFU/g - 4.2×10^4 CFU/g while for dried and exposed roasted pork (DERP) samples ranged from 5.3×10^4 (CFU/g)
49 to too numerous to count (TNTC). The total Enterobacteriaceae count (TEC) and total coliform
50 counts (TCC) values were higher in DERP.

51 Total Vibrio count (TVC) and total mycological count (TMC) were recorded only in DERP. The high microbial
52 loads and diversity of these contaminants from these pork samples is an indication of its low microbiological
53 quality. Thus, the proper hygienic condition is recommended before and after preparation of the pork to prevent
54 it from being a potential source of infections to the public.

55 1 Keywords: roasted pork, pathogenic contaminants, infections.

56 ? , Emem Nsima Ekpe ? & Emem Ibanga Akpan ? Dora Imefon Udoh which linked to numerous food scandals
57 associated with animals such as those surrounding bovine spongiform encephalopathy and food and mouth
58 disease epidemic ??Okonko et al., (2013). Given the danger as well as the complications arising from food-
59 borne infections, this research study focused on the isolation of pathogenic microbial contaminants from roasted
60 pork sold in Uyo metropolis, Nigeria, with views to highlight the public health risk and medical implications of
61 consuming contaminated pork.

63 2 II. Materials and Methods

64 3 a) Collection of Samples

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

76 4 b) Proximate Analysis

77 The sample containers were opened aseptically, and samples were cut using sterile forceps and knife into sterile
78 containers. The proximate analysis was carried out to determine on the pork samples to determine the moisture
79 content, ash content, crude lipid, crude fibre, protein and carbohydrate. The methods of AOAC (2000) were
80 adopted in which the moisture content was determined as the loss in weight that results from drying a known
81 weight of the pork sample at 100°C . The ash content was determined by the ignition of a known weight of the
82 pork sample at 550°C until all carbon has been successfully removed. The crude lipid was derived by hydrolytic
83 methods and the resultant residue was subjected to successive treatments with boiling acid and alkali respectively
84 and at defined concentration; the organic residue was the crude fiber. The crude protein content was determined
85 by the Kjeldahl method and was calculated from the nitrogen content of the pork sample obtained from stepwise
86 digestion of the food substance using chemical reagents (sulphuric acid, sodium hydroxide). Ammonia was the
87 end product obtained and it was measured using standard colorimetric method. The carbohydrate content was
88 determined as nitrogen -free extract (NFE). The percentage carbohydrate was calculated as the difference between
89 100 and the total of all the proximate composition of each sample.

90 5 c) Microbiological Analysis i. Processing and Culturing of 91 Samples

92 The pork samples collected for this study were processed aseptically in the Laboratory. Serial dilution method for
93 pour plate technique described by Fawole and Oso, (2001) was adopted. Each roasted pork sample was ground
94 using a blender (Lab Blender 400 series, UK). Ten (10) grams of each sample was weighed out, and homogenized
95 into 90ml of sterile distilled deionized water and vigorously shaken to dislodge adhered bacteria. Tenfold dilution
96 of the homogenates was made using sterile pipettes and one (1) ml from the aliquot was transferred serially
97 to other test tubes containing 9ml of distilled water up to 10^{-6} . One (1) ml of the diluents of 10^{-4} was
98 aseptically dispensed into sterile Petri dishes containing 15ml of the already prepared molten agar. The media
99 used were Nutrient Agar (Oxoid, USA), MacConkey Agar (Oxoid, USA), Eosin Methylene Blue (Oxoid, USA),
100 Cysteine Lactose Electrolyte Deficient agar (Difco Laboratories, Detroit, Mich), Mannitol salt agar ((Difco

101 Laboratories, Detroit, Mich), Thiosulphate citrate bile-salt agar (Oxoid, USA), and Sabourad Dextrose Agar
102 (Difco Laboratories, Detroit, Mich) plates. A culture of each sample was done in triplicates. All plates were
103 incubated at 37°C for 24 hours in an incubator. Sabourad Dextrose Agar (SDA) plates were kept for 1 week at
104 room temperature for isolation of fungi. The plates were observed and the colonies were counted using colony
105 counter to obtain the total heterotrophic bacteria counts (THBC), total Enterobacteriaceae Count (TEC), total
106 coliform count (TCC), total Vibrio count (TVC) and total mycological count (TMC). The average numbers of
107 colonies were taken since the culture was in triplicate. The number of colonies counted was multiplied by the
108 reciprocal of the dilution factor to determine the microbial load in colony forming unit per gram (CFU/g). The
109 colonies were subcultured to obtain pure colonies. Pure isolates of bacterial colonies were Gram differentiated and
110 biochemically characterized and identified using the standard taxonomic schemes of Holt et al., (1994) Microbial
111 counts results carried out on dried and exposed roasted pork (DERP) showed that THBC for samples from Abak
112 road was 6.4×10^4 CFU/g, samples from Ikot Ekpene road was 5.3×10^4 (CFU/g). Samples from Ikpa road
113 and Use offot road respectively had colonies on the plates that were too numerous to count (TNTC). The TEC
114 ranged from 1.1×10^4 CFU/g - 9.2×10^4 CFU/g with the highest TEC of microbial loads recorded from samples
115 obtained from Nwaniba-Use offot road, Uyo. The TCC ranged from 3.9×10^4 (CFU/g) - 9.7×10^4 (CFU/g), with
116 the highest TCC loads from Abak road. Moreover, samples from Ikot Ekpene road yielded a TVC of 3.0×10^4
117 (CFU/g) while there was no Vibrio count in others. The TMC was also recorded from dried and exposed roasted
118 pork from Abak road, Ikot Ekpene road, and Nwaniba-Use offot road respectively, with the range of 1.3×10^4
119 (CFU/g) - 2.4×10^4 (CFU/g). (Table 4). Microbial counts for freshly prepared and exposed roasted pork (FPTP)
120 samples screened showed the total heterotrophic counts (THBC) ranged from 2.0×10^4 CFU/g - 4.2×10^4 CFU/g.
121 The highest THBC recorded from samples obtained from Abak road, Uyo. Total Enterobacteriaceae count (TEC)
122 ranged from 2.1×10^4 CFU/g - 4.4×10^4 CFU/g and the highest TEC obtained from Ikpa road. Total coliform
123 counts (TCC) ranged from 1.6×10^4 CFU/g to 2.3×10^4 CFU/g. There was no total Vibrio count (TVC) and no
124 total mycological count (TMC) from these samples (Table 3).

125 6 IV. Discussion

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

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

145 *Staphylococcus aureus* is a normal flora of some body parts of man. According to Tauxe (2002) ??009). The
146 pork that has been processed and kept for some days to be sold stand a chance to been contaminated especially
147 when exposing such meat for consumers to see. *Escherichia coli* and *Enterobacter* species have been implicated
148 in the ability to initiate the pathogenic cascade of sepsis leading to septic shock (Prescott et al., 2002). Notably
149 is the fact that *Enterobacter* species are bacteria commonly known to further cause gastroenteritis, meningitis,
150 and infection in the bladder ??Nester et al., 1995). More so, an enterotoxigenic strain of *E. coli* is the most
151 common cause of traveler's diarrhea and some strains of this pathogen can cause a wide variety of infections such
152 as other forms of diarrhea and other gastrointestinal problems especially in a community setting (Donnenberg
153 et al., 2005). Pork or other food products that contain *E. coli* in its infective dose can be a continuous source
154 of infections leading to complications and death especially among children and immunocompromised individuals
155 (Ternhag et al., 2008).

156 The presence of *Vibrio* species is one of the potential sources of diarrhoeal diseases. These organisms are
157 normally found in marine and estuarine environments throughout the world ??McLaughlin, 1995). The major
158 mode of transmission is through contaminated water and food, or person-to-person spread in the overcrowded and
159 unhygienic environment. *Vibrio* species especially *Vibrio cholerae* causes severe watery diarrhea, which can reach
160 up to 20 liters per day ??McLaughlin, 1995;Udoh and Itah, 2012). *Vibrio cholerae* produces a potent enterotoxin
161 called cholera that is responsible for the symptoms of cholera which could cause dehydration and many more
162 diseases ??Nester et al., 1995;Sack et al., 2004). According to WHO (1995), diarrheal diseases have been known

7 V. CONCLUSION

163 and recognized throughout history as one of the prevailing cause of childhood death and more potential life loss
164 than all other causes combined. In developing country, foodborne infection such as diarrheal diseases can have
165 long-term effects especially on children's growth as well as their physical and cognitive development and can lead
166 to many complications and death of both children and adults (Adak et al., 2005).

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

174 The microbiological counts in this study showed the microbial density in both freshly prepared and exposed
175 roasted pork (FPTP) and dried and exposed roasted pork (DERP). Freshly prepared and exposed roasted pork
176 (FPTP) had lesser microbial count as compared to dried and exposed roasted pork (DERP). The level of microbial
177 contamination of the pork samples was further observed as some samples had microbial loads that exceeded the
178 recommended as limit of bacterial counts (10⁵ CFU/g) of the international standards for micro-organisms in
179 foods (ICMSF 2011). Most outstanding were especially observed from DERP samples, notably those from Ikpa
180 road and Nuaniba -Use offot road, in which their enumeration of THBC were too numerous to count (TNTC)
181 exceeding the international standards of (10⁵ CFU/g). To further showed that the DERP samples were highly
182 contaminated pathogenic organisms, *Vibrio* and fungal microbes were also isolated obtained from DERP.

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

202 7 V. Conclusion

203 Roasted pork sold in Uyo metropolis harbor microorganisms. It is very necessary that pork should be in good
204 quality, and this comes as a result of good rearing condition, handling during slaughter, preparation method
205 and transportation. Therefore, pork processors, handlers, and sellers should observe strict hygiene measures
206 so that they may not serve as a source of inoculation of the microorganisms into the meat product. Meat
207 handlers should be educated on the adverse effect of lack of proper personal, and environmental hygiene, and
208 sanitation. Veterinary doctors should inspect the animal before it is slaughtered to establish the fitness of the
209 meat for consumption. Government should set up local regulatory bodies to monitor and regulate the sale of
210 pork. Emphasizing the need of clean environments and placing of the pork in well covered show-case. Consumers
211 should insist on adequate reheating of the pork to destroy vegetative cells. Public health programme is of good
212 necessity to enlighten and educate the general public on the health implications of consuming contaminated meat
213 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.

11

III. Results

[Note: a) Proximate Analysis of Roasted PorkProximate analysis result showed that pork sample had values of
moisture content (52.10%), Ash Year 2018 A]

Figure 1: Table 1 .Table 1 :

2.2

Samples	THBC (CFU/g)	TEC (CFU/g)	TCC (CFU/g)	TVC (CFU/g)
Abak Road	4.0x10 ⁴	3.0x10 ⁴	1.3x10 ⁴	-
Ikot Ekpene Road	3.0x10 ⁴	2.1x10 ⁴	1.6x10 ⁴	-
Ikpa Road	2.0x10 ⁴	4.4x10 ⁴	1.7x10 ⁴	-
Nwaniba -Use Offot	2.4x10 ⁴	2.3x10 ⁴	1.6x10 ⁴	-

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

Figure 2: Table 2) . Table 2 :

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[Note: © 2018 Global Journals]

Figure 3: Table 3 :

7 V. CONCLUSION

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

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

Figure 4: Table 4 :

, it can be transmitted from person to product through unhygienic practices. Therefore, presence of

Figure 5:

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217 assistance and cooperation.

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