ASSESSMENT OF BACTERIOLOGICAL QUALITY OF FRESH MEATS SOLD IN CALABAR METROPOLIS, NIGERIA

Ukut I-OE¹, Okonko IO*², Ikpoh IS³, Nkang AO³, Udeze AO³, Babalola TA⁴, Mejeha OK⁵, Fajobi EA⁶

¹Department of Microbiology, Faculty of Sciences, University of Calabar, Calabar, Nigeria
²Department of Virology, Faculty of Basic Medical Sciences, University of Ibadan College of Medicine, University College Hospital (UCH), Ibadan, University of Ibadan, Ibadan, Nigeria. WHO Regional Reference Polio Laboratory, WHO Collaborative Centre for Arbovirus Reference and Research, WHO National Reference Centre for Influenza.
³Department of Microbiology, Faculty of Sciences, University of Ilorin, Ilorin, Nigeria
⁴Department of Veterinary Microbiology and Parasitology, Faculty of Veterinary, University of Ibadan, Ibadan, Nigeria
⁵Department of Microbiology, Federal University of Technology (FUTO), Owerri, Imo State, Nigeria
⁶Department of Basic Sciences, Federal College of Wildlife Management, New Bussa, Niger State, Nigeria

mac2finney@yahoo.com

ABSTRACT
Ten duplicate samples of fresh meat (beef) were randomly sampled from 2 major markets (Watt and Marian) in Calabar, Nigeria and analyzed microbiologically for the rates of Gram negative bacteria. The mean microbial load on the fresh meat from Watt market ranged between 2.62 x 10⁴ - 4.84 x 10⁴ cfu/g and total coliform count between 1.05 x 10³ - 3.72 x 10³ cfu/g while the fresh meat from Marian market ranged between 2.24 x 10⁴ - 5.01 x 10⁴ cfu/g and total coliform count between 1.23 x 10³ - 3.42 x 10³ cfu/g. A total of 36 isolates belonging to eight genera include Klebsiella pneumoniae [6(16.7%)] which was the most predominant, followed by Enterobacter spp [5(13.9%)], Citrobacter freundii [5(13.9%)], Pseudomonas aeruginosa [4(11.1%)], Escherichia coli [4(11.1%)], Salmonella spp [4(11.1%)], Serratia marcescens [4(11.1%)], and Pseudomonas spp [3(8.3%)]. Proteus vulgaris [1(2.8%)] was less predominant. Statistical analysis of the mean microbial load and total coliform count showed no significant difference between the two markets (P>0.05). This study reveals that fresh meats are often contaminated with bacteria. The presence of higher number of pathogenic Klebsiella pneumoniae, Salmonella and Escherichia coli among others, encountered in fresh meat from conventional beef is alarming. The presence of these organisms in meat foods should receive particular attention, because their presence indicate public health hazard and give warning signal for the possible occurrence of food borne intoxication.

KEYWORDS
Calabar, Escherichia coli, fresh meat, mean microbial load, total coliforms, Gram-negative bacteria.
INTRODUCTION

Food security is a complex issue, where animal proteins such as meats, meat products, fish and fishery products are generally regarded as high risk commodity in respect of pathogen contents, natural toxins and other possible contaminants and adulterants (Yousuf et al., 2008). Food borne infections and illnesses is a major international health problem with consequent economic reduction. It is a major cause of illness and death worldwide (Adak et al., 2005). Recognizing this, the World Health Organization (WHO) developed its Global Strategy for Food Safety (Adak et al., 2005). In the developing world, foodborne infection leads to the death of many children and the resulting diarrheal disease can have long-term effects on children's growth as well as on their physical and cognitive development (Adak et al., 2005). In the industrialized world, foodborne infection causes considerable illness, heavily affecting healthcare systems (Adak et al., 2005). According to Clarence et al., (2009), food borne diseases are diseases resulting from ingestion of bacteria, toxins and cells produced by microorganisms present in food. The intensity of the signs and symptoms may vary with the amount of contaminated food ingested and susceptibility of the individuals to the toxin (Clarence et al., 2009).

Meat is the most perishable of all important foods since it contains sufficient nutrient needed to support the growth of microorganisms (Magnus, 1981). The chief constituents of meat are water, protein and fat, phosphorus, iron and vitamins are also contained in meat. The major primary unit of meat is called carcass. It represents the ideal meat after head, hide, intestine, blood. The edible parts of a carcass include lean flesh, fat flesh and edible glands or organs such as heart, liver, kidney tongue and brain. Meat is considered as the most nutritive source of protein consumed by humans. Age and sex of the animal has a major influence on the quality of meat that is produced from animals (Rao et al., 2009). Most meat have high water content corresponding to the water activity approximately 0.99 which is suitable for microbial growth (Rao et al., 2009).

Meat is considered to be spoiled when it is unfit for human consumption. Meat is subjected to changes by its own enzyme, by microbial action and its fat may be oxidized chemically microorganisms grow on meat causing visual, textural and organoleptic change when they release metabolites (Jackson et al., 2001). In fact, tissue from healthy animal are sterile however, it has been pointed that during slaughter, dressing and cutting, microorganisms came chiefly from the exterior of the animal and its intestinal tract but that more added from knives, cloths, air, carts and equipment in general. External contamination of meat is a constant possibility from the moment of bleeding unit consumption (Lawrie, 1984). Among the factors that affect microbial growth in meat are intrinsic properties (physical and chemical properties of meat) and extrinsic (environmental factors) (Rombout and Wout, 1994), however the factors having the greatest influence on the growth of microorganisms in meat and meat products are the storage temperatures, moisture and oxygen availability (Forest et al., 1985).

Foodborne microbiologic hazards may be responsible for as many cases of illness as possible each year and are thus an important food safety challenge. To lower the incidence of foodborne disease, many experts and stakeholders urge the development of a science- and risk-based food safety system, in which decision makers prioritize hazards and interventions using the best available data on the distribution and reduction of risks (Batz et al., 2005). Such a system requires an understanding of the many risk factors between the point of production and the point of consumption and the ability to systematically target intervention efforts along this "farm-to-fork" continuum (Batz et al., 2005). The preservation of meat as a perishable food usually is accomplished by a combination of preservation methods which greatly lengthen the keeping quality the meat. So, to increase meat quality assurance in accordance with microbial load assessment is deemed necessary (Yousuf et al., 2008).
It has been reported that gram negative bacteria account for approximately 69% of the cases of bacterial food borne disease (Clarence et al., 2009). Turtura (1991) reported that the most frequently coliform identified on meat were *C. freundii*, *E. coli*, *En. agglomeram* and less frequently strains are of the genera Klebsiella, *Shigella sonnie* and Proteus. *E. coli* and *S. aureus* are normal flora in human and animals, their presence in foods are indications of excessive human handling (Clarence et al., 2009). Members of the gram negative bacteria e.g. *E. coli* are widely distributed in the environment contaminated food and water are the major sources by which the bacteria are spread (Clarence et al., 2009). Selected strains can cause a wide variety of infections in hospitals and community setting (Donnenberg, 2005). *Escherichia coli* is commonly used as surrogate indicator, its presence in food generally indicate direct and indirect fecal contamination (Clarence et al., 2009). Bacterial gastrointestinal infections continue to cause illness and death and contribute to economic loss in most parts of the world, including high-income countries that have developed surveillance and control programs (Ternhag et al., 2008).

The possible sources of these bacteria are likely to come from the skin of the animal from which the meat was obtained. Other potential sources of microbial contaminations are the equipment used for each operation that is performed until the final product is eaten, the clothing and hands of personnel and the physical facilities themselves are all implicated (Rombouts and Nouts, 1994). Retail cut could also result in greater microbial load because of the large amount of exposed surface area, more readily available water, nutrient and greater oxygen penetration available (Forest, et al, 1985) hence retail cuts displayed are conducive for microbial growth and proliferation which leads to spoilage of the meat (Ayres, 1995).

However in Nigeria, a number of foods (meat inclusive) have been reported to have high incidence of bacteria (Nkanga and Uraih, 1981; Okonko et al., 2008a, b, c, 2009a, b; Clarence et al., 2009). But there is limited information on the health challenges from food borne diseases from fresh meat retailed within a highly populous community. It was on this basis that it became necessary and essential to give useful information about the bacterial loads in most meat sold in Calabar, Nigeria, which is an indication of this sanitary condition in such area. Although contamination does not necessarily mean food-borne transmission, the possibility of these organisms being a foodborne pathogen should be investigated. This current study therefore, focused on assessing, isolating and identifying gram negative bacteria in fresh meat sold in Calabar metropolis, Nigeria, with a view to highlight the public health risk and implications of consuming such contaminated meat and provides useful information where necessary to the general public and potential approaches for improving the quality assurance and creates awareness among the consumers.

**MATERIALS AND METHODS**

**Study Area**

The study area was Calabar, Cross River State, South-south region of Nigeria. She is one of the most ancient, colonial and cosmopolitan cities in Nigeria.

**Sample Collection**

Ten duplicates samples of different portions of fresh meat (beef) were purchased from two major markets (Watt and Marian markets) in Calabar metropolis, Nigeria. The duplicate samples were aseptically collected in a clean polyethylene bag and transferred immediately to the laboratory for further bacteriological analysis as described by the methods of Fawole and Oso (2001).
Sample Preparation
Ten grams (10g) of each meat sample was weighed out and homogenized into 90 ml of sterile distilled deionized water using a sterile warring blender. Ten fold dilutions of the homogenates were made using sterile pipettes as described by the methods of Fawole and Oso (2001).

Culturing, Enumeration, and Isolation
All the chemicals and reagents used were of analytical grade, obtained from Sigma chemical co. Ltd, England. Media used in this study included: Nutrient Agar (NA) and Peptone Water (PW) as general and enriched media. Other media with selective and differential characteristics used were Mac Conkey agar (MCA), Eosin Methylene Blue (EMB), Kligler Iron Agar (KIA), Citrate Agar (CA), Christensen's Urea Agar (CUA), Mueller Hinton Agar and Mannitol Salt Agar (MSA). All media were prepared according to the manufacturer’s specification and sterilized at 121°C 1 bar for 15 min. From the 10-fold dilutions of the homogenates; 0.1ml of $10^{-2}$, $10^{-3}$ and $10^{-4}$ dilutions of the homogenate was plated in replicate on different media (in duplicates), using pour plate method. The plates were then incubated at 37°C for 24 - 48 h. Mac Conkey agar was used for coliform enumeration while Mannitol salt agar was used for the isolation of S. aureus. Total viable aerobic bacteria count was performed on Nutrient Agar. At end of the incubation periods, colonies were counted using the illuminated colony counter (Gallenkamp, England). The counts for each plate were expressed as colony forming unit of the suspension (cfu/g). Discrete colonies were sub-cultured into fresh agar plates aseptically to obtain pure cultures of the isolates. Pure isolates of resulting growth were then stored at 40°C.

Identification of isolates
Colonies identifiable as discrete on the Mueller Hinton Agar were carefully examined macroscopically for cultural characteristics such as the shape, color, size and consistency. Bacterial isolates were characterized based on microscopic appearance, colonial morphology and Gram staining reactions as well as appropriate biochemical tests for example Kligler’s Iron Agar (KIA) test, Indole production test, Methyl Red (MR) test, Voges-Proskauer (VP) test, Citrate utilization test, Motility Indole Urea (MIU) test, Carbohydrate fermentation test and salt tolerance test as described by Cheesbrough (2003) and Oyeleke and Manga (2008) were carried out. The isolates were identified by comparing their characteristics with those of known taxa, as described by Bergey’s Manual for Determinative Bacteriology (Buchanan and Gibbons, 1974). Statistical analysis: Data were analyzed using the general linear model procedure and ANOVA.

RESULTS
Ten duplicate samples of fresh meats; 5 samples from Marian Market and 5 samples from Watt Market in Calabar, Nigeria were analyzed microbiologically for the incidence of gram negative bacteria. Table 1 shows the estimation of the total viable bacterial counts and total coliform counts in fresh meat on Nutrient agar and Mac Conkey agar. The mean microbial load on the fresh meat from Watt market ranged between $2.62 \times 10^4$ - $4.84 \times 10^4$ cfu/g and total coliform count between $1.05 \times 10^3$ - $3.72 \times 10^3$ cfu/g while the fresh meat from Marian market ranged between $2.24 \times 10^4$ - $5.01 \times 10^4$ cfu/g and total coliform count between $1.23 \times 10^3$ - $3.42 \times 10^3$cfu/g as shown in Table 1.
Table 1: Total Viable Bacterial Count and Total Coliform Count in Fresh Meats Sold in Calabar, Nigeria

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean Total Bacterial Count (CFU/g)</th>
<th>Mean Total Coliform Count (CFU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Watt Market</strong></td>
<td></td>
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<tr>
<td>Mw 1</td>
<td>$4.84 \times 10^4$</td>
<td>$2.97 \times 10^3$</td>
</tr>
<tr>
<td>Mw 2</td>
<td>$3.75 \times 10^4$</td>
<td>$3.72 \times 10^3$</td>
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<tr>
<td>Mw 3</td>
<td>$4.28 \times 10^4$</td>
<td>$2.54 \times 10^3$</td>
</tr>
<tr>
<td>Mw 4</td>
<td>$3.73 \times 10^4$</td>
<td>$2.22 \times 10^3$</td>
</tr>
<tr>
<td>Mw 5</td>
<td>$2.62 \times 10^4$</td>
<td>$1.05 \times 10^3$</td>
</tr>
<tr>
<td><strong>Marin Market</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mw 1</td>
<td>$3.05 \times 10^4$</td>
<td>$1.58 \times 10^3$</td>
</tr>
<tr>
<td>Mw 2</td>
<td>$3.74 \times 10^4$</td>
<td>$3.42 \times 10^3$</td>
</tr>
<tr>
<td>Mw 3</td>
<td>$2.24 \times 10^4$</td>
<td>$1.23 \times 10^3$</td>
</tr>
<tr>
<td>Mw 4</td>
<td>$5.01 \times 10^4$</td>
<td>$2.04 \times 10^3$</td>
</tr>
<tr>
<td>Mw 5</td>
<td>$4.81 \times 10^4$</td>
<td>$2.30 \times 10^3$</td>
</tr>
</tbody>
</table>

Eight genera of bacteria were isolated from the fresh meat samples. The isolates were identified as *Klebsiella pneumoniae*, *Enterobacter spp*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella spp*, *Serratia marcescens*, *Pseudomonas spp* and *Proteus vulgaris* by comparing their morphological and biochemical characteristics with standard reference organisms (Table 2). No *Staphylococcus aureus* was isolated in this study (Table 2).

Table 2: Morphological and Biochemical Characteristics of Gram Negative Bacteria Isolates in Fresh Meats Sold in Calabar, Nigeria

<table>
<thead>
<tr>
<th>Parameters</th>
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<th>III</th>
<th>IV</th>
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<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grams reaction</td>
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<tr>
<td>Cellular morphology</td>
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<td>Motility</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>Catalase test</td>
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<td>+</td>
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<td>+</td>
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<tr>
<td>Oxidase test</td>
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<td>+</td>
<td>-</td>
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<tr>
<td>Indole test</td>
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<td>+</td>
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<td>+</td>
<td>-</td>
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<tr>
<td>Urease activity</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<td>Methyl Red</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td>Voges Proskauer</td>
<td>+</td>
<td>-</td>
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</tbody>
</table>

**Growth on KIA Medium:**
- Slope: Y Y R R Y Y R R R R
- Butt: Y Y Y Y R R Y Y Y Y R R
- Hydrogen Sulphide (H₂S): - - + - - + + - + - +
- Gas production: + + + + - + + + + + +

**Sugar fermentation test:**
- Lactose: A A A - A A/G A - - -
- Sucrose: A A A A/G A A/G A A - A/G A
- Mannitol: A A A - A A N/A - - -
- Maltose: A A A A/G A A/G N/A - - -
- Most probable organism: Kp Es Cf Pa Ec Ss Sm Ps Pv

**Keys:**
- N/A = Not applicable, - = No growth, + = Growth, A/G = Acid production and gas production, A = Acid production only and no gas production, Y = Yellow (Acid reaction), R = Red-pink (Alkaline reaction), Kp = *Klebsiella pneumoniae*, Es = *Enterobacter spp*, Cf = *Citrobacter freundii*, Pa = *Pseudomonas aeruginosa*, Ec = *Escherichia coli*, Ss = *Salmonella spp*, Sm = *Serratia marcescens*, Ps = *Pseudomonas spp*, Pv = *Proteus vulgaris*
Table 3 shows the frequency and percentage incidence of gram negative bacterial pathogens isolated from fresh meats sold at Watt and Marian Markets respectively. It showed that *K. pneumoniacae* [6(16.7%)] was the most predominant pathogens. This was followed by *Enterobacter spp* [5(13.9%)] and *C. freundii* [5(13.9%)], *Ps. aeruginosa* [4(11.1%)], *E. coli* [4(11.1%)], *Salmonella spp* [4(11.1%)], *S. marcescens* [4(11.1%)], and *Pseudomonas spp* [3(8.3%)]. *Pr. vulgaris* [1(2.8%)] was less predominant (Table 3).

It also showed that all the *Enterobacter spp*, *Salmonella spp*, *Pseudomonas spp*, and *Pr. vulgaris* were only isolated from fresh meats sold at Watt market (Table 3). No strain of *C. freundii*, *E. coli*, and *Ps. aeruginosa* was isolated from Watt market (Table 3). Also, only 50% of *K. pneumoniacae*, and *S. marcescens* were obtained from meat sold at Watt market (Table 3). The results went further to show that all the *C. freundii*, *Ps. aeruginosa*, and *E. coli*, were only isolated from the fresh meats sold at Marian market as also shown in Table 3.

No isolates of *Enterobacter spp*, *Salmonella spp* and and *Pr. vulgaris* were isolated from this market. Also, only 50% of *K. pneumoniacae*, and *S. marcescens* were obtained from meat sold at Marian market (Table 3).

Table 3: Frequency and Incidence of Gram Negative Bacterial Pathogens in Fresh Meats Sold in Calabar, Nigeria

<table>
<thead>
<tr>
<th>Pathogens</th>
<th>Total No. (%)</th>
<th>Watt Market (%)</th>
<th>Marian Market (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella pneumoniacae</em></td>
<td>6 (16.7)</td>
<td>3 (50.0)</td>
<td>3 (50.0)</td>
</tr>
<tr>
<td><em>Enterobacter spp</em></td>
<td>5 (13.9)</td>
<td>5 (100.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>5 (13.9)</td>
<td>0 (0.0)</td>
<td>5 (100.0)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>4 (11.1)</td>
<td>0 (0.0)</td>
<td>4 (100.0)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>4 (11.1)</td>
<td>0 (0.0)</td>
<td>4 (100.0)</td>
</tr>
<tr>
<td><em>Salmonella spp</em></td>
<td>4 (11.1)</td>
<td>4 (100.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>4 (11.1)</td>
<td>2 (50.0)</td>
<td>2 (50.0)</td>
</tr>
<tr>
<td><em>Pseudomonas spp</em></td>
<td>3 (08.3)</td>
<td>3 (100.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>1 (02.8)</td>
<td>1 (100.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>36 (100.0)</strong></td>
<td><strong>18 (50.0)</strong></td>
<td><strong>18 (50.0)</strong></td>
</tr>
</tbody>
</table>

Figure 1 shows the overall frequency and incidence of gram negative bacterial pathogens isolated from fresh meats sold at Watt and Marian Markets respectively.

Figure 1: Frequency of Occurrence of Gram Negative Bacterial Pathogens in Fresh Meats Sold in Calabar, Nigeria.
In Watt market, *Enterobacter* spp [5(27.8%)] was the most predominant pathogens in fresh meats as shown in Figure 2. This was followed by *Salmonella* spp [4(22.2%)], *Klebsiella pneumoniae* [3(16.7%)] *Pseudomonas* spp [3(16.7%)] and *Serratia marcescens* [2(11.1%)]. *Proteus vulgaris* [1(5.6%)] was less predominant (Figure 2).

In Marian market, *Citrobacter freundii* [5(27.8%)] were the most predominant pathogens (Figure 2), followed by *Pseudomonas aeruginosa* [4(22.2%)], *Escherichia coli* [4(22.2%)], and *Klebsiella pneumoniae* [3(16.7%)]. *Serratia marcescens* [2(11.1%)] was less predominant (Figure 2).

**Figure 2: Incidence and Distribution of Gram Negative Bacterial Pathogens by Locations**

**DISCUSSION**

Fresh meat samples from both locations (Watt and Marian markets) yielded marked growth of bacteria. The presence of these organisms on meat parts could be attributed to the fact that meat contains an abundance of all nutrients required for the growth of bacteria in adequate quantity. The high total viable counts recorded in this study showed the microbial diversity (differences in form or species) in these markets, condition of the market and the hygienic practice employed by meat sellers and butchers. This determined the variation of bacterial contamination. On comparing the bacterial contamination between the Watt and Marin markets, the result obtained is on the high side. This is an indication of recontamination in food handling and hygiene techniques (Clarence et al., 2009). Similar values were reported by Yousuf et al. (2008) and Okonko et al. (2008c,d, 2009a,b).

A total of 36 isolates comprising of 8 different genera of gram negative bacteria were isolated in this study with an average incidence rate of 50% in each market. This showed that both markets contributed equally to the microbial diversity reported in this study. The bacteria isolates were identified as *K. pneumoniae, Enterobacter* spp, *C. freundii, Ps. aeruginosa, E. coli, Salmonella spp, S. marcescens, Pseudomonas spp* and *Pr. vulgaris* by comparing their morphological and biochemical characteristics with standard reference organisms (Buchanan and Gribbons, 1974; Cheesbrough, 2003). Microorganisms isolated from fresh meat samples in this study have been earlier found in foods, environment and other places, and their pattern is similar to previous reports (Nkanga and Uraih, 1981; Agbeyegbe and Uraih, 1982; Enabulele and Uraih, 2009; Sobukola et al., 2009; Clarence et al., 2009; Oyeleke, 2009; Okonko et al., 2008a, b, c, d, 2009).
Agbeyegbe and Uraih (1982) study reported high prevalence rate of *E. coli* in raw meat samples. Enabulele and Uraih (2009) reported *E. coli* prevalence rate to be 85.65% in a study with the fresh meat samples from abattoir and traditional open market each, recording 100% *E. coli* prevalence. Clarence et al. (2009) and and Oyeleke (2009) reported the presence of *S. aureus, E. coli, Bacillus, Pseudomonas* and *Klebsiella* in meat pie and yoghurts respectively. The findings of this study also agrees to reports of Okonko et al. (2008a, b, c, d, 2009), were they isolated almost similar organisms from water, seafood products and seafood processors. It also disagrees with Ghaderpoori et al. (2009) who reported *Klebsiella sp., Streptococcus faecalis* and *P. aeruginosa* and no average indicator organism (*E. coli*) in 88% of rural water in Saqzez, Iran.

The presence of these organisms in fresh meats depicts a deplorable state of poor hygienic and sanitary practices employed in the slaughtering, processing and packaging of fresh meats. From the results obtained, fresh meats sample were contaminated with high level of *K. pneumoniae, Enterobacter sp., C. freundii, Ps. aeruginosa, E. coli, Salmonella spp, Serratia marcescens, Pseudomonas spp* and *Pr. vulgaris*. This agrees to previous reports by Clarence et al. (2009) who reported *S. aureus, E. coli, Klebsiella sp* and *Pseudomonas* spp in meat pie and Okonko et al. (2009a) who reported *Enterobacter sp., S. aureus, E. coli, Proteus sp, Salmonella sp., Citrobacter sp, Klebsiella sp, Pseudomonas sp., and Serratia sp.* in a study on seafood products. Okonko et al. (2009b) also reported the presence of *S. aureus, Enterobacter sp., P. aeroginosa,* and *E. coli* in palms of all the frozen seafood processors/handlers and water used by them. Actually they consider the detectable presence of pathogens like *Salmonella* sp as an indicator of adulteration (Yousuf et al, 2008). Moreover, the faecal coliforms as *Escherichia coli* are generally considered as indisputable indicators of faecal contamination from warm blooded animals (Yousuf et al., 2008).

Though, Kabir (2009) did not report Streptococci, Bacilli, Micrococi and Salmonella in his study on stored meat samples from probiotics-fed broilers, higher percentage of *Staphylococci, E. coli, Pseudomonas,* and others (unidentified) were reported. Nkanga and Uraih (1981) reported high prevalence rate of *S. aureus* in meat samples from traditional market in Benin City, Nigerian. Okonko et al. (2008c, d) in their study reported that all shrimps sampled harboured similar organisms as found in this present study. Yousuf et al. (2008) reported presence of *S. aureus, Salmonella sp., Shigella sp, Flavobacterium sp. and Vibrio sp.* in the muscle of locally available tiger shrimp (*Penaeus monodon*) and giant water prawn from Bangladesh, Iraq while Okonko et al (2008b) reported *E. aerogenes* and *S. aureus* in similar studies. In the United States, the food vehicle for 75 (41%) foodborne outbreaks was ground beef (Schroeder et al., 2005).

Most of the organisms found in this study are those commonly found in soil and water. Though, no *Staphylococcus aureus* was isolated in this present study as reported in all previous work mentioned above, the presence of *E. coli* (11.1%) and *Enterobacter spp* (13.9%) in this fresh meat samples is an indication of faecal contamination of the meats. This might be due to possible contamination of fresh meats or meat products itself during sales or unhygienic handling of the meats right from the slaughtering, butchering plants and processing or due to contamination from the skin, mouth, or nose of the handlers which can be introduced directly into foods by process line workers, with lesions caused by *S. aureus* on hands and arms coming into contact with the food, or by coughing and sneezing (Sobukola et al., 2009; Okonko et al., 2008a,b,c,d,2009a,b).

The isolation of *Enterobacter* spp. may be as a result of poor environmental conditions due to dust and contamination of the water used during slaughtering, because *Enterobacter* spp. are also inhabitants of dairy products, as reported by Talaro and Talaro (2006). *Salmonella spp* (11.1%), another organism found in the meats is also a pathogenic organism of public health significance and concerns (Okonko et al., 2009a,b). The isolation of *Salmonella* sp. in this study is of practical impact. This organism might have contaminated the meats as a result of handling by meat sellers. This result agrees to previous reports by El-Gohany (1994) that foods of animal origin (minced
meat) either cooked or uncooked were predominantly contaminated with *E. coli*, and Waites and Arbuthnott (1999), who reported 50% *E. coli* contamination in minced meat, sausage rolls and pies. This is also in accordance to the assertion of Okonko et al (2008d, 2009a, b) that improper handling and improper hygiene might lead to the contamination of ready-to-eat foods and this might eventually affects the health of the consumers. This was illustrated by the presence of the indicator organisms.

Incidences of *E. coli*, *Enterobacter spp* and other index of poor sanitary quality found in this study are in agreement with previous studies. *E. coli* O157 outbreaks due to plants and animal produce have become increasingly common (Schroeder et al., 2005). While half of produce-associated outbreaks were due to kitchen-level cross-contamination, which calls for further prevention efforts targeting food preparers, the other half were due to produce already contaminated with *E. coli* O157 before purchase (Schroeder et al., 2005).

*E. coli*, which are normal flora of the human and animal intestine, have been identified as a leading cause of food borne illness all over the world. *E. coli* and *E. coli* 0157: H7 strain has previously been isolated from meat samples (Hussein, 2007). *E. coli* 0157: H7 strain was not detected in any of the fresh meat samples examined. However, diarrhea caused by enterotoxigenic *E. coli* (ETEC) is highly prevalent in young children in developing countries as well as in travelers. It spreads through contaminated water and food (Qadri et al., 2005). The potentially high mortality associated with *E. coli* and *E. coli* 0157: H7 strain infection, therefore make its presence in any food material worrisome and of serious public health concern as most of the outbreaks recorded has been traced to consumption of beef contaminated with the *E. coli* 0157:H7 strain (Hussein, 2007). In spite of the wide knowledge of the organism and its interaction, there seem to be no report on the prevalence of the organism in Africa and particularly Nigeria. In the light of these prevailing circumstances, and increased reported cases of *E. coli* 0157 infection outbreak worldwide, it becomes apparent that a thorough study be conducted in this part of the world to ascertain its presence in our food products (Enabulele and Uraih, 2009).

*Salmonella* species such as *Salmonella typhi* is a bacterium that causes typhoid fever (enteric fever), an acute, life-threatening febrile illness (CDC, 2008). The disease is a cause for concern and a major public health problem in developing countries (Asia, Africa); especially in Nigeria due to poor sanitary conditions and lack of or inadequate potable water (Ibekwe et al., 2008). It is mainly transmitted through food or drink or water, contaminated with urine or faeces of infected people or a chronic carrier (CDC, 2008; Ibekwe et al., 2008). Since 1987, *Salmonella enteritidis* has been one of the most frequently isolated salmonellae associated with foodborne outbreaks, which have been linked to consumption of chickens, eggs, and foods that contain eggs and it presents an interesting challenge from an epidemiologic perspective (Zheng et al., 2007).

Infections with nontyphoidal *Salmonella* have increased during the last 3–4 decades, and although a decrease has been reported over the last decade, *Salmonella* infections continue to be a major public health concern in many countries. These salmonellae are zoonotic, and the infections are generally foodborne (Helms et al., 2005). The main reservoir of zoonotic *Salmonella* is food animals, and the main sources of infections in industrialized countries are animal-derived products, notably fresh meat products and eggs (Helms et al., 2005). Rapid spread of a limited number of successful *Salmonella* clones in different sectors of food animal production (swine, broiler chickens, and particularly layer hens) has been suggested as the most important cause of this increase (Helms et al., 2005).

Fresh meats sold to the public in open markets are grossly contaminated with coliform bacteria as well as other bacterial forms. The finding of this study revealed that fresh meat sold at Watt and Marian markets in Calabar, Nigeria are contaminated with pathogenic gram negative bacteria. The possible source of contaminants, are due to the unhygienic manner of handling meat
from the slaughters to the markets. This also implies that these meats are viable source of various diseases. Some these diseases could spread and acquire epidemic status which poses serious health hazards. Since improper handling and improper hygiene might lead to the contamination of fresh meats and this might eventually affects the health of the consumers (Okonko et al., 2008b,c,d, 2009a,b), it is therefore suggested that fresh meat processors and sellers should be educated on the adverse effect of contamination. However, the processors/handlers/sellers should observe strict hygienic measures so that they may not serve as source of chance inoculation of microorganisms and fecal contamination of fresh meats and other meat products.

The presence of indicator and other organisms examined in this study is of special concern and perhaps the greatest danger associated with fresh meats used for food preparation, eating purposes and for other human consumption is contamination by human excrement (Okonko et al., 2008a, b, c, d, 2009a, b). It demonstrates a potential health risk as the organism is pathogenic and causes complications in children (Taulo et al., 2008). The need for microbial assessment of fresh meats and other meat products processed and packaged for human consumption is therefore emphasized and recommended to reduce possible contamination (Okonko et al., 2009a, b). Irrespective of the presence of these gram negative organisms in fresh meat analyzed, it is believed that cooking processes and hygiene could greatly reduce the microbial load to harmless level. Thorough cooking as well as good hygiene is the order to prevent contamination of food eaten raw fare therefore essential (Amann et al., 1995).

This study also reveals that fresh meats are often contaminated with bacteria. The presence of higher number of pathogenic *K. pneumoniae*, *Salmonella* sp. and *E. coli* among others, encountered in fresh meat from conventional beef is alarming. The presence of these organisms in meat foods should receive particular attention, because their presence indicate public health hazard and give warning signal for the possible occurrence of food borne intoxication (Kabir, 2009). Since control of fecal–orally transmitted pathogens is inadequate in many developing countries, in particular, in sub-Saharan Africa (Okeke et al., 2007) and acquired resistance to antimicrobial drugs is becoming more prevalent among *V. cholerae*, *S. enteritidis*, diarrheagenic *E. coli*, and other pathogens in this region (Okeke et al., 2007), it is therefore necessary that we also make the following recommendations from the findings of this study, that: 1.) Meat handlers and sellers should be educated on the adverse effect of lack of proper personal and environmental hygiene and sanitation; 2.) Veterinary doctors should inspect the animals to be slaughter before the meat is sold to the general public; 3.) Good manufacturing practices should be adhered to strictly by butchers and those selling the meat, the water used in washing the meat should be sterile, also the equipment must be washed properly before use; 4.) Further regulatory and educational efforts are needed to improve the safety of produce items (Schroeder et al., 2005); 5) Improvements in detecting and investigating foodborne illnesses were made during the 1990s when CDC implemented the Foodborne Diseases Active Surveillance Network (FoodNet), a component of the Emerging Infections Programs (EIP), and PulseNet (Hoffman et al., 2005). Continued progress on the part of regulators and industry to improve food safety are dependent on local, state, and federal agencies' ability to conduct epidemiologic and laboratory investigations that identify the offending agents and link them with specific foods (Hoffman et al., 2005) and this should be put in place in Nigeria; and 6.) Fresh meats to be used for consumption purposes should be adequately cooked before use and NAFDAC should ensure and enforce strict compliance of the recommended food standards as regards the production and sales of processed and packaged meat products.

REFERENCES


