Bacteriological Qualities of Beef Sold in Abia and Imo States, Nigeria: Implications for the Sustenance of Enteric Diseases

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Abstract: In Nigeria, meat-borne diseases are important contributors to the heavy burden of morbidity and mortality caused by diarrheal diseases. The aim of this research was to assess the bacteriological quality of beef and beef contact surfaces in Abia and Imo States of Nigeria in relation to their implications for sustaining enteric diseases. Two study areas from each State were selected by random sampling: Afor-Ogbe and Owerri in Imo State and Aba and Umuahia in Abia State. Bacteriological quality of the beef and beef contact surfaces was assessed by collecting and analyzing samples at 8 critical sampling locations (CSLs) created at the perceived critical control points. Mean total viable count (TVC) at the study areas were: Afor-Ogbe, 1.81 x 10⁵; Umuahia, 1.79 x 10⁵; Aba, 1.75 x 10⁶; and Owerri, 1.73 x 10⁷; p = 0.00. Mean TVC strongly correlated with mean total coliform count (TCC) at the critical sampling locations in the study areas. The mean prevalence of pathogens in the study areas were: Afor-Ogbe, 65.50%; Aba, 54.20%; Owerri, 52.30%; and Umuahia, 48.80%. It is concluded here that the bacteriological quality of the beef sold in the two States is very poor, and this exposes the beef consumers to high risk of infection. It is recommended here that the government of Nigeria at all levels should urgently enact and enforce laws on meat safety management based on good hygiene principles of Codex Alimentarius Commission.

Keywords: Bacteriological; beef; study areas; pathogens; enteric diseases

1. Introduction

The safety of meat is intimately connected with safety of its processing environment. Nwantaet al. (2008) reviewed the state of Nigeria abattoir operations and waste management; they also discussed the challenges and prospects of the industry with respect to environmental quality and public health. Their findings reveal that the handling of meat in Nigeria is generally unsatisfactory. These findings are in agreement with the findings of Okoliet al. (2006) who did a study on animal food product delivery system in Imo State, Nigeria and asserted that official slaughter points in the state were principally low-grade quality slaughter premises consisting of a thin concrete slab; meat handling was very unhygienic with carcasses dressed beside refuse heaps of over 2 years standing; carcasses were dragged on the ground and transported in taxi boots and open trucks. To buttress the above facts Oluwafemi et al. (2013) did a review of meat processing practices in Nigeria and concluded that there was a clear indication that the current slaughtering, processing and marketing of meat in many parts of Nigeria are not in compliance with the standard quality and hygiene practices and that it may act as source of contamination and ill health for consumers.

The number, distribution and type of microbiological hazards in meat determine the likelihood of that meat to produce disease in humans when consumed. Several studies have been carried out in the past decade to establish these in order to design a robust food safety management system that reduce to the barest minimum the incidence and prevalence of meat borne diseases. In a review of challenges to meat safety in the 21st century, Sofos (2008) states that the most serious meat safety issues resulting in immediate consumer health problems and recalls from the marketplace of potentially contaminated meat products are associated with microbial hazards, and especially bacterial pathogens. Some recent studies in Nigeria have revealed high meat pathogen prevalence in meat handling locations (Tafidaet al., 2013; Eruteya et al., 2014; Okonko et al., 2010; Falolaet al., 2011; Clarence et al., 2009). More recent investigators have also consistently found bacteriological meat safety in some parts of Nigeria to be below standard (Chukwu et al., 2016, Chukwuet al., 2016, Azage & Kilbret 2017, Falekeet al., 2017, FAO/WHO, 2013).

There has not been any bacteriological assessment of the meat sold in Abia and Imo States. The objective of this study is to assess the bacteriological quality of beef sold in Abia and Imo States of Nigeria.

2. Materials and Methods

The study areas were Abia and Imo States of Nigeria. Both States are situated in the southeastern zone of Nigeria. Abia State lies within approximately latitudes 4° 40’ and 6° 14’ north and longitudes 6° 10’ and 8° 14’ east while Imo State lies between latitudes 5° 4’ and 6° 13’ north and longitudes 6° 15’ and 7° 34’ east.
The study population was the beef handling points in Aba and Umuahia in Abia State and Afor-Ogbe and Owerri in Imo State. Five LGAs were selected in the Aba area; namely Aba North, Aba South, Osisioma, Ugwuagbo and Obingwa. Three LGAs were selected in Umuahia Area; namely, Umuahia North, Umuahia South, and Ikwuano. Three LGAs were selected in Owerri area; namely, Owerri West, Owerri North and Owerri Municipal. Also three LGAs were selected in Afor-Ogbe area; namely, Abom Mbaise, Ahia-Azu-Mbaise and Ngor-Okpala.

The research design was a cross-sectional study that assessed the prevalence of meat-borne pathogens at eight critical control points in the beef chain, which has been called critical sampling locations (CSLs) as adapted from Jacxsens et al. (2009) and International Livestock Research Institute (ILRI, 2011). Fig. 1 is a conceptual framework for the CSLs.

**Figure 1:** Conceptual framework for critical sampling locations. Adapted from Jacxsens et al. (2009) and ILRI (2011).

*CSL = Critical Sampling Location

The bacteriological quality of meat in each of eight critical sampling locations had the results as shown in Table 3.

### 2.1 Bacteriological Analysis

The microbiological analysis was carried out in the multipurpose laboratory of Abia State University Teaching Hospital. Culturing was done using Pour plate method outlined by Maturin and Peeler (2001).

 Altogether, 480 samples were collected by swab method. This analysis was done by the methods of Serraino et al. (2012) and Bhandare et al. (2010). Carcass and the meat chain environment were sampled by sterile specimen sponges wetted with 10 ml of buffered peptone water (Oxoid) from sterile Whirl-Pak bags (Sponge-Bag, PBI-International) using a template of 100 cm² surface area. Sponging within the area consisted of 5 passes vertically (up and down was considered one pass) and 5 passes horizontally (side to side was considered one pass) for each large side of the sponge. The sponge was placed into a Stomacher bag and delivered in a cold box (2–6 °C) to the laboratory within 4 hours.

Test tubes containing swabs were shaken on a vortex mixer for 30 seconds for uniform distribution of microorganisms. Tenfold serial dilution of all the samples was prepared using sterile normal saline solution (NSS). Then the samples were processed for viable counting. Total viable count (TVC), total coliform count (TCC) and bacteria isolates were determined using nutrient agar medium and MacConkey agar as described by Maturin and Peeler (2001).

The resulting pure cultures were carefully examined and characterized based on colony morphology, microscopic appearance, gram staining reaction and biochemical tests comprising: triple sugar iron Agar (TSI) test, ureas test, indole production, methyl red (MR), Voges-Proskauer (VP), motility, citrate test (Bhandare et al., 2010)

### 3. Results

The bacteriological quality of meat in each of eight critical sampling locations had the results as shown in Table 3. Table 3 shows the mean total viable count in Afor-Ogbe (study area 1) by the critical sampling location and meat media (water, floor, slab, knife, operator’s finger, and...
meat). The transport facility has the highest mean total viable count (2.25 x 10^5 ±0.42x10^5). This is followed by the open market (2.23 x 10^5 ±0.23x10^5), the abattoir (2.12 x 10^5 ±0.25x10^5), restaurant (1.65 x 10^5 ±0.13x10^5), supermarket (1.58 x 10^5 ±0.17x10^5), suya vendors (1.52 x 10^5 ±0.19x10^5) and home (1.51 x 10^5 ±0.13x10^5), p<0.05.

Table 3.2 shows the mean total viable count in Aba (study area 2) by the critical sampling location and meat media (water, floor, slab, knife, operator’s finger, and meat). The transport facility had the highest mean total viable count (2.18 x 10^5 ±0.39x10^5). This was followed by the open market (2.08 x 10^5 ±0.24x10^5), the abattoir (2.03 x 10^5 ±0.25x10^5), restaurant (1.65 x 10^5 ±0.17x10^5), suya vendors (1.59 x 10^5 ±0.18x10^5), supermarket (1.55 x 10^5 ±0.08x10^5), mamaput (1.49 x 10^5 ±0.10x10^5) and home (1.48 x 10^5 ±0.13x10^5), p<0.05.

Table 3.3 shows the mean total viable count in Umuahia (study area 3) by the critical sampling location and meat media (water, floor, slab, knife, operator’s finger, and meat). The transport facility had the highest mean total viable count (2.25 x 10^5 ±0.37x10^5). This is followed by the abattoir (2.12 x 10^5 ±0.20x10^5), open market (2.09 x 10^5 ±0.14x10^5), restaurant (1.64 x 10^5 ±0.15x10^5), mamaput (1.61 x 10^5 ±0.10x10^5), supermarket (1.60 x 10^5 ±0.16x10^5), home (1.54 x 10^5 ±0.10x10^5) and suya vendors (1.50 x 10^5 ±0.19x10^5), p<0.05.

Table 3.4 shows the mean total viable count in Owerri (study area 4) by the critical sampling location and meat media (water, floor, slab, knife, operator’s finger, and meat). The transport facility had the highest mean total viable count (2.13 x 10^5 ±0.36x10^5). This was followed by the open market (2.02 x 10^5 ±0.26x10^5), abattoir (2.01 x 10^5 ±0.24x10^5), suya vendors (1.65 x 10^5 ±0.14x10^5), restaurant (1.63 x 10^5 ±0.16x10^5), mamaput (1.53 x 10^5 ±0.11x10^5) and home (1.42 x 10^5 ±0.11x10^5), p<0.05.

Table 3.5 shows a comparison of the bacterial loads at the critical sampling locations at the study areas with the standard microbiological criteria. The Microbiological criteria states that the total viable count (TVC) for raw meat should be between 1.00 X 10^5 cfu/cm^2 and 1.00 X 10^6 cfu/cm^2).

<table>
<thead>
<tr>
<th>CSL</th>
<th>Water</th>
<th>Floor</th>
<th>Slab</th>
<th>Knife</th>
<th>Operator’s Fingers</th>
<th>Meat (Muscle)</th>
<th>Total</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Abattoir</td>
<td>2.00 x 10^5</td>
<td>2.40 x 10^5</td>
<td>2.10 x 10^5</td>
<td>1.88 x 10^5</td>
<td>2.45 x 10^5</td>
<td>1.88 x 10^5</td>
<td>12.71 x 10^5</td>
<td>2.12 x 10^5</td>
<td>±0.25x10^5</td>
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<tr>
<td>2. Trans Fac</td>
<td>-</td>
<td>2.55 x 10^5</td>
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<td>-</td>
<td>-</td>
<td>1.95 x 10^5</td>
<td>4.5 x 10^5</td>
<td>2.25 x 10^5</td>
<td>±0.42x10^5</td>
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<td>3. Suya Vendors</td>
<td>-</td>
<td>1.65 x 10^5</td>
<td>-</td>
<td>1.45 x 10^5</td>
<td>1.70 x 10^5</td>
<td>1.29 x 10^5</td>
<td>6.09 x 10^5</td>
<td>1.52 x 10^5</td>
<td>±0.19x10^5</td>
</tr>
<tr>
<td>4. Supermarket</td>
<td>-</td>
<td>1.55 x 10^5</td>
<td>1.75 x 10^5</td>
<td>1.35 x 10^5</td>
<td>1.52 x 10^5</td>
<td>1.75 x 10^5</td>
<td>7.92 x 10^5</td>
<td>1.58 x 10^5</td>
<td>±0.17x10^5</td>
</tr>
<tr>
<td>5. Open Market</td>
<td>-</td>
<td>2.40 x 10^5</td>
<td>2.10 x 10^5</td>
<td>2.00 x 10^5</td>
<td>2.53 x 10^5</td>
<td>2.10 x 10^5</td>
<td>11.13 x 10^5</td>
<td>2.23 x 10^5</td>
<td>±0.23x10^5</td>
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<tr>
<td>6. Home</td>
<td>-</td>
<td>1.55 x 10^5</td>
<td>1.65 x 10^5</td>
<td>1.40 x 10^5</td>
<td>1.38 x 10^5</td>
<td>1.35 x 10^5</td>
<td>7.53 x 10^5</td>
<td>1.51 x 10^5</td>
<td>±0.13x10^5</td>
</tr>
<tr>
<td>7. Restaurant</td>
<td>-</td>
<td>1.85 x 10^5</td>
<td>1.68 x 10^5</td>
<td>1.62 x 10^5</td>
<td>1.64 x 10^5</td>
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<td>8.27 x 10^5</td>
<td>1.65 x 10^5</td>
<td>±0.13x10^5</td>
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<tr>
<td>8. Mamaput</td>
<td>-</td>
<td>1.60 x 10^5</td>
<td>-</td>
<td>-</td>
<td>1.70 x 10^5</td>
<td>1.45 x 10^5</td>
<td>4.75 x 10^5</td>
<td>1.58 x 10^5</td>
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In Aba 15 out of 24 samples (62.5%) tested positive for *Bacillus spp*; 15 out of 24 samples (62.5%) tested positive for *Salmonella spp*; 14 out of 24 samples (58.3%) tested positive for *E. coli*; 14 out of 24 samples (58.3%) tested positive for *Proteus spp*; 13 out of 24 samples (54.2%) tested positive for *Staph aureus*; 10 out of 24 samples (41.7%) tested positive for *Klebsiella spp*; and 10 out of 24 samples (41.7%) tested positive for *Streptococcus spp*. In all, 91 of the 168 samples (54.2%) had pathogenic bacteria in them. Chi squared test showed the differences these bacteria loads not to be statistically significant, p = 0.072.

In Owerri 18 out of 24 samples (75.0%) tested positive for *Staph. aureus*; 17 out of 24 samples (70.8%) tested positive for *E. coli*; 17 out of 24 samples (70.8%) tested positive for *Proteus spp*; 13 out of 24 samples (54.2%) tested positive for *Streptococcus spp*; 13 out of 24 samples (54.2%) tested positive for *Salmonella spp*; 7 out of 24 samples (29.2%) tested positive for *Bacillus spp*; and three out of 24 samples (12.5%) tested positive for *Klebsiella spp*. In all, 88 of the 168 samples (52.3%) had pathogenic bacteria in them. Chi squared test showed the differences in these bacteria loads to be statistically significant, p = 0.000.

Table 6 also reveals that highest concentration of pathogens were found in study area 1 (Afor-Ogbe), and it is 65.50%.
This was followed by study area 2 (Aba) with pathogen concentration of 54.20%. Study area 4 (Owerri) was the next with mean pathogen concentration of 52.30%. The study area with the least mean concentration of pathogens was study area 3 (Umahia) with mean pathogen concentration of 48.80%. \( (p = 0.001) \).

4. Discussion

Tables 3.1 –3.4 reveal that there existed differences in the level of bacterial loads at the critical sampling locations in the study areas. The differences in the bacteria loads in the eight critical sampling locations at the four study areas were statistically significant \( (p < 0.05) \). This finding is in agreement with the findings Clarence et al. (2009), Okonko et al. (2010) and Falolaet al. (2011). There appears to be no significant improvement in the bacteriological qualities of meat sold in Nigeria over the years.

Table 3.5 shows a comparison of the bacterial loads at the critical sampling locations at the study areas with the standard microbiological criteria. The Microbiological criteria states that the total viable count (TVC) for raw meat should be between 1.00 X 10\(^{3}\) cfu/cm\(^2\) and 1.00 X 10\(^{5}\) cfu/cm\(^2\). Any TVC more than 1.00X 10\(^{5}\) was unacceptable. All the mean bacterial loads at the critical sampling locations in the study areas (range: 1.488 X 10\(^{3}\) cfu/cm\(^2\) to 2.200 X 10\(^{3}\) cfu/cm\(^2\) ) were higher than the standard values (range: 1.00 X 10\(^{3}\) cfu/cm\(^2\) and 1.00 X 10\(^{5}\) cfu/cm\(^2\) ) (Heinz and Hautzinger, 2007; The European Commission, 2005; Turtle and Smith, 2009) \( p = 0.00 \). Therefore the meat sold in those markets in the study areas had the potential to cause enteric diseases and therefore unsafe for human consumption.

There were also differences in the bacteria loads at the four study areas, showing the bacteriological qualities of the study areas. These bacteria loads at the study areas were higher than that found by Clarence et al. (2009) in Benin City, Nigeria but lower than that found by Falola et al. (2011) in Lagos Mainland Local Government Area, Nigeria, and Oluwafemi and Simisaye (2006) in Abeokuta and Benin City, Nigeria.

Table 3.6 shows that seven pathogens were isolated from the meat and environmental samples: *Staph. aureus*, *E. coli*, *Proteus spp*, *Streptococcus spp*, *Bacillus spp*, *Salmonella spp*, and *Klebsiella spp*. These have prevalence that range from 12.5% to 91.7% in the different study areas. Even the minimum is unacceptable as the meat containing that level of pathogen can cause serious enteric disease outbreak (Heinz and Hautzinger, 2007; Health Protection Agency, 2009; Esemonuet al., 2012).

4.1 Implications for the Sustenance of Enteric Diseases

*Staph.aureus* is notorious for causing enteric diseases (Adams, 2009). This study is in agreement with that of Adesijiet al. (2011) in Oshogbo, Nigeria. Staphylococcal enterotoxins produce the food intoxication(Fisheret al., 2018; Medved’ova, et. al., 2017). Transmission is mostly anthroponotic (Argudin et al., 2010; Ercoli, et al.,2017). Its presence in the study samples is therefore due to poor hygiene of the meat handlers.

Isolation of *E. coli* poses a serious enteric disease risk. Although the isolates have not been characterized into their pathogenic strains, a plethora of virulent serotypes of Shiga toxin producing *E. coli* (STE C) and Toxigenic *Escherichia coli* (TEC), have been isolated from human foodborne infections (Büet al., 2015; Wu et al., 2011; Bell and Kyriakides, 2009. Consumption of the meat sold in the study areas therefore has a very high risk of enteric illness caused by pathogenic *E. coli*.

*Bacillus spp*, such as *B. cereus*, *B. anthracis*and to a lesser extent *B. subtilis*, are pathogenic in humans and other mammals (Blackburn and McClure, 2009). Consumption of meat sold in the study areas also has a high risk enteric disease caused by pathogenic *Bacillus spp* (Carrollet al., 2019; Bagcioglu et al., 2019).

*Proteus spp* has been suspected of causing acute and chronic gastrointestinal diseases (FDA, 2013, Shi et al., 2016). This reflects the risk of enteric disease inherent in eating the meat sold in these study areas.

Salmonellosis is an important cause of foodborne human gastroenteritis globally and in Nigeria, and meat is an important contributor to the public health disease burden caused by *Salmonella* infection (Molleret al., 2015; EFSA, 2011; van Hookey et al., 2012; Gutet al., 2019, Fung et al., 2018, Berger et al., 2019). And of greater concern is the recent isolation of multiple drug resistant strains of *Salmonella* from meat (Doyle, 2015).

*Klebsiellaspp* is a foodborne pathogen (Zhang et al., 2018; Lu et al., 2017). An outbreak of nosocomial extended -pectrum \( \beta \)-lactamase (ESBL)-producing foodborne disease occurred in Barcelona, Spain in 2008 (Calboet al., 2011). Eating the meat sold in these study areas has a high risk of enteric disease.

*Streptococcus spp* has pathogenic strains that may produce clinical syndrome similar to staphylococcal intoxication (FDA, 2013, Moris, 2013) including diarrhea, abdominal cramps, nausea, vomiting, fever, chills, and dizziness.

The high prevalence of meat-borne pathogens in all the study areas confirms that consumption of the meat sold in the areas may cause enteric infections with their associated chronic sequelae of irritable bowel syndrome (Haagsma et al., 2010; Schwille-Kiuncket al., 2011; Thabane et al., 2010; Thabane and Marshall, 2009), inflammatory bowel disease (Ekici& Dümüen, 2019), reactive arthritis (Hannu, 2011; Townes, 2010), urological dysfunction (Iwashyna, 2010), and haemolytic uremic syndrome (Mayer et al., 2012;Ekici& Dümüen, 2019).

5. Conclusion

The high total viable count which highly correlated with total coliform count and high prevalence of meat pathogens observed at the critical sampling locations depict very poor meat safety management in the two study states which, with all indications, exposes the meat consumers in the study states to high risk of enteric diseases and their chronic sequelae. There is therefore urgent need to improve meat
safety management in the study states through effective public health education, provision of basic infrastructure at the abattoirs, creating enabling environment for meat safety through enactment of relevant laws and policies and their enforcement.

References


