

ANTIBIOGRAM AND BACTERIOLOGICAL ASSESSMENT OF RAW MEAT SOLD IN OWERRI, SOUTHEAST NIGERIA.

ABSTRACT

This study assessed the antibiogram and bacteriological assessment of raw meat sold in Owerri. Samples of fresh beef were taken from beef vendors from three (3) markets; Relief market, Ekeonuwa market and Amakohia market in Owerri, Imo State. Three samples each weighing 100g were aseptically collected in sterile polythene pouches, sealed and transported in ice to the Imo State University Microbiological Laboratory for microbiological analysis within some few hours of collection. The results of this study showed that the total heterotrophic bacteria counts ranged from 3.72×10^5 cfu/g - 4.2×10^5 cfu/g, the coliform bacterial counts ranged from 1.9×10^5 cfu/g - 2.7×10^5 cfu/g while the total salmonella shigella count ranged from 0 cfu/g - 4.12×10^3 cfu/g. Bacteria isolated were *Escherichia coli*, *Salmonella spp.*, *Streptococcus spp.*, *Bacillus spp.* and *Staphylococcus aureus*. Of the 50 bacterial isolates obtained, *E. coli* [15 (27.7)] and *Staphylococcus spp.* [15 (27.7)] were the prevalent isolates. Other bacterial pathogens incriminated in this study were *Salmonella spp.* [9 (18.0)], *Bacillus spp.* [7 (14.0)], and *Streptococcus spp.* [4 (8.0)]. Most of the Gram-positive bacteria were highly susceptible to ceftizoxime and the Gram-negative to gentamicin and cefotaxime. *E. coli* was susceptible to ampicillin/sulbactam (71% susceptibility), cefotaxime (71%), ciprofloxacin (94%), chloramphenicol (94%), ceftizoxime (100%), ofloxacin (82%), and amikacin (100%). *S. aureus* was susceptible to ampicillin/sulbactam (75%), cephalixin (75%), cefotaxime (100%), roxithromycin (75%), lincomycin (75%), and gentamicin (100%). The bacterial quality of fresh beef sold in Owerri has shown that beef sold in Owerri is contaminated with *Staphylococcus spp.*, *Escherichia coli* and *Salmonella spp.* but the prevalence of *Salmonella spp.* was low. This findings shall guide clinicians in the management of food poisoning/enteritis from possible meat consumption. Effective supervision and health education of meat sellers and their abattoirs is highly recommended.

Keywords. Raw meat, bacterial infection, salmonellosis, food poisoning, Nigeria

Introduction

One excellent source of protein in human diet is meat (Kombaet *al.*, 2012). It has long been known for its high nutrients composition hence consumed by many people worldwide. The protein profile of meat has been described as excellent due to the presence of all the essential amino acids required by the body (Collins and Thato, 2011). The protein and vitamins especially vitamin A and B12 in meat is not available in plant sources. Majority of the worlds' population depend on meat as a source of food (Collins and Thato, 2011). There is considerably high food related infections such as diarrhea, typhoid fever and cholera recorded in hospitals and clinics worldwide. In the past people have expressed worry about the role of meat and meat products in food poisoning but available records show that more than 74% of cases of food poisoning worldwide are due to meat dishes (Hobbs and Roberts, 2013). Meat is highly prone to microbial contamination due to its rich source of nutrients which provide a suitable environment for growth of microbes (Steinkraus, 2014).

Dirty environment and unhygienic food handling influence wide spread of bacterial food poisoning (Burgess *et al.*, 2015). Major bacterial pathogens found in meat include *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, *Salmonella*, *Escherichia coli* and *Staphylococcus aureus* (Hobbs and Roberts, 2013). Contamination could come from unhygienic slaughtering, handling and processing conditions or from inherent microflora in normal tissues of animals, air and environment (Bell, 2017).

The family *Enterobacteriaceae* is a large, heterogeneous group of gram-negative rods whose natural habitat is the intestinal tract of humans and animals. They are also found in soil and plant from where they can contaminate the food chain and cause food-borne gastroenteritis. The genera in the family include *Escherichia*, *Shigella*, *Salmonella*, *Yersinia*, *Enterobacter*, *Klebsiella*, *Serratia*, *Proteus* and others.

Common organisms implicated in meat poisoning include *Escherichia coli*, which becomes pathogenic only when they reach tissues outside of their normal intestinal or other less common normal flora sites. Raw beef can be an important vehicle in the transmission of *E. coli* during slaughtering, processing or from cross-contamination as a result of unsanitary food handling practices. Its presence in meat is usually a result of faecal contamination or when the intestinal tract is punctured (Abaidoo and Obiri-Danso, 2018).

For a long time *Staphylococcus aureus* has been known as one of the most important bacteria that causes disease in humans. It is responsible for many skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis (MDH, 2013). An estimated 185,000 cases of foodborne illnesses associated with Staphylococcal food intoxication occurs annually in United States (Mead *et al.*, 2019). Therefore any food which requires handling in preparation may easily become contaminated. *Staphylococcus aureus* also commonly occurs on the skin and hides of animals, and may thus contaminate foods from these animals as a result of cross-contamination during slaughter (Sprenger, 2015).

Salmonella species

Salmonella are nonspore-forming, rod-shaped, Gram-negative and predominantly motile enterobacteria with flagella distributed all around the cell body. They are widely spread in nature and are responsible for illnesses such as typhoid fever, paratyphoid fever and food poisoning (Fabrega, and Vila, 2013). Salmonellosis is type of food poisoning caused by *Salmonella* enteric bacteria. For over 100 years, The most severely affected people are the elderly, infants, and those with impaired immune systems. (CDC, 2014). Salmonellosis continues to be an important cause of foodborne disease in human worldwide although total number of cases has remained fairly constant between 1996 and 2002 (CDC, 2012).

The aim of this study was to determine the antibiogram and bacteriological assessment of raw meat sold in Owerri

Methodology

This study was conducted in Owerri, the capital of Imo state in Nigeria.

Samples of fresh beef were taken from beef vendors from three (3) markets; Relief market, Ekeonuwa market and Amakohia market in Owerri, Imo State. Three samples each weighing 100g were aseptically collected in sterile polythene pouches, sealed and transported in ice to the Imo State University Microbiological Laboratory for microbiological analysis within some few hours of collection

All the media used were prepared according to the manufacturer's guide. Nutrient agar, MacConkey agar, Salmonella-shigella agar and Muller hinton agar were used for the isolation of bacteria. Muller hinton agar was used for antimicrobial sensitivity testing. Identification of pure colonies using morphological characteristics were based on morphological differences, colonies were isolated from their axenic culture. Slides were equally prepared for Gram staining. Samples were also subjected to biochemical tests using Indole test, Methylred test, Voges- Proskauer Test, Oxidase test, Catalase Test, Coagulate Test, Citrate Utilization Test.

Microbiological tests like motility test, antibiotic culture sensitivity were carried out on the samples.

RESULTS

Bacteria Counts of raw meat Samples

Table .1 shows the bacterial counts of each sample. The total heterotropic bacteria counts of ranged from 3.72×10^5 cfu/g - 4.2×10^5 cfu/g, the coliform bacterial counts ranged from 1.9×10^5 cfu/g - 2.7×10^5 cfu/g while the total salmonella shigella count ranged from 0 cfu/g - 4.12×10^3 cfu/g.

Table .1: Bacteria counts of raw meat samples

| Sample | Total Viable Count (CFU/g) | | |
|--------|----------------------------|-------------------|--------------------|
| | THBC | TCC | TSSC |
| A | 4.2×10^5 | 2.7×10^5 | 0 |
| B | 3.7×10^5 | 2.7×10^5 | 4.12×10^3 |
| C | 3.8×10^5 | 1.9×10^5 | 0 |

Keys:

Sample A= Relief market; Sample B= Ekeonuwamarket; Sample C= Amakohiamarket; THBC = Total Heterotrophic Bacteria count

Morphological and Biochemical identification of bacteria isolates

Results from table .2 shows the morphological appearances and biochemical properties of isolated bacteria. Bacterial isolated were *Escherichia coli*, *Salmonellaspp.*, *Streptococcus spp.*, *Bacillus spp.* and *Staphylococcus aureus*

Table .2 Morphological and Biochemical identification of bacteria isolated from raw meat

| S/N | Morphology | Gram staining | Catalase | Citrate | Indole | Methyl red | Voges proskaeur | Oxidase | Motility | Suspected organism |
|-----|---|---------------|----------|---------|--------|------------|-----------------|---------|----------|---------------------------|
| 1 | Yellow, Glassy, Round, Cocci in cluster | + | + | + | - | + | + | + | - | <i>Staphylococcus spp</i> |
| 2 | Cream, Smooth, Short rod in single | - | + | - | - | + | - | - | - | <i>Escherichia coli</i> |
| 4 | Opaque translucent straight rod colony | - | + | - | - | + | - | - | + | <i>Salmonellaspp</i> |
| 4 | Creamy, Smooth, Irregular, Short rod colony | + | + | + | - | - | + | - | + | <i>Bacillus sp</i> |
| 5 | White raised grape-like colony | + | - | - | - | - | + | - | - | <i>Streptococcus spp</i> |

3.3 Prevalence of isolation of bacterial pathogens of subjects

Table .3 shows the prevalence of isolation of bacterial isolates from raw meat. Of the 50 bacterial isolates obtained, *E. coli* [15 (27.7)] and *Staphylococcus spp* [15 (27.7)] were the prevalent isolates. Other bacterial pathogens incriminated in this study were *Salmonella spp* [9(18.0)], *Bacillus spp* [7(14.0)], and *Streptococcus spp* [4 (8.0)].

Table .3 Prevalence of isolation of bacterial pathogens of subjects

| Isolates | Frequency (%) |
|---------------------------|----------------------|
| <i>Escherichia coli</i> | 15 (27.7) |
| <i>Staphylococcus spp</i> | 15 (27.7) |
| <i>Salmonella spp</i> | 9 (18.0) |
| <i>Bacillus spp</i> | 7 (14.0) |
| <i>Streptococcus spp</i> | 4 (8.0) |
| Total | 50 (100.0) |

Susceptibility of isolated Gram-negative and Gram-positive bacteria from raw meat

Table 4 shows the susceptibility of isolated Gram-negative and Gram-positive bacteria from raw meat. Most of the Gram-positive bacteria were highly susceptible to ceftizoxime and the Gram-negative to gentamicin and cefotaxime. *E. coli*, was susceptible to ampicillin/sulbactam (71% susceptibility), cefotaxime (71%), ciprofloxacin (94%), chloramphenicol (94%), ceftizoxime (100%), ofloxacin (82%), and amikacin (100%). *S. aureus* was susceptible to ampicillin/sulbactam (75%), cephalixin (75%), cefotaxime (100%), roxithromycin (75%), lincomycin (75%), and gentamicin (100%).

Table 4 Susceptibility of isolated Gram-negative and Gram-positive bacteria from raw meat

| Antibiotics | Susceptibility to antimicrobial drugs (%) | | | | | | | | | | |
|---------------------------|---|----|----|----|----|----|-----|----|----|-----|-----|
| Gram negativeAS organisms | BA | CF | PC | CH | CP | CI | TE | OF | GM | AK | PF |
| <i>E.coli</i> | 71 | 6 | 71 | 41 | 94 | 94 | 100 | 24 | 82 | 71 | 100 |
| <i>Slmonella spp</i> | 0 | 20 | 80 | 0 | 0 | 80 | 100 | 80 | 0 | 100 | 20 |

| | | | | | | | | | | | | |
|--------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| <i>Pseudomonas spp</i> | 50 | 100 | 100 | 100 | 0 | 50 | 0 | 0 | 50 | 100 | 0 | |
| Gram positive organisms | AS | BA | PR | TE | CF | CP | PF | OF | CX | RF | LM | GM |
| <i>Staphylococcus spp</i> | 0 | 75 | 0 | 100 | 13 | 0 | 0 | 50 | 75 | 75 | 100 | |
| <i>Streptococcus spp</i> | 100 | 0 | 50 | 50 | 100 | 0 | 0 | 0 | 50 | 50 | 50 | 100 |
| <i>Bacillus spp</i> | 50 | 0 | 100 | 100 | 100 | 0 | 50 | 0 | 0 | 50 | 100 | 0 |

Keys: AS = ampicillin/sulbactam (20 µg); BA = co-trimoxazole (25 µg); CF = cefotaxime (30 µg); PC = piperacillin (100 µg); CH = chloramphenicol (30 µg); CP = ciprofloxacin (5 µg); CI = ceftizoxime (30 µg); TE = tetracycline (30 µg); OF = ofloxacin (5 µg); GM = gentamicin (10 µg); AK = amikacin (30 µg); PF = pefloxacin (10 µg); PR = cephalixin (30 µg); CX = cloxacillin (1 µg); RF = roxithromycin (15 µg); LM = lincomycin (2 µg).

Discussion

The presence of microbial population in meat is a challenging problem to the meat industry (Kombaet *al.*, 2012). From this study, high bacteria counts were enumerated from fresh beef samples which indicated that the beef samples were contaminated. Probable sources of contaminations may include the cutting knives, containers, intestinal contents, water, hides, meat handlers, vehicle for transporting carcasses and the meat processing and selling environments. The study showed that beef sold was contaminated with various genera of bacteria with *Staphylococcus spp.* and *Escherichia coli* being the most abundant. The results of this study can be compared with similar studies in Ghana. Soyiriet *al.* (2008) found various levels and numbers of total bacteria count, *Streptococcus spp.*, *Staphylococcus spp.*, *Bacillus spp.* and *Escherichia coli* in beef sold in the Ashaiman Municipality of Ghana. Adziteyet *al.* (2011) also isolated bacteria species (*Escherichia coli*, *Salmonella spp.*,

Staphylococcus spp. and *Streptococcus spp.*) from raw beef sold in five most popular meat shops in the Tamale Metropolis of Ghana. Microbial contamination of beef samples have also been reported in other parts of the world. In Lahore Pakistan, Ahmad *et al.*, (2013) reported high microbial load of *E. coli*, *Staphylococcus aureus* and *Salmonella* in raw meat from abattoirs and retail shops.

Nevertheless the presence of *Salmonella* and *Escherichia coli* which are known foodborne pathogens give cause for public health concern (Soyiriet *al.*, 2008). For purposes of food safety, the Nigeria Standards Board requires that there should be no pathogen in all ready to eat foods but in this study *Staphylococcus spp.*, *Escherichia coli* and *Salmonella spp.* could not pass the test of a zero cfu/g which the Nigeria Standards Board sets for fresh beef (Soyiriet *al.*, 2008).

Conclusion

This study to assess the bacterial quality of fresh beef sold in Owerri has shown that beef sold in Owerri is contaminated with *Staphylococcus spp.*, *Escherichia coli* and *Salmonella spp.* but the prevalence of *Salmonella spp.* is low. The general insanitary conditions at the slaughterhouse, meat shops and poor hygienic practices of the butchers were major contributors to the microbial contamination of the beef. The presence of these microorganisms in the raw beef though not above the permissible limit (10^6 cfu/g) is an indication of public health hazard and gives a signal of a possible occurrence of food borne intoxication and infection if not controlled.

Recommendation

It is therefore recommended that fresh beef from the study area be thoroughly cooked before consumption to prevent food poisoning and

foodborne diseases. Standard hygienic practices such as HACCP system should be followed at all stages of the meat production chain. This requires training, education and supervision of meat handlers on the basic concepts of personal and general hygiene necessary to improve behavioural changes among butchers and ensuring a safe product to the consumer. Veterinary doctors should inspect the animals before and after slaughtering, before the meat is sold to the general public.

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