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 microbiologicalanalysis within some few hours of collection. The results of this study showed that the total heterotropic bacteria counts of ranged from 3.72×10^5cfu/g - 4.2×10^5cfu/g , the coliform bacterial counts ranged from 1.9×10^5cfu/g - 2.7×10^5cfu/g while the total salmonella shigella count ranged from 0cfu/g - $4.12x10^3$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*Staphylococcusspp*[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 *Streptococusspp*[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%), cephalexin (75%), cefotaxime (100%), roxithromycin (75%), lincomycin (75%), and gentamicin (100%~~). The~~). 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 abbatoirs is highly recommended.

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

Introduction

One excellent source of protein in human diet is meat (Komba*et 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 itsrich 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~~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).

S***almonella species***

Salmonella ~~are~~isnonspore-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~~,~~.t~~T~~he 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. Nutrientagar, MacConkey agar, Salmonella-shigella agar and Muller hinton agarwere used for the isolation of bacteria. Muller ~~hinton~~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~~was 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× 10^5cfu/g - 4.2 × 10^5cfu/g, thecoliform bacterial counts ranged from 1.9 × 10^5cfu/g - 2.7 × 10^5cfu/g while the total salmonella shigella countranged from 0cfu/g - 4.12x10^3cfu/g.

Table .1: Bacteria counts of raw meat samples

Sample	Total Viable Count (CFU/g)		
	THBC	**TCC**	**TSSC**
A	4.2x10^5	2.7x10^5	0
B	3.7x10^5	2.7x10^5	4.12x10^3
C	3.8x10^5	1.9x10^5	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	+	+	+	-	+	+	+	-	*Staphylococcus spp*
2	Cream, Smooth, Short rod in single	-	+	-	-	+	-	-	-	*Escherichia coli*
4	Opaque translucent straight rod colony	-	+	-	-	+	-	-	+	*Salmonellaspp*
4	Creamy, Smooth, Irregular, Short rod colony	+	+	+	-	-	+	-	+	*Bacillus sp*
5	White raised grape-like colony	+	-	-	-	-	+	-	-	*Streptococcus spp*

3.3 Prevelance of isolation of bacterial pathogens of subjects

Table .3 shows the prevelance 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 *Streptococussp p*[4 (8.0)].

Table .3 Prevelance of isolation of bacterial pathogens of subjects

Isolates	Frequency (%)
Escherichia coli	15 (27.7)
Staphylococcus spp	15 (27.7)
Salmonella spp	9 (18.0)
Bacillusspp	7 (14.0)
Streptococcus spp	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%), cephalexin (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**
E.coli	71	6	71	41	94	94	100	24	82	71	100
Slmonella spp	0	20	80	0	0	80	100	80	0	100	20

Pseudomonas spp	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**
Staphylococcus spp	75	0	75	0	100	13	0	0	50	75	75	100
Streptococcus spp	100	0	50	50	100	0	0	0	50	50	50	100
Bacillusspp	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 = cephalexin (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 (Komba*et 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. Soyiri*et 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. Adziteye*t 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~~has 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 (Soyiri*et 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 (Soyiri*et 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 (10^6cfu/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.

REFERENCES

Abaidoo R. C. and Obiri-Danso K. (2018). BIOL 503: Environmental Microbiology. KNUST, IDL (MSC Environmental Science): 3.

Aberle E. D., Forrest J. C., Gerrard D. E., and Mills E. W. (2011). Principles of meat science (4th edition). USA: Kendall/Hunt Publishing Company

Adzitey, F., Teye, G. A., Kutah, W. N. and Adday, S. (2011). Microbial quality of beef sold on selected markets in the Tamale Metropolis in the Northern Region of Ghana. Livestock Research for Rural Development; 23

Ahmad, M. U. D., A. Sarwar, M. I. Najeeb, M. Nawaz, A. A. Anjum, M. A. Ali and N. Mansur, (2013). Assessment of microbial load of raw meat at abattoirs and retail outlets. *The Journal of Animal and Plant Sciences*; 23(3): 2013, Page: 745-748

Australia Food Regulation Standing Committee (AFRSC), (2007). Australian standard for the hygienic production and transportation of meat and meat products for human consumption. AFRSC technical report No. 3 CSIRO publishing, Australia; 54-61.

Bell, R.G. (2017). Distribution and sources of microbial contamination on beef carcasses, *Journal of Applied Microbiology*; 88: 292-300

Burgess, F., Little, C., Allen, G., Williamson, K. and Mitchelli, R. T (2015). Prevalence of *Campylobacter*, *Salmonella*, and *Escherichia Coli* on the External Packaging of Raw Meat. *Journal of Food Protection*; 68(3): 469-75

Carrie, R. Daniel, Amanda J. Cross, Corinna Koebnick and Rashmi Sinha, (2011) Trends in meat consumption in the United States. Public Health Nutrition. *National institute of Health Journal*;575-583

Center for Disease Control and Prevention (CDC), (2014). An Atlas of Salmonella in the United States, 1968-2011

Collins, N. A. and Thato, S. (2011). Isolation of enteric bacterial pathogens from raw mince meat in Mafikeng, North-West Province, *South Africa Life Science Journal*;8(S2)

Dinh Tran Nhat Thu, (2016). Meat quality: understanding of meat tenderness and influence of fat content on meat flavor, University of Technology, VNU-HCM; 65-70

Doyle, M. P. (2017). Microbial Food Spoilage – Losses and Control Strategies, (A Brief Review of the Literature), Fri Briefings (www.wisc.edu/fri/).

Fabrega, A. and Vila, J. (2013). Salmonella enterica Serovar Typhimurium skills to succeed in the Host: Virulence and Regulation. *Clinical Microbiology Reviews*; 26 (2): 308–341.

FAO, (2011). Guidelines for slaughtering meat cutting and further processing; Techniques and hygiene practices in slaughtering and meat handling. Animal Production And Health Paper 91

Food and Agricultural Organization of the United Nations, (2012). Animal production and Health

Food Standard Agency (FSA), (2008). Meat Industry Guide (MIG): food hygiene and other regulations for the UK meat industry. Guidance to assist UK meat plant operators whose premises require approval and veterinary control under the European Union Food Hygiene Regulations; 2(14):1-15.

Forsythe, S. J. (2010). The microbiology of safe food. Oxford: Blackwell Science

Hayes, P. R. (2015). Food microbiology and hygiene. London: Elsevier Applied Science Publishers.

Hobbs, B. C. and Roberts, D. (13). Food poisoning and food hygiene. 6th Ed., St. Edmundsbury Press, Burry, Bodmin, Cornwall, London, UK. Pp. 216-220

Jay J. M. (2016). Modern food microbiology (5th Ed.). New York: Van Nostrand Reinhold

King L. K., Awumbila, B., Canacoo, E. A. and Ofosu-Amaah, S. (2010). An assessment of the safety of street foods in the Ga District of Ghana; implications for the spread of zoonoses. *Acta Tropical*; 76 (1): 39-43

Komba E. V. G., E. M. Mkupasi A. O., Mbyuzi, S. Mshamu, D. Luwumbra, Z. Busagwe and A. Mzula, (2012). Sanitary practices and occurrence of zoonotic conditions in cattle at slaughter in Morogoro Municipality, Tanzania: Implications for Public Health. *Tanzania Journal Health Research*; 14 (2): 2-6

Krebs-Smith, S. M. (2018). Progress in improving diet to reduce cancer risk. *Cancer*; 83: 1425–1432.

Lawrie, R. A. (2018). Lawrie‟s meat science (6th Ed.). Cambridge: Woodhead Publishing Limited.

McArdle, J. (2010). Humans are omnivorous. Vegetarian Resource Group.

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J. S., Shapiro, C., Griffin P. M. and Tauxe, R.V., (1999). Food-related illness and death in the United States. *Emergency Infectious Disease*; 5: 607-625.

Meat Technology Update (MTU), (2010). Sources of contamination on beef carcasses during dressing

Minnesota Department of Health (MDH) (2013). Fact sheet. Causes and symptoms of Staphylococcus aureus. Revised February.

Raloff, J. (2013). Food For Thought: Global Food Trends. Science News Online.

Sofos, J.N. (2018). Challenges to meat safety in the 21st century. *Meat Science*; 18: 3-13.

Soyiri, I.N., Agbogli, H. K. and Dongdem, J. T. (2008). A pilot microbial assessment of beef sold in the Ashaiman market, a suburb of Accra, Ghana. African Journal of Food Agriculture Nutrition and Development; 8(1): 91-103

Speedy, A.W. (2013). Global production and consumption of animal source foods. *Journal of Nutrition*;133:4048S–4053

Sprenger, R. A., (2015). Food for thought: Approaches for steering successful meat business into the next century. *Die Fleischere;i* (6) Vii-Xi.

Tomlins, K. (2012). Street food in Ghana: source of income, but not without its hazards. PH Action News, the Newsletter of the Global Post-Harvest Forum.

Tutenel, A.V., , D., Van Hoof, J., Cornelis, M. and De Zutter, L. (2013). Isolation and molecular characterization of *Escherichia coli* O157 isolated from cattle, pigs and chickens at slaughter. *International Journal of Food Microbiology*; 84(1): 63-9

USDA, (2009). Livestock and poultry; World markets and trade

Williams P. G. (2017). Nutrient composition of red meat. http:// ro. uow .edu .au / hbspapers/48.