IDENTIFICATION OF BACTERIA ASSOCIATED WITH FLESH COW MEAT IN ABAK URBAN AREA OF AKWA IBOM STATE, NIGERIA

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Abstract
The study was conducted to identify bacteria associated with flesh cow meat in Abak urban Area of Akwa Ibom State, Nigeria. A cross-sectional study was conducted where a simple random sampling method was used to select butcher shops, and the Abak urban Area abattoir was purposively selected. A total of 50 samples (20 swab samples from abattoir carcass, 10 samples of carcass washing water about 20ml of each, and 10 swab samples each from butcher shop cutting table and cutting knife, respectively) were collected during the study period. The collected samples were processed for anerobic plate count, and the total mean count was found to be 4.51 $\log_{10}$ cfu/cm^2 from abattoir carcass swab samples, 2.2 $\log_{10}$ cfu/ml from water samples, 6.67 $\log_{10}$ cfu/cm^2 from butcher shop cutting table, and 5.95 $\log_{10}$ cfu/cm^2 from cutting knife swab samples.*Brochothrix thermophacta* was dominant bacterial species isolated (35.2%), followed by *Pseudomonad* (18.2%) and *Enterobacterserratia* (10.2%). The study showed that the hygienic status of the abattoir and butcher shops in the Abak urban Area of Akwa Ibom State is poor, and the obtained results of bacterial load are higher than the acceptable limit of the standard. Therefore, the necessary strategies towards hygiene and sanitation of flesh low meat in Abak Urban Area of Akwa Ibom State should be implemented.

Keywords: Identification, Bacteria, Flesh cow meat, Microorganism and Hygienic status.

Introduction

Contamination of food by microbes is a general public health concern around Nigeria as a whole and Abak Urban area in particular. Episodes of food poisoning due to Microbial contamination have led to a large number of food recalls over the years, particularly of meat products that have caused outbreaks of dangerous illnesses.

The organisms spoiling meat may infect the animal either while still alive (endogenous disease) or may contaminate the meat after its slaughter (exogenous disease) (Lawrie and Ledward. 2006). There are numerous diseases that humans may contract from endogenously

infected meat, such as *Anthrax, Bovine tuberculosis, Brucellosis, Salmonellosis, Listeriosis, Trichinosis* or *Taeniasis* (Lawrie and Ledward, 2006; Norrung *et al;* 2009).

Infected meat, however, should be eliminated through systematic meat inspection in production. and consequently consumers will more often encounter meat exogenously spoiled by bacteria or fungi after the death of the animal (Lawrie and Ledward, 2006). One source of infections organisms is *bacteriaemia*, the presence of bacteria in the blood of slaughtered animals. The large intestine of animals contains some 3.3×10^{13} viable bacteria, which may infect the flesh after death if the carcass is improperly dressed (Lawrie and Ledward, 2006). Contamination can also occur at the slaughter house through the use of improperly cleaned slaughter or dressing implements, such as powered knives, on which bacteria persist. A captive bolt pistol's bolt alone may carry about 400,000 bacteria per square centimeter. After slaughter, care with any of the various sources of infection in the abattoir, notably the hides and soil adhering to them, water used for washing and cleaning, the dressing implements and the slaughter house personnel (Lawrie and Ledward, 2006).

Bacterial genera commonly infecting meat while it is being processed, cut, packaged, transported, sold and handled include Salmonella spp, *Shigella spp, E. coli, B. proteus, S. epidermidis and Staph. aureus, Cl. welchii, B. cereus and Faecal streptococci.* These bacteria are all commonly carried by humans, infected bacteria from the soil include Clostridium*botulinum*. Among the molds commonly infecting meat are *Penicillium, Mucor, Cladosporium, Alternaria, Sporotrichium and Thamnidium* (Lawrie and Ledward, 2006).

As these microorganisms colonize a piece of meat, they begin to break it down, leaving behind toxins that can cause enteritis or food poisoning, potentially lethal in the rare case of botulism. The microorganisms do not survive a thorough cooking of the meat, but several of their toxins and microbial spore do. The microbes may also infect the person eating the meat, although against this the microflora of the human gut is normally an effective barrier (Lawrie and Ledward, 2006).

The presence of infectious agents can be detected with a number of test during the production and processing of meat, but testing by itself is not enough to ensure adequate food safety. The industry-standard Hazard Analysis Critical Control Points (HACCP) System provides for a comprehensive quality management framework as a part of which such tests can be conducted. Testing methods applied include phage and serological typing, direct epiflourescence filter technique (DEFT) and plasmid profiling (Lawrie and Ledward, 2006; David *et al;* 2020).

The presence of bacteria on flesh cow meat can deteriorate the quality of the meat and also renders the meat unfit for healthy consumption. It can also reduced the quantity of protein production in the protein-based industry, thereby leading to low protein intake in the humans population. This research is therefore seeks to identify bacteria that is associated with flesh cow meat in Abak Urban Area.

Objectives of the Study

i. To determine the microbial load of flesh cow meat in Abak Urban Area.
ii. To isolate and characterize the bacteria associated with flesh cow meat in Abak Urban Area.

iii. To identify the bacteria obtained from flesh cow meat in Abak Urban Area.
iv. To recommend ways of handling and processing flesh cow meat to ensure their quality, safe and palatability.

Statement of the Problem

Pathogenic bacteria are bacteria that can cause disease. This article deals with flesh cow meat pathogenic bacteria. Although most bacteria are harmless or often beneficial, some are pathogenic with the number of species estimated as fewer than a hundred that are seen to cause infectious diseases. Human health is at high risk, with victims suffering from abdominal pain and diarrhea with more vomiting after consumption of cow meat that is infected with bacteria. If the infected individuals are not treated immediately may lead to death. This can threaten a whole society as there is only one abattoir servicing the whole Abak Local Government Area. This research work will go a long way in identifying the harmful bacteria associated with flesh cow meat and their preventive measures.

Significance of the Study

The significance of this study is to enable the producers of flesh cow meat especially in Abak Urban area to improve the hygiene conditions of the flesh cow meat and to create awareness on the methods of preserving the flesh cow meat, service various pathogenic bacteria and infections are transmitted through food (flesh cow meat), as a result, the prevention of contamination of flesh cow meat will be eradicated or reduced. The government will benefit maximally while relying on the outcome of this study in its bid to ensure health and safety of the indigenes thereby preserving the citizens.

Materials and Methods

Materials

The materials that were used include flesh cow meat, weighing balance, syringe, wire loop, cotton wool, Aluminium foil, Cover slip, Hand glove, Refrigerator, Autoclave, Microscope, Maccarny bottle, incubator, test tubes, blender, Masking tape, unused polythene bags, reagent or culture media.

Study Design and Target Groups

A cross-sectional study design was employed, whereby a simple random sampling of butcher shop was carried out and the Abak abattoir which was the source of meat to the butcher houses of Abak was purposefully selected. Only cow were slaughtered at the Abak abattoir. Meat surface swab samples, water from abattoir and equipment's (cutting tables and knife) swab samples from butcher slops were collected aseptically, processed and analyzed *bacteriologically*.

Sample Collection

A total of 50 samples were collected aseptically from abattoir and butcher houses. Sterile cotton tipped swabs soaked into buffered pepton water were used for swabbing in a template of 5cm x 10cm area of carcasses, cutting table and knife as described in Government Food Authority, Environmental Swabbing (2013) and SOP (2008) respectively.

The samples were properly labelled, kept in refrigerator and transported to laboratory, for bacteriological analysis.

Isolation and Identification of Bacteria

Bacterial isolation was performed using nutrient agar 2.8g/100ml, MacConkey agar 5.2g/100ml, and Dexycholate citrate agar 4.3g/100ml respectively. All the media used in the present study were prepared according to the manufacturer's specification and collected samples were inoculated into plates and incubated at 37°C for 24 to 48 hours (Quinn *et al;* 2004, Scott, 2011).

Colonies identified as discrete on nutrient agar were carefully examined microscopically (using stereo microscope) for cultural characteristics such as the shape, colour, size and consistency. Gram staining as well as appropriate biochemical tests was carried out according to the standard procedure (Oyeleke and Manga, 2008). The isolates were identified by comparing their morphological and biochemical characteristics with standard reference organisms of known taxa as described in Bergey's Manual for Determinative Bacteriology (Bichanan and Gibbons, 1984).

Data Analysis

A database was developed to store qualitative and quantitative data from the cross-sectioned study using Microsoft Excel 2010 spread sheet. STATA version 11 was used to compute descriptive statistics of variables collected during the study. Overall bacterial load was calculated using descriptive statistics of the sample through frequencies and cross tubulations.

Results and Discussion

Aerobic Plate Count

Twenty - swab samples from abattoir carcass, ten swab samples each from cutting knife and cutting tables and ten samples of carcass washing water were taken and analyzed as indicated in Table 1 and 2. the total mean bacterial count $\log_{10}$cfu/cm^2 was found to be 4.51 and 6.36 from Abak Urban Area abattoir and butcher house swab samples, respectively. The total aerobic plate converts (cfu/ml) from water samples were 2.1 x10^2 in round 1, 1.7 x 10^2 in round 2, 3.1 x 10^2 in round 3, and 2.5 x 10^2 in round 4 and the total mean aerobic plate count of water sample accounted 2.2 $\log_{10}$ cfu/ml. The mean bacterial count (log cfu/cm^2) was found to be 5.95 and 6.67 for the samples from cutting knife and cutting table respectively. The aerobic plate count showed with 4.2 and 8.45 $\log_{10}$ cfu/cm^2 minimum and maximum bacterial load respectively.

Table 1: Aerobic Plate counts from abattoir in (log cfu/cm^2)

Round of sample collection	No of observation	Mean ($\bar{x}$) (log_{10} cfu/cm^2	Standard deviation(SD)	Minimum Count (log_{10} cfu/cm^2	Max count (log_{10} cfu/cm^2
R1	5	4.40	0.61	3.6	5.48
R2	5	4.66	0.96	3.3	6.26
R3	5	4.37	0.66	3.5	5.36
R4	5	4.62	0.67	3.46	5.47
Average	-	4.51	0.73	-	-

Table 2: Aerobic Plate counts (APC) from cutting knife and cutting table of butcher shops in log_{10} cfu/cm^2

Code of butcher house	$APCCT_1$	$APCCK_1$	$APCCT_2$	$APCCK_2$	$APCCT_3$	$APCCK_3$
A	6.47	5.6	7.49	5.3	7.17	6.25
B	6.56	6.1	7.75	6.18	6.6	7.5
C	6.68	5.6	5.68	6.14	7.2	6.4
D	5.47	6.3	6.6	5.3	6.56	5.6
E	5.8	5.6	6.38	6.5	7.63	4.9

APCCT = Aerobic Plate Count from Cutting Table in three round (1,2 and 3).

APCCK = Aerobic Plate Count from Cutting Knife in three round (1, 2 and 3)

Bacterial Isolation

- Bacterial contaminants found in the meat samples were *Brochothrix thermophaeta, Pseudomonad, Pantoea, Serratia, Alcaligenes, Enterobacterserratia and Movaxella species. Brochothrix thermophaeta* was the dominant isolate (35.2%), followed by *Pseudomonad* (18.2%).

Table 3: Isolated bacteria from collected samples

Isolated bacteria	Abattoir Carcass (%)	Cutting table (%)	Cutting Knife (%)	Water (%)	Total (%)
Brochothrix thermophaeta	3(27)	5(35)	6(28)	2(10)	20(35.2)
Pseudomonad	3(28.5)	3(21.5)	5(37)	2(12.5)	11(18.2)
Pantoea	1(30)	4(46.9)	2(23.1)	0	7(9.9)
Serratia	1(30)	2(70)	0	0	3(3.8)
Alcaligenes	2(28.3)	2(28.7)	3(43)	0	7(12.8)
Enterobacter serratia	1(30)	4.(47.1)	2(22.9)	0	9(10.2)
Moraxella	1(27)	3(29.2)	2(28.6)	1(15.2)	7(9.9)

Discussion

Abattoir is one of the food industries that contribute to the problem of possible food-borne diseases and health hazards associated with food unless the principles of food borne hygiene practices are implemented (Roberts *et al;* 2009). The current study showed that there was no clear division of slaughtering process: stunning, bleeding, skinning, evisceration, hanging and cutting/deboning. Furthermore, there was no preventive mechanism installed for insects and rodents in Abak urban area Abattoir which is similar with report in (Haileselassic *et al;* 2013).

The aerobic plate count (APC) is used as an indicator of the level of bacteria in meat and is a useful tool in monitoring food safety. To prevent the occurrence of food-borne illness and possible meat spillage. it is important to ensure that foods sold are safe, wholesome, and in good hygienic condition. In this study, 36.4% of the abattoir carcass swab samples were found exceeding the limit (10^5cfu cm^{-2} or 5.0 $\log_{10}$ cfu/cm^{-2}) of total plate count on meat set by WHO (2007). If the bacterial count exceeds the above standard in flesh meat, then the meat is not acceptable and this indirect alarm signals on meat hygiene along meat chain from abattoir to butcher shops. The total mean value in the present study of abattoir carcass swab sample was 4.51 ± 0.74 $\log_{10}$ cfu/cm^2, similar value has been reported by (Siham and Taha, 2009)

from Algeria and (Haileselassic *et al;* 2013) Mekelle abattoir in Ethiopia, which had a mean value of 4.48 ± 0.63 $\log_{10}$ cfu/cm^2 and 5.04 $\log_{10}$ cfu/cm^2, respectively. However, the result of the present study is lower than 5.80 ± 0.17 $\log_{10}$ cfu/cm^2 reported in (Sudhakar *et al;* 2009) at Mumbai abattoir in India. The difference could be due to the hygienic condition perform by workers in the abattoir and it may also be due to countries, states and local government areas differences.

The water used in slaughter house can also contaminate the meat during washing. The water used for cleaning procedures and meat processing in the abattoir must meet drinking water standard (Adebowale, 2010). For this reason an adequate supply of Potable water should be available to meet operational and clean up needs and it should be analyzed frequently to confirm its quality (Canadian Food Inspection Agency, 2010). The total mean value of examined water samples during the study was 2.2 $\log_{10}$ cfu/ml. The present finding is higher than the report of Tarwate *et al;* 1993, who reported a mean value of 2.1 $\log_{10}$ cfu/ml from water in abattoir. However, it is lower than the report of Pius (2013) who had reported a mean value of 4.3$\log_{10}$ cfu/ml in Ibadan. The variability of the results could be duc to the quality of water used in the abattoir for washing carcass and regular monitoring and follow-up.

The high microbial load on the knife and cutting table is an indication of inadequate cleaning. Usually in the study area, knives are washed only with water and there is poor sterilization and continuous use of a single knife despite contact with dirty or contaminated surfaces. The presence the bacterial pathogens in meat. Contact surfaces may contribute to the contamination of meat (Endale and Hailay, 2013). In a similar war, the present study revealed the total mean of APC from butcher house equipment (cutting knife and cutting table). This result is similar with the value obtained from knife in (Pius, 2013) of 6.16 + 1.25$\log_{10}$ cfu/cm^2 in Tanzania. The result of the cutting table is in agreement with findings in (Endale and Hailay, 2013). However, the highest bacterial load, 8.5$\log_{10}$ cfu/cm^2, from the cutting table of butcher shop was reported from the study conducted in Pakistan (Ali *et al;* 2010). The variations of bacterial load observed in different studies might be due to lack of good processing and handling practices and sanitary standard operating procedures of meat along the meat production chain (Pius, 2013).

Among isolated bacteria, *Brochothrix thermophaeta* was the predominant organism followed by *Pseudomonad* and *Serratia* species with minimum load from objectively isolated and identified bacteria in this study. The higher rate of contamination of flesh cow meat with these organisms is an indication of deplorable state of poor hygienic and sanitary practices employed night from the slaughtering house, transportation to butcher shops and processing at the butcher shops (Haileselassic *et al;* 2013).

Conclusion and Recommendations

The results obtained from this study showed that there was high microbial logarithmic mean values (aerobic plate counts) from the samples tested are an indication of poor meat quality, making it a potential sources of food-borne infection caused by *Brochothrix thermophaeta, Pseudomonad and Enterobacterserretia* species and food spoilage. This was due to many factors such as the low level of sophistication, poor hygienic and sanitation procedures conducted at the Abak Urban area abattoir and butcher shops.

From the results. it can be figured out that contamination was present right from the abattoir to the butcher shops where the meat produced in the study site is contaminated before it gets into the hands of consumers. Therefore, it is importance to create awareness about hygiene and sanitation of meat both in abattoir and butcher shop, and appropriate control method of the problems should be designed and implemented. More, further investigation should be carried out to isolate and characterize the bacterial load of flesh cow meat in different study areas in Nigeria.

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