INVESTIGATION ON THE MICROBIOLOGICAL QUALITY OF RAW MEATS SOLD IN SOME PARTS OF IBADAN METROPOLIS, NIGERIA

Folake T. Afolabi[*1], Adetutu R. Arowosebe[2], Stella M. Adeyemo[3]
[1,2*]Department of Microbiology, University of Ibadan, Nigeria.
[3]Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.
*E-mail: folakeojo1@yahoo.com

Abstract
This study was carried out to assess the microbiological quality of fresh raw meats sold at butcher open shops in Ibadan metropolis, Oyo State, Nigeria. 16 samples of pork, goat, beef and chicken meats were obtained from 4 market locations and analyzed using standard microbiological techniques. The total viable count, total staphylococcal count, total enteric count, and total fungal count ranged from 1.0 x10^6 to 4.6 x 10^8, 5.0 x 10^4 to 1.0 x 10^7, 1.0 x 10^3 to 1.96 x 10^8, and 6.0 x 10^3 to 5.0 x 10^5 respectively. The percentage of microbial genera isolated from the meat samples were; Salmonella (7.45%), Shigella (6.21%), Staphylococcus (10.56), Micrococcus (17.39%), Enterococcus (1.24%), Streptococcus (4.35%), Yersinia (0.62%), Proteus (16.15%), Escherichia (7.45%), Paracolons (2.49%), yeast (9.94%), Klebsiella (5.59%), Enterobacter (1.86%), and Lactic acid bacteria (8.70%). 2.49% of the isolates obtained from goat meat showed heamolytic positive while 1.24%, 2.49%, and 0.62% of the isolates obtained from chicken, beef, and pig respectively, were able to lyse blood cells. This study confirmed the presence of probable pathogenic organisms in the raw meats sold in some parts of Ibadan metropolis. It significantly points to the great need to evaluate and monitor the occurrence rate of pathogenic organisms in livestock sold in Nigeria. There is also the need for proper and adequate cooking of food of animal origin prior consumption.

Keywords: raw meat; isolates; pathogenic; pork; goat; beef; chicken

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1. INTRODUCTION

Raw meat is defined as any type of uncooked muscle tissue of an animal that is used for food. In the meat production industry, the term "meat" refers to mammalian flesh, while the word "poultry" and "seafood" are used for differentiating between the tissue of birds and aquatic creatures (Duffy, 2006).

Meat is known for its nutritive values, that is why it is being consumed by many people worldwide. Eaton and Konner (1985) in their work reported that meat and its products contribute about "a third" of the energy that humans need.

The protein profile of meat contains amino acids that and it has been described as excellent because of the presence of the essential ones needed by the body. It has also been proved that proteins and vitamins (A and B_{12}) in meat can not be substituted by plant sources which further justified the nutritive importance of meat (Bradeeba and Sivakumaar, 2012).

Notwithstanding the major role meat play in our meals, it can also serve as a rich medium of growth for harmful microorganisms. Meat infected with microorganisms is the cause of many food-borne diseases (WHO, 1997). The source of these pathogenic microorganisms may be the animals themselves or from outside. The surroundings where these animals are kept as well as the way they are processed after slaughtering can also result in contamination with microorganisms (Adeyemo, 2002). Meat infected with microorganisms is normally poor in quality (Mukhopadhyay *et al*., 2009).

Raw meat is a medium for bacteria growth; this is as a result of its high moisture contents. It is rich in protein, fermentable carbohydrate, favorable pH and other growth factors (Magnus, 1981).Spoilage of meat can occur if the meat is not treated within hours or days and this makes the meat to become unappetizing, poisonous or infectious. Spoilage of meat can occur if the meat is not treated within some hours or days and this result in the meat becoming unappetizing, poisonous or

infectious. Spoilage is caused by infection and decomposition of meat by microorganisms that are borne by the animal itself, through the handlers, equipments and the environment in which the animals are being rared. Meat can be kept edible for some time if proper hygiene is maintained during production and processing and also if appropriate food safety, food preservation and food storage procedures are put in place. (Lawrie and Ledward, 2006). Microbial contamination of the sterile muscles of healthy animals during slaughter, fabrication, processing and handling also occurs as a result of contamination from sources which include; air, water, faeces, soil, feed, hides, lymph nodes, intestines, processing equipments_ (Pietzsch and Kawerau, 1984; Bell, 1997).

Meat is not only susceptible to spoilage, but it is also frequently implicated in the spread of food-borne illness, various biochemical changes and microorganisms are associated with meat, during the process of slaughter, processing and preservation (Olaoye and Onilude, 2010). The rich source of nutrients of meat provides both pathogenic and non-pathogenic microbes a suitable environment for growth (Steinkraus, 1994). The widespread distribution of meat products makes the consequences of contamination with food poisoning microorganisms a problem (Macrae *et al*., 1993). Most food-borne related illnesses are attributed to one of five major groups of pathogenic bacteria. These five groups are; *Salmonella, Shigella, Clostridium botulinum, Clostridium perfringens, Bacillus cereus,* and *Staphylococcus aureus*. There are other pathogens such as; *Yersinia enterocolitica, Escherichia coli, Listeria monocytogenes,* and *Campylobacter jejuni* that have been added to the lists. *Salmonella* infections in humans often result from the ingestion of contaminated foods, such as poultry, beef, pork, eggs, milk, seafood, and flesh produce. It is known that retail shop meat also contain higher microbial load because of the large amount of surface area exposure, readily available water, nutrient and greater oxygen penetration (Forest *et al.*, 1985). There are considerable human health consequences with foodborne infections ranging from protracted illness to death and patients with impaired immunity are at greater risk. Thus, this study aimed at ascertaining the current prevalence rate of pathogenic isolates from meat samples sold in Ibadan metropolis, Oyo State, Nigeria. This work will also aid in mapping out preventive strategies against some microbial infections.

2. MATERIALS AND METHODS

Sample sites and collection

The samples collected were fresh raw meats which included; pork, goat, beef, and chicken meats. The samples were collected from different butcher open shops at 4 different market locations in Ibadan metropolis, Oyo State, Nigeria. The market locations are; Bodija, Dugbe-Mokola, Aleshinloye, and Oje-Bode, and these market location collection points are used to group the raw meats collected.

A total of 16 fresh raw meats samples were collected from the 4 market locations. Meat samples were obtained as offered to the consumer and taken to the laboratory of the Microbiology Department in University of Ibadan for microbial analyses.

Isolation procedure

Isolation of the microorganisms was done according to the modified method of Harrigan and McCance (1976), where 10g of the meat sample were weighed and then shredded by hand covered with sterile hand gloves and added into a 90ml sterile distilled water, which was then homogenized by hand shaking thoroughly. It was serially diluted using serial dilution technique and the different diluents factor were plated out on different media, which were then incubated at their appropriate temperature.

Characterization of bacterial isolates

The bacterial test organisms were checked for purity, subjected to various morphological, biochemical and sugar fermentation tests to confirm their probable identity. The results

were recorded and their probable identities were confirmed with reference to Bergey's Manual of Determinative Bacteriology (Deming and Junge, 2001).

Pathogenicity testing of the organisms

DNase and blood heamolysis tests were done to check for the pathogenicity strength of the isolates. For DNase test, the isolates were cultured on a DNase agar plate and incubated for 24hours after which it was flooded with conc. HCL. A clear zone around the inoculum indicates that the isolate is DNase positive while for blood heamolysis testing, nutrient agar medium was sterilized and cooled to 45^0C, and then 5% human blood was added and mixed thoroughly. It was then poured into petri dish and allowed to solidify and dried. An overnight growth isolate was then streaked on the dried blood agar plate and incubated for 24hours. The result was recorded based on partial, complete, or no haemolysis (Olutiola *et al*., 1991).

Proximate analyses of the meat samples

Samples were analyzed chemically according to the Official Methods of Analysis described by the Association of Official Analytical Chemist (A.O.A.C., 2005). All analyses were carried out in triplicate.

3. RESULTS AND DISCUSSION

Frequency of occurrence

A total of 161 isolates covering about 12 genera were isolated from the meat samples sold in Ibadan metropolis, and were characterized as *Salmonella*, *Shigella*, *Staphylococcus*, *Micrococcus*, *Enterococcus*, *Streptococcus*, *Yersinia*, *Proteus*, *Escherichia*, *Paracolon*, *Klebsiella* and *Enterobacter*. The highest predominant species is *Micrococcus* followed by *Proteus* sp., while *Escherichia* sp. and *Salmonella* sp. have the same percentage of occurrence of 7.45%. Table 1 shows the percentage of occurrence of the isolates across the four locations.

Table 1. Frequency of occurrence of the isolates obtained from the raw meats from the four locations

Isolates	No (%) of occurrence	Bodija	Dugbe	Aleshinloye	Oje
Staphylococcus aureus	1 (0.62)	1	-	-	-
Micrococcus luteus	20 (12.42)	6	3	6	5
Enterococcus faecalis	2 (1.24)	2	-	-	-
Streptococcus agalactiae	2 (1.24)	2	-	-	-
Streptococcus pyogenes	1 (0.62)	1	-	-	-
Micrococcus varians	8 (4.97)	1	1	4	2
Staphylococcus saprophyticus	9 (5.59)	-	4	3	2
Staphylococcus epidermidis	7 (4.35)	-	2	1	4
Streptococcus mitis	4 (2.49)	-	2	2	-
Yersinia kristensenii	1 (0.62)	1	-	-	-
Salmonella typhi	3 (1.86)	3	-	-	-
Shigella flexnerii	1 (0.62)	1	-	-	-
Proteus mirabilis	16 (9.94)	4	3	3	6
Escherichia coli	8 (4.97)	2	3	2	1
Proteus vulgaris	8 (4.97)	1	6	1	-
Salmonella paratyphi	9 (5.59)	2	6	-	1
Proteus rettgeri	1 (0.62)	1	-	-	-
Paracolon spp.	4 (2.49)	4	-	-	-
Yeast	16 (9.94)	4	10	-	2
Shigella boydii	7 (4.35)	-	2	3	2
Escherichia freundii	4 (2.49)	-	4	-	-
Klebsiella pneumoniae	9 (5.59)	-	1	6	2
Shigella sonnei	1 (0.62)	-	-	1	-
Enterobacter intermedius	3 (1.86)	-	-	3	-
Lactic acid bacteria	14 (8.70)	-	-	7	7
Shigella dysenteriae	1 (0.62)	-	-	-	1
Proteus penneri	1 (0.62)	-	-	-	1

Micrococcus luteus has the highest percentage (12.42%) of occurrence which is followed by *Proteus mirabilis* (9.94%), while *Staphylococcus aureus, Streptococcus pyogenes, Yersinia kristensenii, Shigella flexnerii, Proteus rettgeri, Shigella sonnei, Shigella dysenteriae* and *Proteus penneri* has the lowest percentage of occurrence of 0.62%.

Isolation of microorganisms

The prevalence of *Salmonella* species in this study confirmed the microbial analysis of meat samples done by Garedew *et al.* (2015), where they also observed the same rate of prevalence. Their study confirmed a high isolation rate of *Salmonella* from meat samples that were analysed when compared with other samples they analysed in their study. Their study also confirmed that the *Salmonella* obtained from hand swabs resulted from workers in the butcher shops that did not develop the habit of regular hand washing after using the toilet, after touching dirty surfaces and before handling meat and equipment. Thus, the contamination of these meat samples with *Salmonella* species can come from the poor hygienic habit of the personnel handling the meat. The extent of contamination of retain-beef and related meat products with *Salmonella* sp. in Zaria, Nigeria, has also been evaluated by Tafida *et al*. (2013), who tested 435 retailed beef and related meat products for the presence of *Salmonella* species. Ten of the samples tested for *Salmonella* isolates were found to be raw beef samples having the highest isolation rate.

The presence of *Escherichia coli* in this study could be due as a result of handling processing such as killing equipment and the water used in washing the carcass. *E. coli* is commonly used as an indicator, the presence of *E coli* in food indicate direct and indirect faecal contamination (Clarence *et al*., 2009). During the past two decades, severe outbreaks of gastrointestinal illness have occurred due to food borne pathogenic *E. coli*, especially 0157:H7 strain (Armstrong *et al.* 1996). Cattle are reported as the primary reservoir of *E. coli* 0157:H7, however, the organism has also been isolated from domestic animals such as chicken, pig, sheep and goat (Aibinu *et al*., 2007; Ojo *et al*., 2009). In Southwestern Nigeria, Ojo *et al*., (2009) isolated *E. coli* 0157:H7 strains in the feaces of sheep, goats, pigs and cattle sampled from farms, markets and abattoirs. Aibinu *et al*, (2007) also isolated *E. coli* O157 from cattle, pig, chicken sheep and humans in Lagos and Ogun States in Nigeria.

Enterococcus feacalis and *Enterobacter* sp. seen in this study were also reported by Sharma and Chattopadhyay (2015) as present in 90% occurrence of the raw meat samples sold in Kolkata, India. However, this high level of their occurrence is contrary to the findings observed in this study. Study by Tanimoto *et al*. (2005) reported the prevalence of *E. feacalis* in raw chicken meat sold in Japan, and isolation of this organism was also reported by Simjee *et al*. (2002). *Enterococcus* sp. has been known to be contaminant of various foods, especially those of animal origin. Despite the fact that foods containing *Enterococci* have a long history of safe use, these organisms are not considered as Generally Regarded as Safe (GRAS) organisms. This *Enterococcus* sp. has also been reported by Hayes *et al.* (2003), who found the species to be the most dominant bacteria on 971 of the 981 samples (99%) of all the meat samples obtained from the state of Lowa. This is, however contradicting to the percentage of occurrence of this species in this study. The occurrence of such bacterial isolate on meat samples could be determined by the storage and handling conditions adopted. Cerveny *et al*. (2009) stated that storage conditions affect the type of microbes found in meat and meat products.

The high predominance of Gram-negative bacteria observed in this study has also been reported by other researchers. Clarence *et al*. (2009) reported that 69% of cases of bacterial food borne diseases are caused by Gram-negative bacteria. The prevalence of *Salmonella* and *Shigella* species from the raw meat in this study is in contrast to the report by Omorodion and Odu (2014) where no *Salmonella* and *Shigella* were reported in the meat samples sold in Port Harcourt, Nigeria.

The presence of *Salmonella* sp., *Proteus mirabilis*, *Staphylococcus aureus* and *Streptococcus pyogenes* has been reported by Okonko *et al*. (2010) to be isolated from poultry feeds sold in Nigeria. The presence of these microorganisms in the animal feeds can serve as a source of contamination to the animal that ingested them during feeding, which could be one of the reasons why these isolates were seen in the raw meats.

In this study, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Yersinia kristensenii*, *Shigella flexnerii*, *Proteus rettgeri*, *Shigella sonnei*, *Shigella dysenteriae*, and *Proteus penneri* have the lowest percentage of occurrence of 0.62%, while *Micrococcus luteus* showed the highest percentage of occurrence of 12.42% followed by *Proteus mirabilis* (9.94%). Turtura (1991) reported that the most frequent coliform identified on meat include *E. coli*, and less frequent strains are the genera of *Klebsiella*, *Shigella* and *Proteus*. This is in consonant with the findings in this study, although *E. coli* in this study had a low percentage of occurrence of 4.97%. However, this low occurrence of *E. coli* in this study may be as a result of the culture medium used for its isolation from the raw meats.

Total Viable Count

The degree of microbial distribution was high in the meat samples. Result showed that the total viable count, total staphylococcal count, total enteric count, and total fungal count range from 1.0 x10^6 to 4.6 x 10^8 CFU/g, 5.0 x 10^4 to 1.0 x 10^7 CFU/g, 1.0 x 10^3 to 1.96 x 10^8 CFU/g, and 6.0 x 10^3 to 5.0 x 10^5 CFU/g respectively. This result is seen in table 2. This agrees with the study of top animal feeds done by Okonko *et al*. (2010), where the microbial load is more than 10^8 CFU/g. Similar total bacterial count ranging from 1.0 x 10^7 to 9.4 x 10^7 (CFU/g) was also reported in a study on commercially produced yoghurt by Oyeleke (2009). This current finding of high microbial load seen in the raw meats was also in agreement with that of Fasanmi *et al*. (2010) who reported the presence of high mean values of microbial load of table scrapings from meat stalls in Ibadan metropolis, Nigeria. A high mean value of microbial load (5.5 x 10^7 CFU/g) found in hanging meat and 6.5 x 10^7CFU/g in the minced meat was also reported by Tesfay *et al*. (2014).

Table 2. Total microbial count of the of the meat samples (CFU/g)

Samples	Location	TVC	TSC	TEC	TFC
Chicken	Bodija	1.0 x 10^6	2.4 x 10^5	-	-
	Dugbe	1.0 x 10^7	5.0 x 10^4	5.4 x 10^5	-
	Aleshinloye	1.3 x 10^7	1.9 x 10^6	3.5 x 10^4	4.0 x 10^5
	Oje	4.4 x 10^6	2.0 x 10^5	1.0 x 10^3	1.3 x 10^5
Goat	Bodija	4.6 x 10^8	3.0 x 10^6	1.9 x 10^8	-
	Dugbe	2.2 x 10^8	5.0 x 10^6	3.0 x 10^7	6.0 x 10^4
	Aleshinloye	3.0 x 10^4	2.4 x 10^6	2.9 x 10^7	2.0 x 10^3
	Oje	1.4 x 10^6	2.8 x 10^6	1.0 x 10^5	4.0 x 10^5
Beef	Bodija	2.2 x 10^8	9.2 x 10^5	3.5 x 10^5	-
	Aleshinloye	3.0 x 10^7	4.8 x 10^6	3.0 x 10^5	4.0 x 10^4
	Dugbe	1.3 x 10^7	1.1 x 10^6	1.3 x 10^7	8.0 x 10^3
	Oje	1.2 x 10^7	2.5 x 10^6	-	6.0 x 10^3
Pork	Bodija	1.4 x 10^8	6.9 x 10^5	3.2 x 10^7	-
	Aleshinloye	1.8 x 10^8	1.0 x 10^7	2.0 x 10^6	1.0 x 10^4
	Dugbe	3.4 x 10^7	2.3 x 10^6	4.0 x 10^5	4.0 x 10^5
	Oje	4.8 x 10^7	1.0 x 10^6	1.0 x 10^5	5.0 x 10^5

TVC; Total viable count
TSC; Total Staphylococcal count
TEC; Total enteric count
TFC; Total fungal count
(-); No growth

The high contamination of this meat may be due to high exposure to dusts from the environment. The high microbial load of enteric and staphylococcal bacteria observed in this study could be as a result of poor hygiene practice by the workers during the meat processing and selling (Garedew *et al*., 2015). The high level of total viable count is more than the acceptable level of 10^5 CFU/g count recommended by Meat Standard Committee (2002) in the microbiological testing for process monitoring in the meat industry. Thus, it has reached the marginal level where control and critical action is required.

The microbial load of pork, chicken and beef sold in Port- Harcourt metropolis, Nigeria, as reported by Omorodion and Odu (2014) was found to range from 8.6 x 10^5 to 2.6 x 10^6 CFU/g. This result is lower than the result of total viable count observed in this study, but their fungal count is closely related to the one in this study.

Pathogenicity testing

The pathogenicity of some of the isolates was done to check for potential virulent organisms. 2.49% of the isolates obtained from goat meat showed heamolytic positive, while 1.24%, 2.49%, and 0.62% of the isolates obtained from chicken, beef, and pig respectively, were able to lyse blood cells. These groups of organisms, which mostly are from the genera of *Staphylococcus, Streptococcus, Shigella,* and *Salmonella,* have been reported by many researchers to be virulent. Thus, their presence in these meat samples pose serious health threat to the consumers.

Plasmid profiling

Ten of the isolates were chosen across the four locations for plasmid profiling analysis, and the result as seen in plate 1 shows that four out of them were found to contain plasmids of different numbers and sizes. *Salmonella paratyphi* contains one plasmid of about 15.846kbp molecular weight, *Shigella dysenteriae* contains one plasmid of about 16.394kbp, *Shigella sonnei* contains also one plasmid of about 15.846kbp, and *Staphylococcus saprophyticus* contains two plasmids of about 16.394kbp and 10.184kbp. These high molecular weights of the plasmids found in these isolates may have contributed to the virulent capacity of the organisms, as plasmids have been known to carry virulent genes.

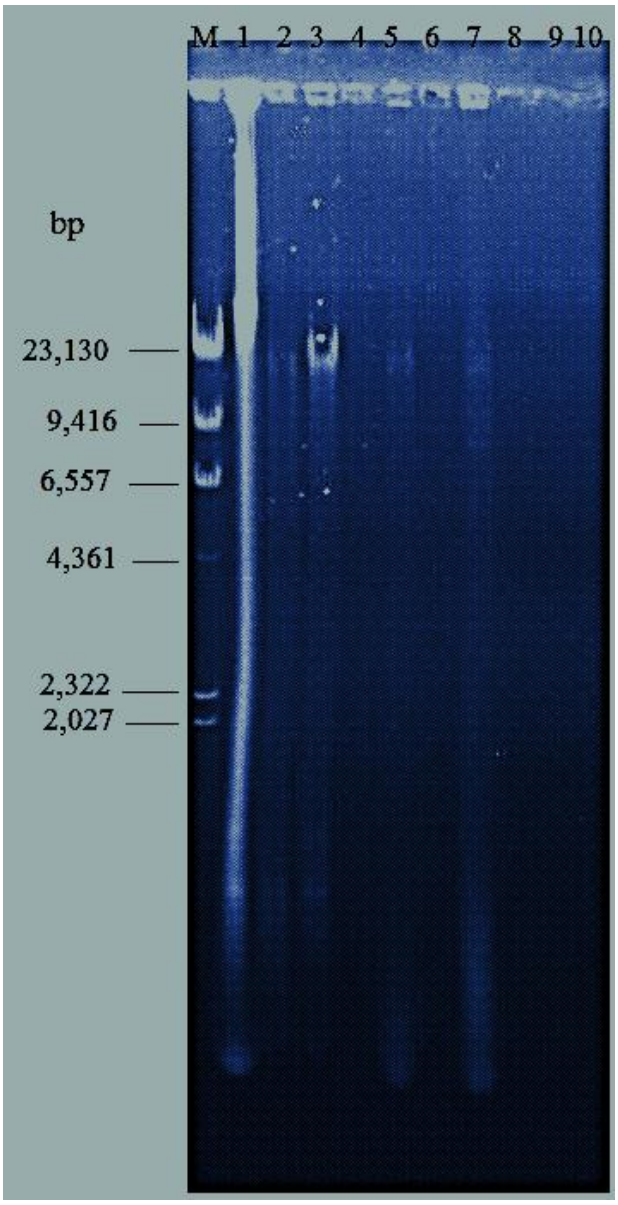

Plate 1. Plasmid profiling of the selected isolates
Keys; M=DNA Marker, 1. *Paracolon* sp. 2. *Salmonella paratyphi* 3. *Shigella dysenteriae* 4. *Proteus penneri* 5. *Shigella sonnei* 6. *Enterococcus feacalis* 7. *Staphylococcus saprophyticus* 8. *Micrococcus luteus* 9. *Micrococcus luteus* 10.*Staphylococcus saprophyticus*

Table 3. Proximate analyses (%) of the meat samples

Samples	pH	Percentage (%) Moisture Content	Crude protein	Crude fat	Crude fibre	Ash
Chicken	*6.43	68.86±0.04	24.96±0.02	4.82±0.04	0±0.0	1.36±0.01
Goat	6.56	67.4±0.03	20.14±0.01	11.22±0.02	0±0.0	1.24±0.01
Beef	5.85	72.19±0.03	18.81±0.04	7.71±0.01	0±0.0	1.29±0.01
Pork	6.63	46.24±0.04	16.57±0.01	36.87±0.04	0±0.0	0.32±0.02

* Values are means of triplicates

Proximate analyses of the meat samples

The proximate analysis of the four meat samples as seen in table 3, revealed that beef contained the highest moisture content (72.19%), followed by chicken (68.86%), which also has the highest crude protein content (24.96%) and Ash content (1.36%). Pork contained the lowest moisture content (46.24%), crude protein content (16.57%) and ash content (0.32%) but has the highest crude fat content of 36.87%. From the proximate analysis of the meat samples, no significant difference could be observed between the mean moisture content and crude protein of chicken and goat meat. However, there was significant difference in the mean crude fat of all the meat samples, but no significant difference in their ash content. These components and other factors of the meat provide excellent growth media for a variety of microflora, some of which are pathogens as reported also by Jay *et al*. (2005). These constitutes of meats has also been reported by Heinz and Hautzinger (2007). The pH of the raw meat samples range from 5.85 to 6.63. This pH range serves as the optimum pH for the growth of the microorganisms seen in this study, as reported by many Researchers. Freese *et al*. (1998) also indicated that pH above 4.4 and 5.0 would promote growth of pathogens.

In this current study, the floor of the butcher shops was not constructed with materials that can be easily cleaned. Also, there were cracks on the floor and the walls of the shops were not painted white. Also, about one-third of the butcher shops did not have ceilings; most of their tables were dirty. The condition of these butcher shops did not conform to World Health Organization Standard (WHO and FAO, 2009) that recommended that structures within establishments should be built of durable materials and must be easy to maintain, clean and should easily be disinfected. WHO and FAO recommended the following conditions to be met to protect the safety and suitability of food; that surfaces of floors and walls should be constructed with impervious materials that will have no toxic effect on the materials that it will be used for, floors of the place must be constructed in a way that adequate drainage and cleaning can be carried out easily, walls should have a smooth surface, ceiling and fixtures should be constructed and furnished to minimize the built up of dirt, condensation and shedding of particles (WHO and FAO,2009).In this study, it was observed that the workers in the butcher shops did not wear aprons when they were handling and selling the meats, except for chicken shops were hot water were used to clean the chicken during slaughtering. In the butcher shops, the workers were seeing handling money with bare hand as they were selling the meat to customers. In some of the shops, there was no wash hand basin. The workers did not have knowledge of good hygienic practices as there is a probability that the butchers had taken no proper training in food safety. Research has shown that poor personal hygiene is one of the factors that are responsible for the contamination of foods. Continous handling of food and money also increases the risk of cross contamination (Ukwuru and Gabriel, 2012).

4. CONCLUSIONS

In conclusion, the presence of high microbial load in raw meats suggests the need for

improved hygiene practices. Thus, there is a need to check the hygienic condition of the animal house and personnel treating the animal during rearing and slaughtering. There were also potential virulent microorganisms in a number of raw meats sold in Ibadan, Oyo State Nigeria. Hence, the need for urgent and constant epidemiological surveillance, with strict enforcement of good manufacturing practices.

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