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Bacteriological assessment of raw meats sold in Lagos, Nigeria

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A B S T R A C T

The high nutritive value of meat, having both essential macro- and micro-nutrients, makes it an important part of a balanced diet for most people. However, meat is also a suitable medium for growth of microorganisms. This study evaluated the bacteriological quality of raw meat sold in Lagos, Nigeria. Ten fold dilutions of twenty meat samples were plated using the spread plate technique. Total viable bacterial count (TVBC), total Enterobacteriaceae count (TEC), total coliform count (TCC) and Escherichia coli (ECC) were determined using plate count agar, violet red bile glucose agar, Mac Conkey agar and ethylene methylene blue (EMB) agar, respectively. Enterococci spp were counted on Slanetz Bartley medium. Staphylococcus aureus and Micrococci were enumerated on Mannitol salt agar (MSA) and Baird Parker agar, respectively. Salmonella-Shigella agar was used for the isolation of Salmonella spp. Biochemical tests were performed for further identification of isolates. TVBC ranged from 1.44 x 10^4 cfu/g to 4.38 x 10^4 cfu/g; TEC ranged from 1.02 x 10^3 cfu/g to 2.45 x 10^3 cfu/g; TCC had a rage of 1.24 x 10^3 cfu/g to 2.76 x 10^3 cfu/g. TSAC ranged from 1.25 x 10^2 to 2.17 x 10^2 cfu/g. Salmonella counts ranged from nil to 1.20 x 10^2 cfu/g. S. aureus had the highest percentage occurrence while P. aeruginosa had lowest. This study revealed that raw meats sold in some parts of Lagos are of poor bacteriological quality. Hygienic practices of meat sellers need to be improved upon while organizing public enlightenment programmes is also crucial.

Key words: *Bacteria; food-borne; quality; safety; public health; food handling; sanitation.*

Abbreviations: ECC = Escherichia coli count; EMB = Ethylene methylene blue; MSA = Mannitol salt agar; TEC = Total Enterobacteriaceae count; TCC = Total coliform count; TSAC = Total Staphylococcus aureus count; TVBC = Total viable bacterial count.

Introduction

Food security is a complex issue, where animal proteins such as meats, meat products, fish and fishery products are generally regarded as high risk commodity in respect of pathogen contents, natural toxins and other possible contaminants and adulterants (Yousuf et al., 2008). Food borne infections and illnesses is a major international health problem with consequent economic reduction. It is a major cause of illness and death worldwide (Adak et al., 2005). Foodborne infections lead to diarrheal diseases which usually have long-term effects on children's growth as well as on their physical and cognitive development, as well as the death of many children and (WHO, 2004).

Meat is considered the most nutritive source of protein consumed by humans. It is a perishable food. It contains sufficient nutrient needed to support the growth of microorganisms (Magnus, 1981). Water, protein and fat, phosphorus, iron and vitamins are also contained in meat and which are the chief constituents. Carcass is the major primary unit of meat and represents the ideal meat after head, hide, intestine and blood. Lean flesh, fat flesh and edible glands or organs such as heart, liver, kidney tongue and brain are the edible parts of a carcass. The preservation of meat as a perishable food usually is accomplished by a combination of preservation methods which greatly lengthen the keeping quality of the meat.

Meat is considered spoilt when it is unfit for human consumption. It is subjected to changes by its own enzyme, by microbial action and its fat may be oxidized chemically. Microorganisms grow on meat causing visual, textural and organoleptic change when they release metabolites (Jackson and Mcgowan, 2001).

Bacterial gastrointestinal infections continue to cause illness and death and contribute to economic loss in most parts of the world, including high-income countries that have developed surveillance and control programs (Ternhag et al., 2008). The possible sources of these bacteria are likely to come from the skin of the animal from which the meat was obtained. Other potential sources of microbial contaminations are the equipment used for each operation that is performed until the final product is eaten, the clothing and hands of personnel and the physical facilities themselves art all implicated (Rombouts and Nouts, 1994).

A number of foods have been reported to have high incidence of bacteria in Nigeria (Okonko et al. 2009a, b; Clarence et al., 2009; Bello and Osho, 2012; Bello et al., 2013; Bello et al., 2014). However, there is limited information on the microbiological risk associated with fresh meat retailed within such a highly populous community like Ikorodu. This study was carried out to determine the bacteriological quality of meat vended in Ikorodu, Lagos, Nigeria.

Materials And Methods

Sample Collection

Twenty meat samples were purchased from stalls across Ikorodu markets. These were collected into separate sterile plastic bags and transported to the laboratory immediately after collection in ice-chest and tested upon arrival. Samples that could not be transported immediately were stored at $4^{O}C$ for no longer than 4 hours (Elmali and Yaman, 2005).

Bacteriological Analysis

Preparation of samples for analysis

A ten-gram sample was weighed, introduced into a mixer with a sterile spatula under aseptic conditions, and then homogenized by adding 90 ml peptone (water 0.1%). One mil portion of each homogenate was used to prepare ten-fold dilutions up to 10^{-6} with peptone water (Agaoglu et al., 2000).

Total Viable Bacterial Count (TVBC)

Spread plate technique was used to inoculate agar plates. Aerobic mesophiles were determined using plate count agar (Oxoid CM 325); plates were incubated at $30^{O}C$ for 24 to 48 hours.

Total Enterobacteriaceae Count (TEC)

Enterobacteriaceae were isolated and enumerated on Violet Red Bile Glucose Agar. Plates were incubated at $37^{O}C$ for 24 to 48 hours. Pink-red colour colonies with precipitation were taken into consideration.

Total Coliform Count (TCC) and Escherichia coli

Coliform and Escherichia coli were enumerated on Mac Conkey agar and ethylene methylene blue (EMB) agar , respectively. Plates were incubated at $37^{O}C$ for 24-48 hours. Pink red colonies with precipitation on Mac Conkey agar were enumerated as coliforms while colonies with greenish metallic sheen on EMB agar were counted as E. coli. Indole, methyl Red, Voges-Proskaner and Citrate (IMViC) tests were performed on colonies that showed shiny-metallic green to identify E. coli.

Enterococci spp

Enterococci spp were counted on Slanetz Bartley medium after incubating aerobically at $37^{O}C$ for 24-48 hours. The red colonies grown on this medium were taken into considerations.

Total Staphylococcus aureus Count and Micrococci

Staphylococcus aureus and Micrococci were enumerated on Mannitol salt agar (MSA) and Baird Parker agar, respectively. Plates were incubated at $37^{O}C$ for 24 to 48 hours. Yellow colonies on MSA were regarded as Staphylococcus aureus while small brown-black colonies without zones on Baird Parker agar were considered as Micrococcus spp. Catalase and coagulase tests were used for identification of Staphylococcus aureus (Addo et al., 2007).

Bacillus cereus

Bacillus cereus was isolated on Mannitol egg-yolk polymyxin (MYP) agar aerobically at $30^{O}C$ for 24 to 48 hours. Typical colonies of Bacillus cereus were rough and dry with a bright pink background surrounded by an egg yolk precipitate. These were selected for further examinations (Gram stain, catalase test, motility test, nitrate reduction, VP reaction etc).

Salmonella spp

A twenty-five-gram sample was incubated in 225 ml buffered peptone water at $37^{O}C$ for 24 hours. Subsequently, 0.1ml was inoculated into Rappport Vassiliadis broth and incubated at $43^{O}C$ for 24 to 48 hours. Streak plates were prepared on Salmonella-Shigella agar and incubated at 24 to 48 hours. Pink-red colonies with black centers were

inoculated onto triple-sugar iron agar and lysin iron agar. Biochemical tests were performed for the identification of Salmonella spp.

Results And Discussion

Table 1 shows the bacteriological quality of raw meats sold in Ikorodu, Lagos, Nigeria. Twenty samples were analyzed. Each sample showed varying bacteriological qualities. The highest TVBC was encountered in sample 1 with count of 4.38×10^4 cfu/g while the lowest count was encountered in sample 18 with counts of 1.44×10^4 cfu/g. TEC ranged from 1.02×10^3 to 2.45×10^3 cfu/g as obtained in samples 11 and 1, respectively. Sample 1 had the highest TCC while the lowest occurred in sample 9 with counts of 2.76×10^3 cfu/g and 1.24×10^3 cfu/g, respectively. TSAC ranged from 1.25×10^2 to 2.17×10^2 cfu/g. Salmonella did not occur in some of the samples. Salmonella counts ranged from nil to 1.20×10^2 cfu/g as in sample 12. Samples 1, 4. 9, 11, 13, 18 and 19 were free of Salmonella.

Table 2 shows the occurrence of individual bacterial species in the different samples. Staphylococcus aureus was found to be present in all twenty meat samples while the occurrence of other bacterial species varied in the different samples. The percentage occurrence of bacterial species from raw meat samples sold in Ikorodu, Lagos, Nigeria is shown in Figure 1. S. aureus had the highest percentage occurrence of 19.23% while P. aeruginosa had the lowest (2.56%). Micrococcus spp was next to S. aureus with percentage occurrence of 16.67%; followed by Enterococcus spp (15.38%) and Klebsiela spp (11.54%). Salmonella spp and E. coli had same perentage occurrence of 14.1% and Proteus spp (6.41%).

Table 1: Bacteriological quality of raw meats sold in Ikorodu, Lagos, Nigeria.

Sample	Bacterial counts in CFU/g				
	TVBC	TEC	TCC	TSAC	Salmonella Count
1	4.38×10^4	2.45×10^3	2.76×10^3	2.17×10^2	-
2	4.10×10^4	2.40×10^3	2.48×10^3	2.00×10^2	3.00×10^2
3	3.97×10^4	1.44×10^3	2.45×10^3	1.49×10^2	2.47×10^2
4	4.20×10^4	1.95×10^3	2.25×10^3	1.27×10^2	-
5	3.50×10^4	1.20×10^3	2.14×10^3	1.46×10^2	1.44×10^2
6	2.97×10^4	1.12×10^3	1.95×10^3	1.96×10^2	2.40×10^2
7	3.00×10^4	1.50×10^3	1.90×10^3	1.44×10^2	2.72×10^2
8	2.48×10^4	1.45×10^3	1.43×10^3	1.44×10^2	2.65×10^2
9	1.83×10^4	1.15×10^3	1.24×10^3	1.27×10^2	-
10	2.00×10^4	1.25×10^3	1.97×10^3	1.37×10^2	2.00×10^2
11	2.45×10^4	1.02×10^3	1.65×10^3	1.39×10^2	-
12	2.94×10^4	1.11×10^3	1.52×10^3	1.40×10^2	1.20×10^2
13	1.48×10^4	1.42×10^3	1.59×10^3	1.26×10^2	-
14	2.24×10^4	1.48×10^3	1.60×10^3	1.25×10^2	1.85×10^2
15	2.47×10^4	1.54×10^3	1.40×10^3	2.40×10^2	1.22×10^2
Sample	Bacterial counts in CFU/g				
	TVBC	TEC	TCC	TSAC	Salmonella Count
16	3.00×10^4	1.04×10^3	1.35×10^3	2.22×10^2	1.27×10^2
17	2.35×10^4	1.49×10^3	1.29×10^3	1.96×10^2	1.55×10^2
18	1.44×10^4	1.64×10^3	1.54×10^3	2.62×10^2	-
19	1.75×10^4	1.60×10^3	2.23×10^3	1.45×10^2	-
20	2.32×10^4	1.95×10^3	2.54×10^3	2.57×10^2	2.25×10^2

Table 2: Occurrence of bacterial species in different meat samples purchased in Ikorodu, Lagos, Nigeria

Sample	Bacterial Isolates
1	Staphylococcus aureus, Escherichia coli, Klebsiella spp, Enterococcus spp,
2	Staphylococcus aureus, Klebsiella spp, Salmonella spp, Micrococcus spp
3	Staphylococcus aureus, Micrococcus spp, Escherichia coli, Salmonella
4	Escherichia coli, Klebsiella spp, Enterococcus spp , Staphylococcus aureus Proteus spp
5	Staphylococcus aureus, E. coli, Klebsiella spp, Enterococcus spp, Micrococcus spp
6	Staphylococcus aureus, Micrococcus spp, Escherichia coli, Klebsiella spp, Salmonella
7	Psuedomonas aeroginosa, Staphylococcus aureus, Salmonella, Micrococcus spp ,
8	Staphylococcus aureus, Enterococcus spp, Salmonella, Proteus ssp
9	Enterococcus spp, Proteus spp, Micrococcus spp, Staphylococcus aureus
10	Staphylococcus aureus, Klebsiella spp, Salmonella, Micrococcus spp ,
11	Staphylococcus aureus,Enterococcus spp,
12	Micrococcus spp, Enterococcus spp, Salmonella spp, Escherichia coli, Staphylococcus aureus
13	Staphylococcus aureus, E. coli, Klebsiella spp, Enterococcus spp,
14	Micrococcus spp, Staphylococcus aureus , Klebsiella spp, Salmonella
15	Staphylococcus aureus, ,Enterococcus spp, Escherichia coli
16	Escherichia coli, Enterococcus spp, Salmonella spp, Proteus spp
17	Staphylococcus aureus, Micrococcus spp, Escherichia coli, Salmonella
18	Psuedomonas aeroginosa, Staphylococcus aureus, Klebsiella spp, Micrococcus spp
19	Staphylococcus aureus, Micrococcus spp, Escherichia coli, Enterococcus spp
20	Micrococcus spp, Enterococcus spp, Proteus ssp, Salmonella spp, Staphylococcus aureus

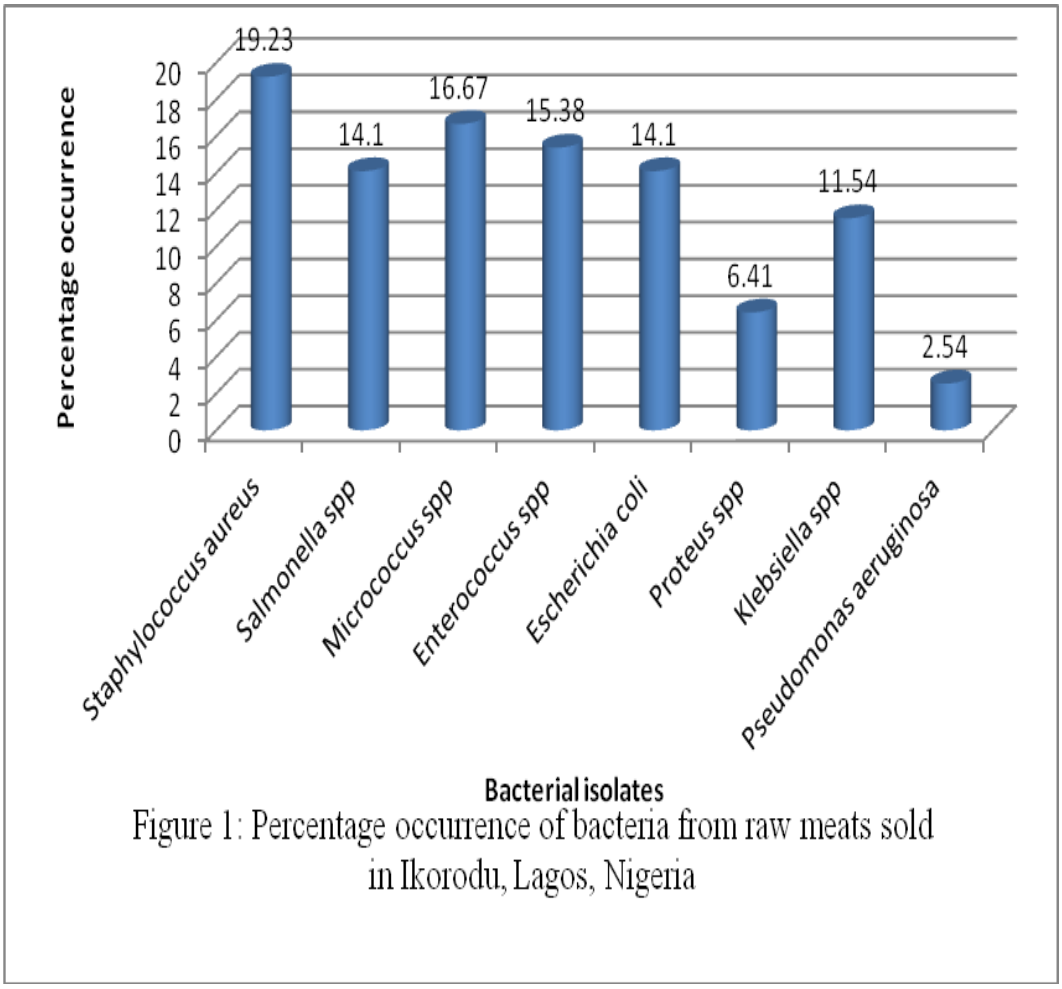

Figure 1: Percentage occurrence of bacteria from raw meats sold in Ikorodu, Lagos, Nigeria

Table 3: Biochemical characteristics of bacterial isolates from raw meat sold in Ikorodu, Lagos, Nigeria

Parameter	S.aureus	Salmonella Spp	Micrococcus spp	Enterococcus spp	E.coli	Proteus spp	Klebsiella spp	P.auroginosa
Gram's reaction	+	_	+	+	_	_	_	_
Catalase test	+	-	+	_	+	-	+	+
Coagulate test	+	-	-	+	_	-	_	_
Citrate Utilization test	_	_	+	+	+	+	+	+
Oxidate test	_	_	-	+	_		+	+
Urease test	+	_	-	N/A	_	+	+	N/A
Indole test	_	_	-	-	_	+	+	_
Glucose	+	+	+	+	+	+	+	N/A
Lactose	+	+	-	+	+	+	N/A	N/A
Sucrose	+	_	+	+	+	+	+	N/A
Mannitol	+	+	_	+	+	_	_	N/A
Maltose	+		+	+	+	+	_	N/A
Cellular morphology	Cocci	Rod	Cocci	Cocci	Straight rods	Rod	Rod	Rod
Growth in Mannitol salt	Bright yellow	N/A	N/A	N/A	N/A	NA	N/A	N/A
Growth in MacConkey agar	N/A	N/A	N/A	Pink	Red/Pink	NA	Mucoid	Pale
Growth in blood agar	Creamy white	N/A	N/A	Creamy	Circular	NA	Large white	Greenish

Keys: + = Positive; - = Negative; N/A = Not applicable

A total of 82 isolates comprising of 8 different genera of bacteria were obtained in this study. The percentage occurrence of bacterial species from raw meat samples varied from one market to the other. This showed that all markets contributed equally to the microbial diversity reported in this study. The bacteria isolates were identified as S. aureus, P. aeruginosa, Micrococcus spp, Enterococcus spp, Klebsiela spp, Salmonella spp, E. coli and Proteus spp by comparing their morphological and biochemical characteristics with standard reference organisms.

The presence of these organisms in meat could be attributed to the fact that meat contains abundant nutrients required for the growth of bacteria. The high total viable counts recorded showed the microbial diversity on meats sold in these markets, and as a reflection of the environmental (Clarence et al., 2009). Some of the microorganisms isolated from fresh meat samples in this study have been earlier found in foods form other places (Enabulele and Uraih, 2009; Sobukola et al., 2009; Clarence et al., 2009; Oyeleke, 2009; Okonko et al., 2009). Nkanga and Uraih (1981) reported high prevalence rate of S. aureus in meat samples from traditional market in Benin City, Nigeria. E. coli and S. aureus are normal flora in human and animals, their presence in foods are indications of contaminations with faecal matters and excessive human handling (Clarence et al., 2009; Bello et al., 2013a).

The isolation of Enterococcus spp may be as a result of poor environmental conditions due to dust and contamination of the water used during slaughtering. This organism might have contaminated the meats during the process of handling by meat sellers. This is also in accordance to the assertion of Okonko et al. (2009a, b) that improper handling and improper hygiene might lead to the contamination of ready-to-eat foods and this might eventually affects the health of the consumers (Bello et al., 2013a, b; Bello et al., 2014).

However, the processors/handlers/sellers should observe strict hygienic measures so that they may not serve as source of chance inoculation of microorganisms and fecal contamination of fresh meats and other meat products. The presence of indicator and other organisms examined in this study is of special concern and perhaps the greatest danger associated with fresh meats used for food preparation, eating purposes and for other human consumption is contamination by human excrement It demonstrates a potential health risk as the organism is pathogenic and causes complications in children.

Conclusion

It was revealed in this study that raw meat sold in Ikorodu, Lagos, Nigeria are of poor bacteriological quality. It showed that fresh meats are often contaminated with pathogenic bacteria and their presence meat foods should receive particular attention, because their presence indicate public health hazard and give warning signal for possible occurrence of food borne intoxication.

Recommendations

Meat handlers and sellers should be educated on the adverse effect of lack of proper personal and environmental hygiene and sanitation. Fresh meats to be used for consumption purposes should be adequately cooked before use and NAFDAC should ensure and enforce strict compliance of the recommended food standards as regards the production and sales of processed and packaged meat products. Veterinary doctors should inspect the animals to be slaughter before the meat is sold to the general public. Good manufacturing practices should be strictly adhered to by butchers and those selling the meat. The water used in washing the meat should be sterile, also the equipment must be washed properly before use. Further regulatory and educational efforts are needed to improve the safety of produce items. Continued progress on the part of regulators and industry to improve food safety are dependent on local, state, and federal agencies' ability to conduct epidemiologic and laboratory investigations that identify the offending agents and link them with specific foods; this should be adopted and executed in developing countries that are yet to adopt them.

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