Isolation and Identification of Pathogenic Bacteria Associated with Raw Meat from Different Locations in Ado-Ekiti

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Abstract: **Raw meat is any type of uncooked muscle tissue of an animal used as food. Meat is a complex niche that has chemical and physical characteristics that support plethora of microorganisms' growth. Eighteen (18) samples of fresh and spoilt meat (beef, mutton and chicken) were randomly collected from three (3) different locations in Ado-Ekiti metropolis. Samples were analysed for bacteriological contamination using standard Microbiological methods. Associated pathogenic bacteria recovered from the isolated raw meat samples include *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Bacillus cereus, Staphylococcus epidermidis and Proteus mirabilis* with percentage occurrence of 24.2%, 17.7%, 16.1%, 12.9%, 12.9%, 11.3% and 4.9% respectively. The mean aerobic plate count in order of 10^6 (cfu/mL) was high for spoilt raw meat (Mutton) samples at 1.46 than 1.40 reportedfor fresh raw meat (Mutton) samples.**

Keywords**: Meat, Pathogenic bacteria, Contamination, Isolation, Antimicrobial sensitivity.**

I. INTRODUCTION

Meat is one of the best sources of proteins, vitamins and minerals, which are essential nutrients required for proper growth and maintenance(Orpin *et al*., 2018). Meat has been defined as the flesh of animals which are suitable as food (Forrest, 2001). They include all processed or manufactured products which might be prepared from these tissues i.e. meat may be fresh, cured, dried or otherwise processed. Different types of meat can be gotten from animals, *e.g.* pork meat (pig), mutton (sheep),poultry meat(Chicken) and beef (cow).

Meat is a complex niche that has chemical and physical properties which allow the colonization and development of a variety of microorganisms. It is one of the highly perishable foods due to its high nutritional contents, continuous enzymatic action and the presence of microorganisms (bacteria, yeasts and moulds) which may result in oxidative rancidity, discolouration, mouldiness, off flavour, sliminess etc. The principal source of these deteriorative and depreciating changes is plethora of microorganisms which in turn render the meat unacceptable and unfit for human consumption (Forrest *et al*., 2001).

Microorganisms commonly associated with meat include psychrophiles of the genera Pseudomonas, Lactobacillus, Moraxella, Acinetobacter, Microbacteria, Brochotrix, Klebsiellaand Vibrio (Gill and Newton, 1998). Others include Mesophiles like *Salmonella species*, *Escherichia coli*, and *Clostridium perfringens*, the Thermophiles that comprises *Streptococcus faecalis*; and members of the genera Flavobacterium, Bacillus, Leuconostoc, Proteus, Micrococcus and Achromobacter (Ajiboye *et al*., 2011).

The microbial load in meat becomes higher with continued favorable growth conditions that include acidity, pH, temperature, moisture, nutrient requirement and competition. Understanding and proper management of these factors brings about increased meat shelf life.Best preservative methods for meat include drying, salting and high temperature (Orpin *et al*., 2018), these procedure sometimes make meat preservation more difficult than other kinds of food (Ajiboye*et al*., 2011). In recent times, literatures have reported that large percentage of all raw meat diets (commercial or homemade) are contaminated with bacteria. About 20-44% of commercial raw food is contaminated with Salmonella. Also, animals that eat raw meat can shed these bacteria in their feces reported by Finley *et al*. (2008) where approximately half the dogs that consumed contaminated raw food shed Salmonella in their feces for up to 7 days. Other bacteria found in raw meat diets include *Esherichia coli* 0157:H7 and *Clostridia species*. These bacteria are risks, not only to the animals eating the diets, but also to other pets and people inthe household, particularly young, old, or immune suppressed people or animals (LeJeune and Hancock, 2001).

Bacterial contamination had been reported in meat and its products. A 2011 study by the Translational Genomics Research Institute showed that nearly half (47%) of the meat and poultry in U.S. grocery stores were contaminated with *Staphylococcus. aureus*, with more than half (52%) of those bacteria resistant to antibiotics. A 2018 investigation by the Bureau of Investigative Journalism and The Guardian found that around 15% of the US population suffers from foodborne illnesses every year. The investigation also highlighted unsanitary conditions in US-based meat plants, which included meat products covered in excrement and abscesses "filled with pus" (Wasley, 2018).

Studies have shown that the meat products were contaminated at various stages of preparation in Calabar, Lagos and Ebonyi (Iroha *et al*., 2011, Odey *et al*., 2013, Bello *et al*., 2016), while Falegan *et al*. (2017) and Orpin *et al*. (2018) reported

contamination of suya (sliced roasted beef) in Ado-Ekiti Metropolis, Ekiti State and Dutsinma Local Government Area, Kastina State respectively. Since there is dearth of information of raw meats in Ado-Ekiti, this study was to determine the degree of bacterial contamination of raw meat sold to customers at backyard meat stalls and open wet market in Ado-Ekiti.

II. MATERIALS AND METHODS

2.1 Collection of samples

A total of Eighteen (18) samples of fresh and spoilt meat (beef, mutton and chicken) were randomly obtained from 3 different locations; viz, Atikankan, Irona and Oja Oba. 100 g of meat samples were collected into clean, dry and sterile polythene bags and transported to the laboratory for microbiological analysis. This was done within an hour and 96 hours (4 days) of collection for raw and spoilt meat respectively.

2.2 Sample analysis

The samples were aseptically cut into thin smaller pieces using sterile knife. The analytical portions were placed in separate sterile plastic bags and homogenised in 250 mL of distilled water, this was used as stock. Serial dilutions were achieved up to five fold (10^{-5}) for each prepared sample using 1 mL from stock homogenate and 9 mL of sterile distilled water for the serial dilution experiment. This was carried out in order to obtain discrete colony.

Nutrient agar,(2.8 g) was dissolved in 100mL of distilled water to prepare five plates. The dissolved agar was autoclaved at 121^0C for 15 minutes after sealing with cotton wool and foil paper to prevent contamination. Then it was allowed to cool down at 45^0C and poured into sterile petri dishes. We took pre-serially diluted samples(0.1 mL) and dispensed onto prepared solidified nutrient agar plates by spread plate method. This was allowed to set firmly for five (5) minutes afterward inoculated plates were incubated at 37^oC for 24 hours. Bacteria colonies observed after 24 hours were then sub-cultured into freshly prepared Nutrient agar in sterile petri dishes to obtain discrete colonies and were subsequently identified.

2.3 Microscopy and Colonial Identification

The cultural characteristics suchas size, pigment, margin, elevation, form and surface of the isolates on the agar plate were observed. Biochemical tests performed include gram staining, catalase, coagulase, citrate, urease and antibiotic sensitivity tests.

2.4 Gram's Reaction

The smears of the isolates were prepared on clean grease-free slides and heat-fixed. Two drops of Crystal violet was added for 60 seconds, followed by Gram's iodine for 60 seconds. The slides were rinsed with water and decolorized using alcohol for 15 seconds and rinsed with water. The decolorized slide was counter stained with Safranine for 60 seconds. It was rinsed off using water. The slides were air-dried and viewed under the microscope using oil immersion lens.

2.5 Antibiotic sensitivity test

Antibiotic sensitivity test by the Kirby Bauer's disc diffusion method was performed on the isolates using commercially available antibiotic discs on Mueller-Hinton Agar (MHA). Immediately after standardization, a sterile cotton swab was immersed into bacteria suspension and a lawn culture was performed on the surface of MHA plate. Commercially available antibiotic discs were arranged on the surfaces of inoculated plates. The plates were incubated at 37^oC for 16–18 hours. After incubation, the diameter of zones of inhibition was measured for each antimicrobial agent and it was compared with the National Committee for Clinical Laboratory Standards (NCCLS) chart.

2.6 Data Analysis

The number of viable bacteria cells in a sample of meat was estimated using colony forming unit (cfu/mL) while simple descriptive statistics was used to determine the frequency of occurrence of bacterial isolates.

III. RESULTS

The results obtained from this study are presented in the tables below. Table 1shows the morphological characteristics of bacterial isolates from fresh and spoil meat samples obtained from raw meatretail outlets in Ado-Ekiti, Ekiti State. The bacterial isolates were observed to have smooth and rough edges, creamy and whitish colours, and raised and flat elevations, and cocci and rod- like shapes. Their biochemical test ranges from negative to positive. Hence, a total of seven bacteria were identified namely, *Staphylococcus aureus, Staphylococcusepidermidis, Proteusvulgaris, Proteus mirabilis, Klebsiella Pnuemoniae, Bacillus cereus and Escherichia. coli.*

The frequency of occurrences of bacterial isolates in all meat samples are presented in Table 2, where sixty-two (62) isolates were recovered. Highest percentage occurrence was observed for *Staphylococcus aureus with* 24.2% gotten from five (5) and Eight(8) isolates from fresh and Spoilt meat samples respectively; 17.7%for *Escherichia coli* arising from five(5) and Six(6) isolates from fresh and Spoilt meat samples respectively*;* 16.1% *for Klebsiella pneumonia* gotten from four(4) and six(6) isolates gotten from fresh and Spoilt meat samples respectively*;* 12.9% *for Proteus vulgaris* arising from three(3) and five(5) isolates from fresh and Spoilt meat samples respectively*;* 12.9%for *Bacillus cereus* arising from three (3) and five(5) isolates from fresh and Spoilt meat samples respectively*;* 11.3% for *Staphylococcus epidermidis* gotten from three (3) and four (4) isolates from fresh and Spoilt meat samples respectivelyand4.9% for *Proteus mirabilis* arising from one(1) and three (3) isolates from fresh and Spoilt meat samples respectively.

From Table 3, both fresh and spoilt Mutton meat from Oja - Oba market had the highest aerobic plate count of 1.40 $x10^6$ and 1.46 $x10^6$cfu/mL respectively compared to Atikankan and Irona markets while, both fresh and spoilt Beef meat from Irona market had the lowest aerobic plate count of 0.88 $x10^6$and 1.12 $x10^6$ cfu/mL respectively compared to Atikankan and Oja-Oba markets. Mutton meat had the highest meanaerobicplate count of 1.28 $x10^6$ and 1.46 $x10^6$ cfu/mL in both fresh and spoilt samples respectively while Beef meat had the lowest Mean aerobic plate count of 1.09 $x10^6$and 1.31x 10^6 cfu/mL in both fresh and spoilt samples respectively. The mean aerobic plate count increased in all spoilt meat samples than in fresh meat samples.

The result of conventional antibiotic susceptibility test was presented in Table 3. *Staphylococcus aureus* was susceptible to Streptomycin (15.00 mm), followed by Norfloxacin (12.00 mm) intermediately and resistant to other antibiotics used. *Escherichiacoli* was only susceptible to Levofloxacin (14.00 mm) and resistant to other antibiotics used. *B. cereus* was the most susceptibe isolate followedby *Proteus mirabilisProteu vulgaris* was the most resistant, followed by *Esherichia. coli* and then *Staphylococcus aureus*

.Table 1: Morphological and biochemical properties of bacteria isolated in both fresh and spoilt raw meat samples (chicken, beef and mutton)

Isolates	Colony morphology	Gram reactions	Biochemical tests			
			Coagulase	Catalase	Citrate	Urease
Staphylococcus aureus	Convex, rough and creamy cocci in clusters	Positive	Positive	Positive	Negative	Negative
Staphylococcus epidermidis	Raised, rough and whitish cocci in singles and pairs	Positive	Negative	Positive	Negative	Positive
Proteus vulgaris	Flat, smooth and creamy rods in singles and pairs	Negative	Negative	Positive	Positive	Positive
Proteus mirabilis	Raised, rough and creamy long rods in singles and pairs	Negative	Negative	Positive	Positive	Positive
Klebsiella pnuemoniae	Flat, rough and creamy rods in clusters	Negative	Negative	Positive	Negative	Negative
Bacillus cereus	Convex, smooth and milky long rods in clusters	Positive	Negative	Positive	Negative	Negative
Escherichia coli	Raised, rough and creamy short rods in singles	Negative	Negative	Positive	Negative	Negative

Table 2: Frequency of occurrences of bacterial isolates in meat samples from the three locations

Isolates	Fresh samples			Total (T1)	Spoilt samples			Total (T2)	Overall Total	Percentage
	Chicken	mutton	Beef		chicken	Mutton	Beef			
Staphylococcus aureus	1	2	2	5	2	3	3	8	15	24.2
Staphylococcus epidermidis	1	1	1	3	2	1	1	4	7	11.3
Proteus vulgaris	1	1	1	3	1	2	2	5	8	12.9
Proteus mirabilis	-	-	-	0	1	1	1	3	3	4.9
Klebsiella pnuemoniae	1	1	2	4	2	2	2	6	10	16.1
Bacillus cereus	1	1	1	3	1	2	2	5	8	12.9
Escherichia coli	1	2	2	5	2	2	2	6	11	17.7
									62	100

Table 3: Aerobic plate count of meat samples

Meat samples	Fresh				Spoilt			
	Atikankan (cfu/mL)	Irona (cfu/mL)	Oja-Oba (cfu/mL)	Mean aerobic plate count (cfu/mL)	Atikankan (cfu/mL)	Irona (cfu/mL)	Oja-Oba (cfu/mL)	Mean aerobic plate count (cfu/mL)
Chicken	1.24 x 10^6	1.20 $x10^6$	1.36 $x10^6$	1.26 $x10^6$	1.40 $x10^6$	1.32 $x10^6$	1.60 $x10^6$	1.44 $x10^6$
Beef	1.40 $x10^6$	0.88 $x10^6$	1.00 $x10^6$	1.09 $x10^6$	1.60 $x10^6$	1.12 $x10^6$	1.20 $x10^6$	1.31 $x10^1$
Mutton	1.12 $x10^6$	1.32 $x10^6$	1.40 $x10^6$	1.28 $x10^6$	1.20 $x10^6$	1.58 $x10^6$	1.60 $x10^6$	1.46 $x10^6$

Table 4: Antibiotic sensitivity test of the bacteria isolates

	Antibiotic sensitivity									
Isolates	RD (mm)	AMX (mm)	S (mm)	NB (mm)	CH (mm)	CPX (mm)	E (mm)	LEV (mm)	CN (mm)	APX (mm)
Escherichia coli	7.00	0.00	0.00	0.00	0.00	10.00	0.00	14.00	0.00	0.00
Staphylococcus aureus	0.00	10.00	15.00	12.00	0.00	0.00	5.00	11.00	10.00	0.00
Proteus mirabilis	0.00	14.00	0.00	15.00	14.00	15.00	0.00	14.00	16.00	0.00
Proteus vulgaris	0.00	10.00	0.00	0.00	6.00	8.00	0.00	0.00	6.00	0.00
K.lebsiella pneumoniae	0.00	0.00	0.00	0.00	12.00	10.00	0.00	0.00	8.00	0.00
Bacillus cereus	32.00	0.00	26.00	0.00	30.00	23.00	28.00	0.00	0.00	0.00
Staphylococcus epidermidis	0.00	0.00	9.00	0.00	18.00	0.00	0.00	10.00	0.00	0.00

KEY: CPX Ciprofloxacin (5 mg)

RD Rifampicin (10 mg)
CN Gentamycin (10 mg)
AMX Amoxil (30 mg)
S Streptomycin (10 mg)
LEV Levofloxacin (5 mg)
E Erythromycin (10 mg)
NB Norfloxacin (10 mg)
APX Ampiclox (25 mg)
CH Chloramphenicol (10 mg)
Susceptible (S) = ≥ 14
Intermediate (I) = 12 – 13
Resistant (R) = ≤ 11

IV. DISCUSSION

Recent increase in the consumption of meat and its products arises from reasons including high protein contents, vitamins, minerals, lipids and savory sensation (Orpin *et al*., 2018). High bacterial load in food indicates the general quality of the food and the degree of spoilage it might have undergone. However, the greatest risk to human health is due to the consumption of raw or meat not properly cooked and meat products according to the findings of Yagoub (2009).Seven (7) different organisms were isolated fromboth fresh and spoilt raw meat. This is in congruent with earlier studies on raw and processed meats in Calabar, Lagos and Ado-Ekiti Nigeria (Odey *et al*., 2013, Bello *et al*., 2016, Falegan *et al*., 2017). Exposure to environmental conditions and inappropriate handling may be related to the growth and proliferation of bacteria in the meat stalls in the markets. Less-formed deep layer bacterial colony units observed might probably be due to the fact that bacteria need longer time to produce enough protease enzymes to be able to penetrate the meat.

In this study, the most prominent bacteria isolated from the fresh and spoilt meat samples was *Staphylococcus aureus*, followed by *Escherichia coli* and *Klebsiella pneumonia*. This is in congruent with earlier reports that isolated *Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella spp, Klebsiella pneumoniae* and *Staphylococcus epidermidis* from raw and processed meat product (Suya) in varying part of Nigeria (Iroha *et al*., 2011, Moshood *et al.* 2012, Odey *et al*., 2013, Bello *et al*., 2016, Falegan *et al*., 2017 and Orpin *et al*., 2018). These in turn might be majorcauses of gastro-intestinal disorders, food poisoning and food borne diseases. Highest percentage occurrence of *Staphylococcus aureus* (24.2%), followed by *Escherichia coli* (17.7%) and *Klebsiella pneumonia*(16.1%) is similar to report from Moshood *et al*. (2012) where they observed similar trend from Balangu (roasted meat product) sold in Bauchi. This might arise from unhygienic conditions of butchers as humans are carriers *of Staphylococcus aureus* (on the skin, nose and throat), using non-portable water for the rinsing of meat after slaughtering, unhygienic storage facility and undercooking of meat respectively.

Defective storage temperature of meats and prolonged holding at warm temperatures affect microbiological quality and safety of the meat (Prakash *et al*., 2012). However, mean aerobic plate count in fresh raw meat samples also revealed contamination by bacterial; this is of great public health concern. This may be due to the fact that meat handlers (butchers) processed meat on unhygienic wooden logs in addition to the fact that, they are not accustomed to wearing gloves. This might culminate in transmission of food borne diseases arising from bacterial contamination.

Staphylococcus aureus, *Escherichia coli*, *Bacillus cereus*, *Proteus mirabilis* and *Proteus vulgaris* resistant to certain antibiotics is similar to earlier studies on Suya (sliced roasted beef) sold in Ado-Ekiti Metropolis, Ekiti State and raw meat sold in Abakaliki, Ebonyi State, Nigeria (Falegan *et al*., 2017, Iroha *et al*., 2011). Resistance to these antibiotics might be due to its misuse and use of sub-standard antibiotics in growing animals to treat bacterial infection; this thus causes public health concern like protracted illness and even death in vulnerable patients like ones with impaired immunity.

This study presented the degree of contamination status of raw meat sold in Ado-Ekiti market/abattoir and as well demonstrated the role of this meat as a reservoir of antibiotic resistant bacteria that can be transmitted to humans, thereby

constituting a public health concern in the long run. We hereby suggest the application of stringent hygiene practices before and during meat processing and prudent use of antibiotics in animal husbandry which are essential for the control of further emergency of antibiotic resistance.

V. CONCLUSION

The result illustrates the high rate of superficial bacterial contamination of meat. This may be due to poor condition of slaughtering of cattle and handling of their carcasses, as well as unhygienic practices by handlers within Ado-Ekiti. The high prevalence of potential pathogenic bacteria in meat represents a potential health hazard to the people and the society.

VI. RECOMMENDATIONS

To safeguard the public against the risks of food borne bacterial infections, there is need to advocate for practicing good sanitation and meat handling techniques in the butcher retail shops. There is also the need to educate butchers and meat vendors on the adverse effects of meat contamination on public health. However, the meat vendors should observe strict hygienic measures such as daily washing of their tables before and after sales. This should be done by consistent sterilization of all knives and cutlasses used in the cutting of meat meant for public consumption. Nevertheless, the public must be enlightened on the need to properly cooked meat before consumption as the undercooked meat will allow more proliferation of pathogens after some days. Routine inspection of slaughter houses should be made mandatory by appropriate authorities.

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