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Bacteriological Quality and Antibiotic Susceptibility Pattern of Pathogens Isolated from Meat Sold in Abattoir and Retail Outlets in Ogoja Urban, Cross River State, Nigeria

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Abstract: The bacteriological quality of fresh beef meat sold in abattoir and some retail outlets in Ogboja and Ishibori Markets were investigated using standard microbiological procedures. The antibiotic susceptibility test of the isolates was also determined using standard diffusion method. The result of total aerobic count on the fresh meat from the abattoir ranged between $3.0x10^5 – 4.0x10^5$ cfu/g, while that of Ogboja retail outlet was $3.2x10^5 – 6.9x10^6$ and Ishibori retail outlet $2.8x10^5 – 5.2x10^6$. A total of seven bacteria were isolated, these include: *Pseudomonas* species, *Enterobacter* Species, *Salmonella* species, *Proteus* species, *Staphylococcus* species, *Streptococcus* species and *Escherichia coli*. The result on percentage frequency of distribution revealed that *Streptococcus* species had a higher percentage of 34.1% widely distributed while *Enterobacter* species had the Lower percentage of 3.5%. The result of antibiotic susceptibility test also revealed that *Escherichia coli* and *Pseudomonas* species were resistant to most of the antibiotic used while *Staphylococcus* was mostly susceptible to the tested antibiotics. And of all the antibiotics used Ampicillin was resistance to all the isolates. Due to the high presence of pathogenic microbial contamination there is need to Educate meat processors and sellers on good hygiene practices.

Key words: Bacteria · Quality · Beef · Meat and Abattoir

INTRODUCTION

Food Security is a complex issue, where animals proteins such as meat, meat products, fish and fishery products are generally regarded as high risk commodity to infection and oxidation [1]. These food borne infections and the consequent illnesses are some of the major international challenges that lead to high mortality and economic loss [2].

Beef is a meat that is produced from cattle. It is produced and consumed worldwide, with South America, Africa, Asia and Australia being the highest consumers of the product [3]. Its production therefore is increasing like that of many other commodities, as it is needed by both individuals and operators of fast food centres [4].

Abattoir a place where livestock including cattle are slaughtered and processed [5]. A number of slaughter facilities found in an abattoir have been suspected to be a source of contamination during the slaughtering processes. It has been reported that abattoir is not 100% hygienic [6]. In most developing countries, their traditional methods of handling, processing and marketing of meat undermine quality whereas poor sanitation leads to considerable loss of product as well as the risk of food - borne disease [5].

In addition, improper handling and improper hygiene may lead to the contamination of fresh meats and eventually affects the health of the consumers [7]. Also, sellers are often poorly educated, unlicensed, untrained in food hygiene and they work under crude unsanitary conditions with little or no knowledge about the causes of food borne disease [8].

In Nigeria particularly in rural communities and small towns, slaughtering of animals usually takes place under very unhygienic conditions. The state of health of animals prior to slaughtering and the prevailing circumstances in the slaughter can contribute to the quality of meat from such animals [9]. Processing procedures and monitoring

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of critical points in the meat production are not fully developed. Abattoir has also become a source of infection and pollution, attracting domestic and wild carnivores and rodents due to inadequate slaughtering and disposal facilities [10]. Apart from incorrect processing procedures, marketing practices of meat consumed by most populace is a major area of concern [11].

Previous Studies have documented contamination of raw meat by bacterial pathogens. Some notable Pathogens that can serve as contaminants of meats include: *Salmonella, Vibrio cholerae, Escherichia coli and Listeria species*. Epidemiological reports suggest that meat product is one of the major causes of diarrheal illness which account for 36% of mortality cases.

Reduction of risk for human illness associated with raw product can be better achieved through controlling point of potential contamination in the field, during harvesting, during processing or distribution or in retail markets, food service facilities or at home [12].

Presently, little or no inspection/supervision is being carried out by veterinary and public health officers on the operations of abattoir and many of them are located in isolated areas across the metropolis. Since there is increasing demand for beef and beef products, there is therefore need to investigate the bacteriological quality and antibiotics susceptibility pattern of pathogens isolated from meat sold in abattoir and retail outlets in Ogoja Urban.

MATERIALS AND METHODS

Study Area: This study was carried out in Ogoja, in the northern Senatorial district of Cross River State. Ogoja is a Local Government Area in Cross River State, Nigeria. It's headquarters is Ogoja town in the North East of the Area near the A4 highway at 6° 39' 17 "N 8° 47' S I" E location in Nigeria. It has an area of 972KM^2 and a population of 171,901 at the 2006 census. The postal code of the area is 550. The town was one of the provinces during pre-colonial independence. It shares boundaries with Benue State to the North, Ebonyi State to the West, to the East by Camerouns. It consists of many tribes, which includes Ishibori, Igoli as the central town, Mbube being one of the Major tribes.

Sample Collection and Preparation: Thirty samples of freshly slaughtered meat were purchased from the abattoir and from the various retail outlets (Ogboja and Ishibori Markets) within Ogoja Urban in the morning and later in the late afternoon. The samples were aseptically collected into sterile plastic containers with covers and were labeled accordingly and stored in a cooler (Igloo USA) packed with ice pack. The samples were immediately transferred to Laboratory Unit of General Hospital Ogoja, for further bacteriological analysis as described by the methods of [13]. The various samples were prepared by macerating 10gms portion of each of the meat sample in 90ml of 0.1% peptone water as diluents and homogenized for about 2 minutes, then a 10 fold serial dilution was prepared, using sterile pipettes as described by [13].

Determination of Total Aerobic Count: Total aerobic count was performed using pour plate method. The various samples were analyzed for total aerobic counts using nutrient agar as the medium for inoculation. Aliquot of 1ml each of the 10 fold dilution (10^4 for all morning samples and 10^5 for late afternoon samples) was transferred to a sterile petri-dishes and molten agar poured onto it. The plate was gently swirled for uniform mixing of the sample and the agar in the plate and was allowed to set. The plates were incubated at 37°C for 24/hrs, after incubation, the plates that have countable colonies were removed and counted using the colony counter. However, the exact number of colonies counted were multiplied by the dilution factor and expressed as colony forming unit per ml (cfu/ml) [13].

Isolation and Identification of Bacterial Pathogens: This was done by streaking on selective media MacConkey agar, *Salmonella/ Shigella* agar for the most common meat bacterial pathogens, this includes *Salmonella* spp, *Escherichia coli*, *Staphylococcus* spp and *Streptococcus* spp. Samples were then inoculated onto nutrient agar and then incubated at 37°C for 24/hrs. After incubation the plates were examined for microbial growth, again, sub-cultures were made to get discrete colonies. Pure discrete colonies gotten were then stored in agar slants and refrigerated for further biochemical and other investigations. The Isolates obtained were identified based on established conventional cultural morphological and biochemical characterization [14].

Gram Staining: With the method employed by [14], a smear was prepared from each of the culture on a grease free microscope slide, allowed to dry and fix through the upper part of burnsen burner flame. The smear was covered with crystal violet stain for 60 seconds, then the stain was washed off with clean water. The smear was flooded with logul's iodine for 60 seconds and washed off with clean water. It was decolourised with acetone/alcohol

and washed off immediately with clean water the smear was then covered with neutral red stain for 2 minutes and washed off with clean water. The slide was hanged on the wooden rack to dry, slides were observed under 100x oil immersion objective lens. Gram positive bacteria appeared purple blue, while gram negative ones appeared pink to red.

Biochemical Test: The following biochemical tests were carried out; Catalase Test and Coagulase test [14], Sugar fermentation test [15], Motility test [14].

Detection of *Salmonella* Species: To determine salmonella species, 0.1ml of the meat sample dilution was inoculated on already prepared plate of *Salmonella/Shigella* agar (SSA) by streaking technique and the plate was incubated aerobically at 37°C for 24/hrs. Microbial growth was observed the next day and discrete colories were sub cultured into fresh nutrient agar plates aseptically to obtain pure decrete colonies. Colonies identifiable as discrete on the nutrient agar were carefully examined macroscopically for cultural characteristic such as shape, color, size and consistency. Gram staining reaction was done. Appropriate biochemical test was done and the result recorded.

Isolation of *Pseudomonas* Species: *Pseudomonas* species were identified by inoculating O.1ml of the serial diluted (10^{-5}) meat sample onto already prepared MacConkey agar, by streaking method and was incubated aerobically at 37°C for 24 hours. The microbial growth observed the next day was greenish colour. Fresh nutrient agar was prepared, allowed to solidified after which colonies from the MacConkey plate was subculture on the nutrient agar to obtain discrete pure colonies. Gram staining was done and appropriate biochemical test was done and results recorded accordingly.

Detection of *Enterobacter S*pecies: *Enterobacter* species was isolated using MacConkey agar, freshly prepared was allowed to set and, dried. 0.1ml of the meat sample (10^{-5}) of the serial dilution was inoculated onto the MacConkey agar plate by streaking technique and aerobically incubated at 37°C for 24 hours. Pink to red large colonies were observed the next day. Discrete colonies were subcultures onto fresh nutrient agar plates aseptically to obtain discrete pure colonies. Macroscopical examination was done for cultural characteristic such as shape, size, colour and consistency. Gram staining technique was done and biochemical test done and results recorded accordingly.

Isolation of *Proteus* Species: This was isolated by inoculating 0.1ml of the meat sample (10^{5}) serial dilution on already prepared blood agar and incubated aerobically at 37°C for 24hrs. The next day, the microbial growth produced a characteristic swarming growth over the surface of blood agar. Freshly prepared nutrient agar was prepared and sub-culture onto it to obtain discrete colonies. Suspected colonies were gram stained and biochemical test done, results recorded accordingly.

Detection of *Escherichia coli:* Isolation and identification of *Escherichia coli* was done using 0.1ml of the meat sample dilution ($10^{-}5$), by streaking method on already prepared MacConkey agar and was incubated aerobically at 37°C for 24hrs. at the end of the incubation periods, colonies were observed. Discrete colony was sub culture onto fresh nutrient agar to obtain pure discrete colonies. Gram staining technique was done to determine the Gram's reaction.

Detection of *Staphylococcus* Species: Isolation and identification of *Staphylococcus* species was done by inoculating 0.1ml of the meat sample on the already prepared chocolate agar (heated blood) by streaking method; and incubated aerobically at 37°c for 24hrs, slightly raised colonies, creamy, was observed the next day. A smooth creamy colony was sub cultured on fresh nutrient agar to obtain pure cultures. The discrete colonies on the nutrient agar were examined for morphological cultural characteristics, Gram staining was done and as well as biochemical tests as seen in Table 3.

Antibiotic Susceptibility Test: The antibiotic susceptibility test was performed against a wide range of antibiotics. The standard procedures of clinical and laboratory standard institute (CLSI) were strictly followed throughout the test procedure. All the identified isolates; *Escherichia coli*, *Staphylococci* species, *Salmonella* species, *Enterobacter* species, *Pseudomonas* species, *Proteus* species and *Streptococcus* species cultures were tested for Tarivid (5µg) Ciproflox (5µg) Peflacine (5µg) Augumentin (25µg) Gentamycin (10µg) Levofloxacin (5µg) erythromycin (15µg) Ampiclox (20µg) Nalidixic acid (30µg) Ampicillin (10µg) Norbactin (10µg) Streptomycin (10µg) Chloramphenicol (30µg); using the standard diffusion methods of determining susceptibility (Kirby Bauer) on nutrient agar already seeded with the bacterial isolates and incubated at 37°C for 24/hrs. Plate were examined for zones of inhibition around the antibiotic disc and measured in millimeter (mm) [16]. The results were reported as susceptible, intermediate or resistant.

Preparation of 0.5 McFarland Turbidity Standard: Turbidity standard equivalent to 0.5 McFarland was prepared by adding 1ml of concentrated tetraoxosulphate (VI) acid to 99ml of distilled water and dissolving 0.5g of dehydrate barium chloride ($BaCl_2.2H_2O$) in 50ml of distilled water in separate reaction flasks respectively. Barium chloride solution (0.6ml) was added to 99.4ml of the tetraoxosulphate (VI) acid solution in a separate test tube and the reaction mixture mixed well to form 0.5 McFarland turbidity standard. Small portion of the turbid solution was transferred to a capped test tube similar to the tube used for preparing the test microorganisms and stored at room temperature [14]. All test bacteria was standardized individually before use to 0.5 MacFarland turbidity standards.

RESULTS AND DISCUSSION

Meat is the most perishable of all food hence it contains sufficient nutrient needed to support the growth of microorganisms [17]. The result of this study shows that the meat had a high microbial load from the retail outlet samples collected from Ogboja and Ishibori as compared to that collected in the morning from the abattoir. This is an indication of recontamination in food handling and hygiene techniques [18].

Total Aerobic Counts at the Various Meats Outlets

Table 1: Aerobic Counts of Bacteria on Meat Sample

Location Abattoir	Morning Bacterial cfu/g	Evening Cfu/g
B 1	3.0×10^5	-
B 2	3.2×10^5	-
B 3	3.5×10^5	-
B 4	4.0×10^5	-
B 5	3.6×10^5	-
B 6	3.5×10^5	-
OGBOJA RETAIL OUTLET		
Ro. 1	4.0×10^5	6.9×10^6
Ro. 2	4.4×10^5	5.8×10^6
Ro. 3	3.2×10^5	4.7×10^6
Ro. 4	4.2×10^5	4.9×10^6
Ro. 5	3.3×10^5	4.2×10^6
Ro. 6	3.6×10^5	5.0×10^6
ISHIBORI RETAIL OUTLET		
Ro. 1	3.6×10^5	5.0×10^6
Ro. 2	2.8×10^5	4.2×10^6
Ro. 3	2.9×10^5	4.0×10^6
Ro. 4	3.0×10^5	5.0×10^6
Ro. 5	4.0×10^5	5.2×10^6
Ro. 6	4.2×10^5	4.8×10^6
Key:		
B	=	Butcher
Ro	=	Retail Outlet

Isolation and Identification of Bacteria from Fresh Beef Meat Samples

Table 2: Morphological and Biochemical Characteristics of Bacteria Isolates

Test	*Staphylococcus Species*	*Streptococcus Species*	*Escherichia Coli*	*Salmonella Species*	*Enterobacter Species*	*Proteus Species*	*Pseudomonas Species*
Colonial morphology	Flat circular milky colonies	Mucold colonies with partial haemolysis	Pink colonies	Pale colonies	Large dry colonies	Confluent colonies with swarming ends	Colonies fluoresces green
Cellular morphology	Cocci in clusters	Cocci in short chain	Short rods in singles	Short rods in singles	Short rods in singles	Rods in pair and singles	Rods
Gram stain	+	+	-	-	-	-	-
Lactose fermentation	-	-	+	-	+	+	-
Motility	-	-	+	+	+	-	+
Catalase	+	-	-	-	-	-	-
Coagulase	+	-	-	-	-	-	-
Glucose	-	-	-	+	-	+	-
Urease	-	-	-	-	-	+	+
Indole	-	-	+	+	-	-	+
Oxidase	-	-	+	-	-	-	-

Key: + Positive
- Negative

Table 3: Percentage Frequency of Occurrence of Bacteria Isolates Obtained from the Meat Samples from Abattoir and Retail Outlets

Isolated bacteria	Total No.	Abattoir (Morning)	Ogboja Retail outlet (Morning)	Ogboja Retail outlet Late afternoon	Ishibori Retail Outlet (Morning)	Ishibori Retail Outlet Late afternoon	Percentage Occurrence (%)
Streptococci species	29	5	6	6	6	6	34.1
Escherichia coli	5	0	0	3	0	2	5.9
Staphylococcus species	26	6	5	5	4	6	30.6
Pseudomonas species	5	1	1	1	1	1	5.9
Proteus species	7	1	1	1	2	2	8.2
Salmonella species	10	0	2	2	2	4	11.8
Enterobacter species	3	1	1	1	0	0	3.5
Total	85	14	16	19	15	21	

Antibiotics Susceptibility Studies Against Isolated Pathogens inPercentage (%)

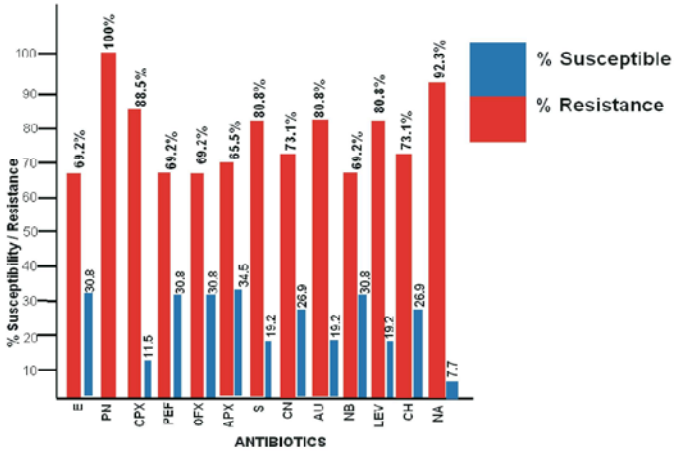

Fig. 1: Antibiotics Susceptibility and Resistance Pattern of *Staphylococcus* Isolated from Abattoir and Retail Outlets in Ogoja Urban.
KEY: E=Erythromycin (15µg) PN=Ampicillin (10µg) CPX=Ciprofloxacin (10µg) PEF=Peflacine (5µg) OFX=Tarivid (5µg) APX =Ampiclox (20µg) S=Streptomycin (10µg) CN=Gentamycin (10µg) Au= Augumentin (25µg) NB=Norfloxacin (10µg) LEV=Levofloxacin (5µg) CH=Chloramphenicol (30µg) Na =Nalidixic Acid (30µg)

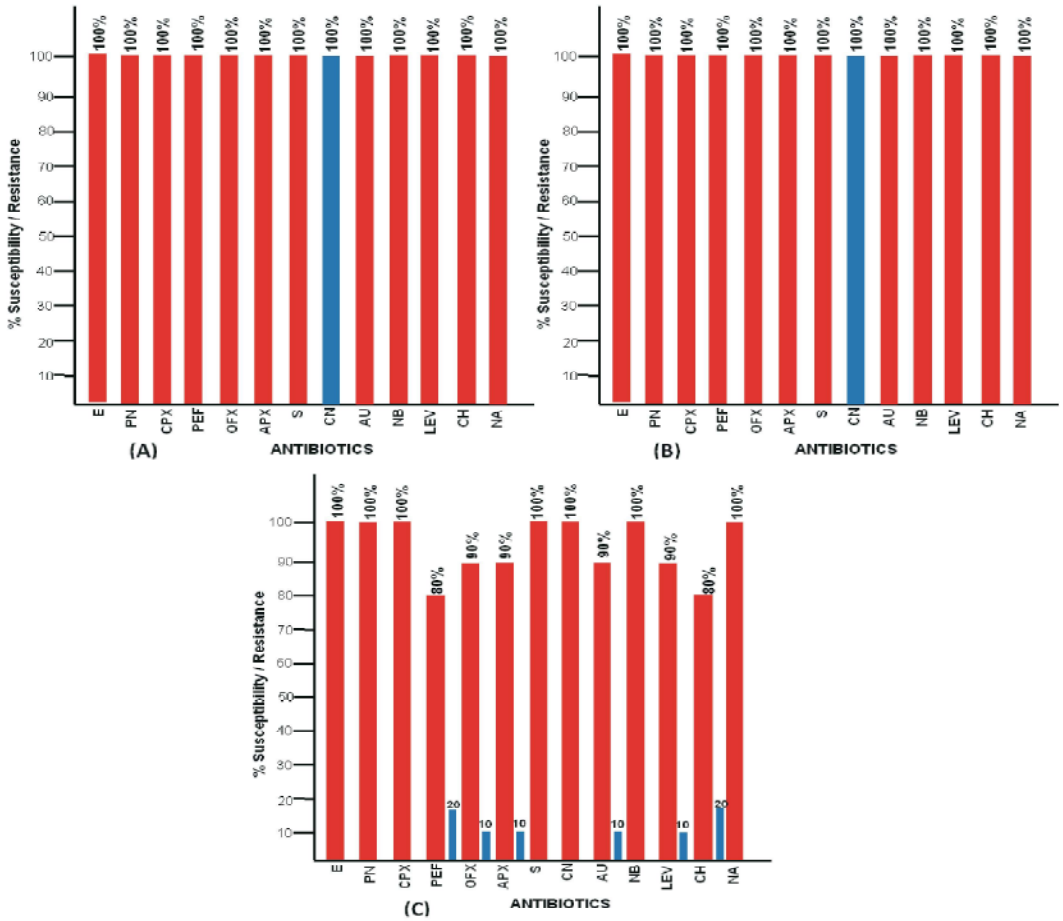

Fig. 2: Antibiotics Susceptibility and Resistance Pattern of *Pseudomonas* species (A), *Escherichia coli (*B) and *Salmonella* species (C) Isolated from Abattoir and Retail Outlets in Ogoja Urban

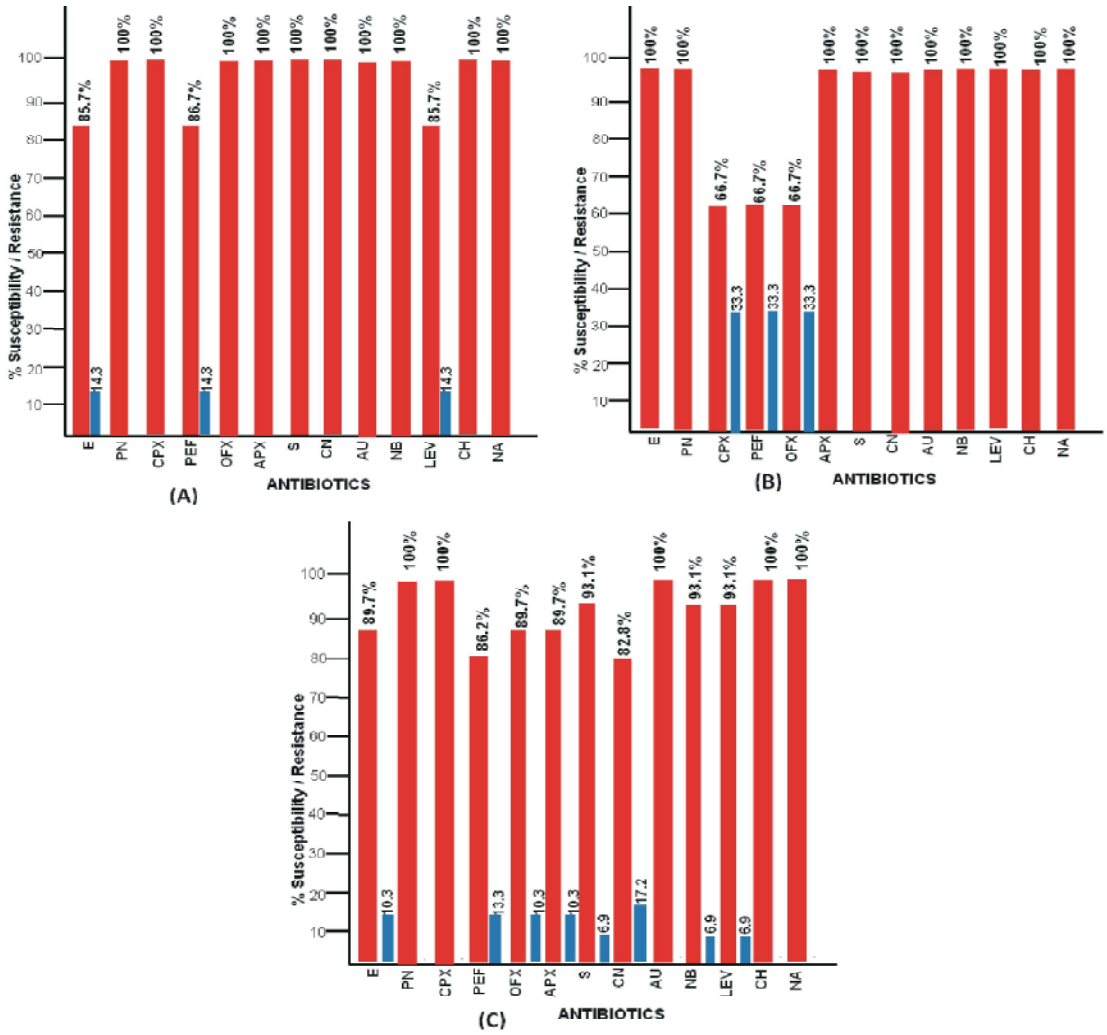

Fig. 3: Antibiotics Susceptibility and Resistance Pattern of *Proteus* species (A), *Enterobacter* species (B) and *Streptococcus* species (C) Isolated from Abattoir and Retail Outlets in Ogoja Urban.

Table 1, shows the estimation of total aerobic bacteria count on nutrient agar, the total aerobic count ranges from $2.8x10^4$ – $6.9x10^6$ cfu/g. Different types of bacteria were isolated from the meat samples at different times of the day, the bacteria isolated include *Streptococcus* species, *Salmonella* species, *Staphylococcus* species, *Proteus* species, *Pseudomonas* species, *Escherichia coli* and *Enterobacter* species.

Staphylococcus species and *Streptococcus* species were mostly isolated from both morning and evening samples from the various retail outlets and the abattoir; the bacteria isolated from the samples that were collected in the morning were also found in the samples collected evening of the same day, as also reported by [19].

Samples were not collected from the abattoir in the evening because they only slaughter and distribute out to sellers in various retail outlets in the morning. Samples that were taken from Ishibori had the least total aerobic count of $2.8x10^5$ cfu/g in the morning and Ogboja had the highest total aerobic count $4.4x10^5$ in the morning. While samples taken in the evening from Ishibori had $4.0x10^6$ as the least and Ogboja had the highest total aerobic count of $6.9x10^6$ cfu/g, from observation Table 1 result shows that the total aerobic count for the various samples from the different retail outlets increased with time.

The high bacteria counts from the meat sample in Ogboja retail outlets from this study could be attributed to the filthy environment hence the market especially the meat sellers point of business is closer to the bushy area with dirty stream that run by and dumping of waste in it.

Also the length of time that the sellers display or expose this meat to the harsh weather is a factor that contributed to the high bacteria load on samples collected from the retail outlets in the evening period. This is in accordance with the report of [20]. They reported in their study that high bacteria level on meat sample may be as a result of exposing the meat for a longer time in the market at high temperature. [21] also stated that, the longer meat surface is exposed to the environment the higher the microbial load. While the low total aerobic count in the morning could be that the environmental conditions were not favourable for the bacteria to grow, also the minimal processing operations within the morning hours. This finding is in line with the work of [10] who stated that, animal product may be microbiologically contaminated by organisms living in them naturally or organisms entering them from the surrounding such as those resulting from processing operations.

The possible sources of contaminations are due to the unhygienic practices of the meat handlers, right from the abattoir through transportation and the markets environment, as reported by [22].

The presence of these isolates in this fresh meat sample is an indication of faecal contamination of meat. Hence the isolation of *Salmonella species, Staphylococcus species, Streptococcus species and Escherichia coli* is worrying because certain strains of these bacteria cause food-borne infection [19]. Also [20] stated that careless sneezing and coughing among butchers can led to contamination of the product. The presence of the isolated bacteria on meat and other food has been widely reported in other parts of the world. This result is in line with that of [23] who reported the presence of *Escherichia coli, Streptococcus species, Salmonella species and Staphylococcus species* on beef sold in Tamale metropolis.

Escherichia coli and *Pseudomonas* Species were resistance to most of the antibiotic used in his study, but were susceptible to Gentamycin, this is in accordance to [24]. Of the entire antibiotic used, Ampicillin was resistance to all the isolates. While *Staphylococcus* was almost susceptible to all the antibiotics used. The varying zones of inhibition obtained may be due to their different diffusion rates [25].

CONCLUSION

Staphylococcus species, Escherichia coli, Proteus species, Enterobacter species and Pseudomonas species were isolated from meat sold in abattoir and retail outlets within Ogoja Urban though the microbial load was a little on the high side, this meat is not spoiled but due to the presence of pathogenic microbial contamination, meat sold in Ogoja may not be considered as safe products seeing the poor meat processing and sellers in a highly polluted environment.

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