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Isolation, Identification and Antibiogram of Bacterial Pathogens Gotten from Cow Meat Obtained from Different Market Sources

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Abstract

Food safety is of increasing concern among consumers of meat especially with reference to food borne microbes. In Nigeria, the consumption and sales of meat products are increasing. The aim of this work was to determine the microbial loads cow meat obtained from different market sources and antibiotic resistance of the isolates. Cow meat samples were randomly collected from local markets in five (5) different LGA in Ibadan, Oyo State, Nigeria and were subjected to microbial isolation and identification procedures using the serial dilution and pour plate methods and were identified using the colonial, microscopic, cultural, morphological and biochemical characteristics. The isolates were subjected to antibiotic susceptibility testing using the Kirby- Bauer disk diffusion method. Results obtained revealed that the predominant bacterial pathogen isolated was *Escherichia coli* followed by *Staphylococcus aureus, Salmonella* spp, *Campylobacter jejuni, Clostridium perfringes, Yersinia enterocolitica, Aeromonas* spp, *Pseudomonas aeruginosa and Shigella flexneri. S. aureus* and *Salmonella* spp were resistant to 80 % of all the antibiotics. *C. jejuni,* and *S. flexneri* showed resistance to 60% of the antibiotics while *C. perfringes* and *P. aeruginosa* showed resistance to 50 % of the antibiotics whereas, *E.coli* and *Aeromonas* spp. showed resistance of 40%. The lowest resistance of 30 % was recorded in *Y. enterocolitica.* The multiple antibiotic resistance index (MARI) of the isolates recovered in the present study indicates multidrug resistance in nature. The result of the study showed contamination of cow meat probably due to poor sanitary environment and dirty places during slaughtering, transportation and processing.

Keywords: Microbial load, Cow meat, Isolates, Antibiotic susceptibility, Multiple antibiotic resistance index

1.0 Introduction

In Nigeria, the consumption and sales of meat products are increasing. The most widely consumed meat is beef, the flesh of mature cow that normally weigh from 450 to 540 kg (1,000 to 1,200 pounds). Meat, the flesh or other edible parts of animals (usually domesticated cattle, swine, and sheep) used for food, including not only the muscles and fat but also the tendons and ligaments. Meat has a high water content corresponding to the water activity approximately 0.99 which is conducive for bacterial growth (Rao *et al.*, 2009).

Meat is valued as a complete protein food containing all the amino acids necessary for the human body. Cow meat is composed of about

20-45% protein; others are water, fat, phosphorus, vitamins and zinc (USDA, 2011). The fat of meat, which varies widely with the species, quality, and cut, is a valuable source of energy and also influences the flavor, juiciness, and tenderness of the lean. Parts such as livers, kidneys, hearts, and other portions are excellent sources of vitamins and of essential minerals, easily assimilated by the human system.

Pathogens are always a threat to livestock and domestic animals due to their exposure to the contaminated environments. Most meat contamination is from the heavily colonized areas of the animal, such as the skin (fleece) and the gastrointestinal tract, and the type and numbers found will reflect both the animal's indigenous microflora and its environment.

The processing of cow meat begins from buying of the cow and then taken to abattoirs for killing and processing as described by Komba et al. (2012). Meat can be further contaminated or cross-contaminated by various pathogenic bacteria after the slaughter process, such as during chilling, cutting, deboning, and slicing processes (Duffy *et al*., 2001). Thus, all processing conditions are important factors that can affect microbiological quality (Ukut *et al.*, 2010).

Antimicrobial drugs are used medically in animals and humans to treat bacterial infections, and they can also be added to commercial livestock and poultry feed at sub-therapeutic dosages to promote growth (Van den Boogard *et al*., 2001). During the twentieth century, antibiotics significantly reduced mortality associated with infectious diseases; however, their widespread and repeated use in animal farming has resulted in the emergence of bacterial multidrug resistance (MDR); as a result, the presence of antibiotic-resistant populations transforms infections that were once treatable into potentially life-threatening events. Antibiotics slow the growth of organisms without resistance mechanisms, but they have no or little effect on resistant infections, allowing them to survive and flourish in the host.

The safety of raw and processed meat has indeed become a great concern to public health officers' due to the degree of antibiotic resistant bacteria isolated from them. In spite of increased consumer demand for food safety standards for beef in Nigeria, there are still poor hygiene and sanitary practices along the food production chain, which contributes to unacceptable levels of microbial load in meat. This poses a health risk to consumers (Mtenga *et al.,* 2000).

This study therefore, was designed to determine the extent of bacterial contamination of cow meat processed and sold in markets within Ibadan, Oyo State, Nigeria, as well as their antibiotic susceptibility patterns. It is expected that the information obtained from this study, would help educate the public on possible vehicles for meat contamination and its link to outbreaks of multidrug resistance (MDR) bacteria.

2.0. Materials and Methods

2.1. Sample Collection

Five cow meat samples were collected from randomly selected retail markets located in different local governments in Ibadan, Oyo State. About 100 grams of meat samples were collected in clean, dry and sterile polythene bags and transported to the laboratory within 1-2 hours of collection for microbiological analysis or refrigerated at 4°C till further analysis were carried out and processed no later than 48 hours after purchase. Sample type, source and other relevant data were recorded for each sample.

The materials and reagents used in this study include: Sterile bags, sterile knife, conical flasks, test tubes, weighing balance, disposable petri dishes, pipette, cotton wool, laboratory mortar and pestle, spatula, sterile distilled water, sterile nutrient broth, nutrient agar, nutrient broth, MacConkey broth, eosin methylene blue (EMB) agar and MacConkey agar

2.2. Preparation of Sample Homogenate.

At the laboratory, the samples were aseptically cut into thin smaller pieces using a sterile knife. Twenty-five grams of the cut cow meat sample was weighed using a weighing balance (S. Mettler), it was then transferred into a laboratory mortar and pestle. It was pounded for 10 minutes. The pounded cow meat was added to a blender jar capacity 500 ml and 225 ml peptone water (0.1%) and was mixed for 2 minutes. Ten-fold dilutions were prepared under aseptic conditions from each sample using 0.1% peptone water as diluents, this resulted in a dilution of 10^{-1} decimal dilutions of 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} was then prepared by serial dilution.

2.3. Serial Dilution and Isolation of Microorganism

A ten-fold dilution was prepared using 0.1% of peptone water as diluent. Assembly of five test-tubes was labeled. Each test-tube according to dilution factor (10^{-1},10^{-2}, 10^{-3},10^{-4}, 10^{-5}). A 9 ml portion of peptone water was added to each test-tube and 1ml of the sample homogenate was dispensed into the first test-tube (10^{-1}) using a sterile syringe, the solution was mixed gently and properly. Further dilution was carried out on the remaining 4 test tubes. Dilutions 10^{-3} and 10^{-5} were inoculated into nutrient agar, nutrient broth, EMB and MacConkey broth, MacConkey agar through the pour plate method. The plates were incubated at 37 ^{o}C for 24 hours. After 24 hours, the plates were retrieved and the number of colonies were counted. The differential media plates were also observed for growth. The color, size, shape and elevation of the colonies were observed.

2.4. Microscopy and Colony Morphology

Identification, characterization and identification of the colony isolates was achieved by initial morphological examination of the colonies in the plate (macroscopically) for colonial appearance, size, elevation, form, edge, consistency, color, odor, opacity, and pigmentation and the results were recorded. Gram's staining from the colonies provided a preliminary identification of the pathogenic bacteria

2.5. Biochemical Characterization

Biochemical characterization of the bacteria was done by performing specific tests such as Catalase test, oxidase test, Indole test, Methyl red, Voges Proskauer, Citrate tests, Sugar fermentation tests, and Coagulase test.

2.6. Identification and Characterization of Isolated Bacteria

The various bacteria colonies were identified based on their colonial, morphological and biochemical characteristics

2.7. Antibiogram of Isolated Bacteria

Antibiogram potential of the isolates were determined according to the modified Kirby-Bauer disc diffusion method using Nutrient agar following Clinical Laboratory Standards Institute guidelines (CLSI Performance Standards for Antimicrobial Susceptibility Testing).

Antibiotic susceptibility test was carried out using Gram- positive and Gram- negative standard antibiotic disk. Antibiotics used are Gram –ve discs including; Septrin (30µg), Sparfloxacin (30µg), Gentamicin (30µg), Augmentin (30µg), Chloramphenicol (30µg), Ciprofloxacin (30µg), Amoxicillin (30µg), Perfloxacin (30µg), Tarivid (30µg), Streptomycin (30µg) and Gram +ve discs including; Perfloxacin (30µg), Gentamicin (30µg), Ampiclox (30µg), Zinacef (30µg), Erythromycin (30µg), Ciprofloxacin (30µg), Amoxicillin (30µg), Rocephin (30µg), Streptomycin (30µg), Septrin (30µg).

Nutrient agar plates were prepared and seeded with bacterial inoculum using the pour plate method. After gelling, antibiotic discs were placed on the surface of the agar using sterilized forceps. The antibiotic discs were gently pressed to be in contact with the surface of the agar. The inoculated plates were carefully inverted and incubated for 24 hours at 37^{0}C. After incubation,

the plates were observed for zones of inhibition. A metric ruler was used to measure the diameter of the zone of inhibition for each antibiotic used. The values obtained for the zones of inhibition from the individual antibiotics were compared with the standard table of interpretation of zones of inhibition (in mm) for Kirby-Bauer antibiotic susceptibility test to determine the sensitivity zone. The measurement obtained from the individual antibiotics were compared with the standard table of interpretation of zones of inhibition (in mm) for Kirby-Bauer antibiotic susceptibility test to determine whether the tested bacteria species is sensitive or resistant to the tested antibiotics according to Clinical and Laboratory Standard Institutions (CLSI) recommendations

2.8. Determination of Multiple Antibiotic Resistance Index (MARi).

The MAR index for the resistant bacteria isolates was determined according to the procedure described by Krumperman (1983). This is essentially to determine the degree of bacterial resistance to antibiotics. The indices were determined by dividing the number of antibiotics to which the organism were resistant to (a) by the number of the antibiotics tested (b), Resistance to three or more antibiotics is taken as MAR and MAR greater than 0.2 indicates a high risk source of contamination.

3.0. Results and Discussion

3.1. Total Bacteria Counts

The total bacteria count of the isolates from samples are shown in Table 1. Sample B was more predominant with a total viable count (TVC) of 8.5 $x10^{-5}$ cfu/ml while the total coliform count (TCC) was $2.4x10^{-5}$ cfu/ml, Sample D follows closely with a TVC of 6.2 $x10^{-5}$ and TCC of 1.35 $x10^{-5}$ cfu/ml. Sample A has a TVC of 4.4 $x10^{-5}$ and TCC of $1.23x10^{-5}$, Sample E has a TVC of 3.5 $x10^{-5}$ and a TCC of $1.6x10^{-5}$. Sample C has the least count with the TVC of 2.6 $x10^{-5}$ cfu/ml, conversely, the TCC was very high at $7.3x10^{-5}$ cfu /ml as presented in Table 1.

Table 1: Total Bacterial Counts

Sample	NA Total viable counts cfu/g	MAC Total coliform counts cfu/g
A	4.4×10^{-5}	1.23×10^{-5}
B	8.5×10^{-5}	2.4×10^{-5}
C	2.6×10^{-5}	7.3×10^{-5}
D	$6.2\ x10^{-5}$	$1.35\ x10^{-5}$
E	$3.5\ x10^{-5}$	$1.6x10^{-5}$

Legend: NA- Nutrient Agar, MAC- MacConkey Agar

3.2. Cultivation and Cultural Presentation of Isolates.

The colonies observed were grown on EMB agar, MacConkey agar, Nutrient agar, Nutrient broth. Their shape, elevation, surface, mode of spread, color and texture were observed. The predominant bacterial pathogen isolated was *Escherichia coli* found in all the cow meat samples, followed by *Staphylococcus aureus*, *Salmonella* spp, *Campylobacter jejuni*, *Clostridium perfringes, Yersinia enterocolitica*, *Aeromonas* spp, *Pseudomonas aeruginosa and Shigella flexneri*

3.3. Identification of Isolates.

After being subjected to morphological, cultural and biochemical tests (including Gram staining, sugar fermentation, catalase test, Vogeus Proskeur test, oxidase test, coagulase test and sugar fermentation test). The isolates were characterized to species level using the Bergey's manual. The following isolates were identified; *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp, *Campylobacter jejuni*, *Clostridium perfringes, Yersinia enterocolitica*, *Aeromonas* spp, *Pseudomonas aeruginosa and Shigella flexneri*

Table 2. Antibiotic Resistance Profile of Test Organisms to Standard Antibiotic

	Diameters zone of inhibition in mm															
Test organism/ Antibiotics	*S. aureus*		*Salmonella spp*		*C. jejnui*		*E. coli*		*C. perfringes*		*Y. enterocolitica*		*Aereomonas spp*		*S. flexneri*		*P. aeruginosa*	
Septrin	-	R	10	S	10	S	10	S	10	S	7	R	10	S	-	R	6	R
Ciprofloxacin	8	R	5	R	-	R	10	S	1	R	10	S	10	S	-	R	10	S
Perfloxacin	12	S	-	R	12	S	12	S	12	S	12	S	-	R	10	S	5	R
Gentamicin	-	R	-	R	10	S	10	S	10	S	10	S	10	S	7	R	5	R
Ampiclox	-	R	-	R	5	R	-	R	12	S	-	R	5	R	-	R	-	R
Zinacef	5	R	-	R	-	R	-	R	10	S	12	S	10	S	6	R	12	S
Amoxicillin	7	S	-	R	7	R	5	R	10	S	10	S	5	R	-	R	10	S
Rocephin	-	R	-	R	5	R	-	R	6	R	10	S	-	R	5	R	-	R
Streptomycin	10	S	-	R	10	S	15	S	5	R	-	R	15	S	10	S	6	R
Erythromycin	-	R	10	S	10	S	5	R	8	R	10	S	10	S	5	R	5	R
Chloramphenicol	7	R	-	R	10	S	10	S	2	R	-	R	12	S	-	R	-	R
Sparfloxacin	-	R	5	R	12	S	-	R	-	R	-	R	5	R	12	S	-	R
Augmentin	-	R	5	R	6	R	4	R	10	S	-	R	10	S	10	S	12	S
Tarivid	-	R	7	R	10	S	-	R	-	R	10	S	7	R	5	R	10	S

Legend; R—Resistant, S—Susceptible, - --- No activity

3.4. Antibiotic Profile of Test Isolates:

Table 2 presents the antibiotic profile of the test isolates using standard antibiotics. Zones of inhibition with values lower than 10mm are designated resistant while values ≥10 are designated as sensitive. *S. aureus* was resistant to all the antibiotics used except Perfloxacin and Streptomycin with a percentage resistance of 80%. *Salmonella* spp was resistant to all antibiotics used except Septrin, and Erythromycin with a resistant factor of 80%. *Campylobacter jejuni* was resistant to six of the antibiotics used which include, Ciprofloxacin, Augmentin, Amoxicillin, Ampiclox, Zinacef, Rocephin and with a resistance factor of 60%.

However, *Escherichia coli* was sensitive to six of the antibiotics used including Septrin, Chloramphenicol, Ciprofloxacin, Gentamicin, Perfloxacin, and Streptomycin with a percentage resistance of 40%. Similarly, *Clostridium perfringes* showed high resistance to Chloramphenicol, Streptomycin, Ciprofloxacin, Rocephin and Erythromycin with percentage resistance of 50%. *Yersinia enterocolitica* was also sensitive to Ciprofloxacin, Perfloxacin, Tarivid, Amoxicillin, Zinacef, Rocephin and Erythromycin with a resistance factor of 30%. Also, *Aeromonas* spp. was resistant to Sparfloxacin, Perfloxacin, Tarivid, Ampiclox, Zinacef and Erythromycin with 40% resistance factor. *Shigella flexneri* was sensitive to Chloramphenicol, Augmentin, Perfloxacin, and streptomycin with 60 % resistance factor and *Pseudomonas aeruginosa* was sensitive to Ciprofloxacin, Augmentin, Tarivid, Amoxicillin, and Zinacef with a percentage resistance of 50%.

Table 3: Multiple Antibiotic Resistance Index

	MAR Index	
Test isolates	**A**	**B**
Escherichia coli	0.4	0.5
Staphylococcus aureus	0.6	0.6
Salmonella spp	0.9	0.8
Campylobacter jejuni	0.3	0.4
Clostridium perfringes	0.5	0.4
Yersinia enterolitica	0.5	0.3
Aeromonas spp	0.4	0.4
Shigella flexneri	0.6	0.8
Pseudomonas aeruginosa	0.6	0.7

Legend: A – Gram –ve antibiotics, B- Gram +ve antibiotics

The result of the antibiotic susceptibility profile shows that all the isolates have a high degree of antimicrobial resistance to the antibiotics used. Antibiotic resistance has been a major menace to the human race. The antibiotic resistance pattern of the bacterial isolates shows the high incidence of multi-drug resistant bacterial contaminants in meat. According to Ali *et al.* (2010), the observed high resistance of some of the isolates could be attributed to the use of antibiotics to treat cows or their addition in cow feed and water, which surely can precipitate resistance development by such isolated bacteria species against known antibiotics. Antimicrobial resistance among food borne bacterial pathogens tends to occur in food/animals before it occurs in humans (White *et al.*, 2002).

The multiple antibiotic resistance index (MARI) of the isolates (Table 3) recovered in the present study indicates multidrug resistance in nature. The MARI value > 0.2 is suggesting multidrug resistance due to high-risk application and contamination of antibiotics (Joseph *et al*,.2017)

Handling of the animals, misuse of antibiotics, and other factors might be responsible for the differences certainly and not only geographical location. Overall, the high prevalence of *E. coli* and *S. aureus* occurrence over other isolates can be explained by the members of normal flora in animals; however, occurrence of *E. coli*, *Salmonella* spp., *Shigella* spp., and *Staphylococcus aureus* is a pointer to high burden that have potential risk to animals and human health (El- Jakee *et al*., 2008). It is a known fact that *Staphylococcus aureus* and *Pseudomonas* spp. are of pathogenic and public health importance (Cheesbrough, 2000).

This states the role of raw food as is the case of cow meat as a reservoir of antibiotic resistant bacteria which can be transferred to humans thereby causing gastrointestinal disorders and food borne illness which can be life threatening. It is imperative that basic hygienic practices be incorporated in abattoirs and retail meat outlets to ensure food safety.

4.0 Conclusion

This study reveals that cow meat is a very dangerous food if the appropriate hygiene measurement is not applied during slaughtering, cleaning, transportation, packing and marketing. It could be contaminated with many bacteria that could pose a life threat on public health level. For safety purposes, this study recommends that there is an urgent necessity to minimize the contamination of meat sold in market places by

proper sanitation and inspection practices. Proper amenities must be provided in abattoirs, especially those located in rural areas. There should be routine periodic microbial quality tests conducted also in the various abattoirs by public health officers while initiating and sustaining enlightenment campaigns on relevant advanced technique(s). Regular disinfection of the abattoirs is advocated to reduce or eliminate possible pathogenic organisms that could cause food borne diseases or illnesses. Abattoirs should be sited far away from residential areas to reduce cross transmission, while all categories of workers in the abattoirs should be reminded via seminars, workshops, posters and/or signboards, on the need for safe hygienic practices. Training should be given to meat handlers and butchers regarding food safety practices and proper inspection procedures should be strictly adhered to minimize the contamination of raw meat and meat products sold in the marketplace.

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