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Research Article

Antibiotic Resistant Organisms in Retail Beef Sold in Ovia North and South Local Government Areas of Edo State Nigeria

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Abstract

The emergence of antimicrobial-resistant organisms in retail beef is associated with the use of antibiotics in food producing animals. This is of major public health significance arising from the risk of these bacteria entering the food chain. The antimicrobial resistance of organisms obtained from retail beef sold in markets in Ovia North and South Local Government areas of Edo state, Nigeria was determined. Six samples of retail beef were obtained randomly from beef vendors in five specified markets in Ovia Local Government Areas of Edo state. A total of forty six isolates were obtained from the sampled retail beef using standard isolation techniques. The isolates present included *Klebsiella* spp., *Staphylococcus* spp., *Proteus* spp., *Pseudomonas* spp. and *Escherichia coli*. The antibiotic susceptibility test revealed the presence of antibiotic resistant organisms among the sampled retail beef. Antimicrobial resistant organisms should be continuously monitored to improve the management of the risks associated with antibiotic resistance transferred from retail beef to humans.

Keywords: Antibiotic resistance; Retail beef; Microorganisms

Introduction

The culinary name for meat from bovines, especially cattle is beef. Beef can be gotten from cows, bulls, heifers or steers. Beef is amajor source of protein and an important source of vitamins for most people in many areas of the world. It is also the third most widely consumed meat in the world, accounting for about 25% of meat production, after pork and poultry at 38% and 30% respectively [1]. As a result of the extensive and semi-extensive animal production system in Nigeria, Beef is one of the main supplies of meat in Nigeria [2].

Possible sources of contamination of retail meat include slaughtering of sick animals, cleaning of meat with dirty water, unhealthy condition of butcher and handlers, contamination by flies, processing of animals close to sewage or refuse dumps and slaughtering of animals using contaminated equipment's such as knife and other utensils [3,4]. As a result of its high nutritive value, microorganisms can easily grow on beef. Most meat has a high water content which is suitable for microbial growth [5]. The microbial load in beef increases as long as favorable growth conditions exist.

The occurrence of antibiotic resistant organisms in retail beef is associated with the use of antibiotics in food producing animals [6-8]. There is an increase inhuman infection involving antimicrobial-resistant bacteria. For example, animals are recognized as reservoirs for human intestinal pathogenic *E. coli* and a source for human extra intestinal pathogenic *E. coli* [9]. As a result of antimicrobial agents commonly used for food-producing animals in farms, human infections involving antimicrobial resistant extra intestinal pathogenic *E. coli* transferred from animals could be more difficult to treat [10]. Therefore, the monitoring of bacterial resistance to antimicrobial agents is essential especially in developing countries like Nigeria that lack adequate data regarding antibiotic resistance. The aim of this study was to determine possible antibiotic resistant organisms obtained from retail meat from five different markets in Ovia Local Government areas of Edo state.

Materials and Method

Sample collection

A total of thirty retail beef samples were obtained randomly from vendors in five specified markets in Ovia Local Government Areas of Edo state. Six samples were randomly obtained per market. The samples were aseptically packaged and transported to the laboratory for microbiological analysis.

Microbiological evaluation of samples

A preliminary enrichment step was used for the samples. Meat samples (a gram each) were suspended in 9 ml of peptone water. After incubation for about 18 hours at 37°C, the enrichment culture was plated with onto selective agar medium (MacConkey, Eosin methylene blue, Mannitol salt and Sabouraud dextrose agar) before another incubation at 37°C for 24 hours. Plates were observed for clear growth of organisms. Isolates were identified and characterized based on their cultural characteristics and biochemical reactions. Standard microbiological/biochemical methods were used in the identification of bacteria [11]. The identification test included gram reaction, citrate, urease, oxidase, methyl red and Voges Proskauer test.

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Antibiotic susceptibility test

Antibiotic susceptibility patterns of the microbial isolates were tested against a number of antibiotics which include Ceftazidime (30 µg), Cefuroxime (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Ofloxacin (5 µg), Amoxicillin/clavulanate (30 µg), Nitrofurantoin (30 µg), Ampicillin (10 µg), Ceftriaxone (30 µg), Erythromycin (5 µg), Ofloxacin (300 µg) using Kirby and Bauer disc diffusion methods of determining susceptibility [12].

Results

Out of the 30 samples examined, different number of isolates was obtained from each of the markets. Table 1 shows the location of the markets and number of isolates from samples obtained in the markets.

The isolated bacteria obtained from the meat samples from all the markets include *Klebsiella* sp., *Staphylococcus* sp., *Proteus* sp., *Pseudomonas* sp. and *Escherichia coli.* Table 2 shows the frequency distribution of isolated organisms from the examined samples in the different markets. *Klebsiella* sp. had the highest occurrence among the bacteria isolated from the samples in all the markets.

Table 1: Location and number of isolates from samples obtained in the markets.

Location	No. of isolates	Frequency of isolates %
Iguobazua	17	37
Okada	8	17.4
Ugbogi	6	13
Usen	6	13
Utese	9	19.6
Total	46	

Table 2: Frequency distribution of isolated organisms from the examined samples in the different markets.

Bacterial isolates	IGUOBAZUA	Okada	Ugbogi	Usen	Utese	Total No.	Frequency %
Klebsiella sp.	5	6	2	2	7	22	47.8
Staphylococcu spp.	3	-	2	-	-	5	11
Proteus sp.	2	2	-	1	1	6	13
Pseudomona spp.	4	-	1	1	1	7	15.2
E.coli	3	-	1	2	-	6	13

Using the Clinical Laboratory and Standard Institute (CLSI guidelines) for determining susceptibility, the antibiotic susceptibility test showed the presence of isolates resistant to antibiotics tested (Tables 3-8).

Discussion

This study evaluated the microbial quality of retail meat in markets in Ovia north and south local government areas of Edo state. The high number of isolates enumerated in the retail beef samples from the markets indicated that the samples were contaminated. This study showed contamination of the samples analyzed with different kinds of micro-organisms. The micro-organism with the highest rate of occurrence in the markets sampled was *Klebsiella* sp. while the least one was *Staphylococcus* sp. Micro-organisms can easily be introduced in either the pre or post processing stages of meat. This study is a reflection of the unhygienic practices of meat processing in developing countries. Knives, hands and clothing's of the workers, abattoir environment, slaughter slabs, physical facilities can serve as intermediate sources of contaminants. During handling, contamination could come from containers, other contaminated meat, air and personnel resulting in an increase in the microbial load of retail fresh beef samples. Previous reports show that retail cut could also result in an increase in microbial load as a result of the large exposed surface area, more readily available water, nutrient and greater oxygen available [13,14]. Hence, smaller retail cuts displayed in the markets creates a good environment for microbial growth and proliferation which leads to the spoilage of the meat [15]. *Klebsiella* sp. was the most prevalent organism in the study. Previous reports show the presence of bacteria in meat samples [16-18]. The study report a high number of Gram negative bacteria compared to the Gram positive bacteria in the retail meat samples. This supports previous studies that reported a high incidence of Gram negative bacteria in meat samples [17-20]. The presence of these organisms in the retail samples is indicative of a public health hazard and gives a signal of possible occurrence of food borne intoxication and infection. This also implies that these contaminated meats are good sources of various diseases which could spread and acquire epidemic status posing serious health hazards.

Table 3: Distribution of antibiotic resistance among Gram-negative bacteria isolates from Usen market. Zone of inhibition (mm) of antibiotics used.

Bacterial isolates	GEN	CPR	OFL	CRX	CAZ	AMP	AUG	NIT
E.coli	21	-	-	-	-	-	-	14
Klebsiella sp.	NG	-	-	-	18	-	-	-
E.coli	17	-	-	-	13	-	-	-
Klebsiella sp.	15	-	-	-	-	-	-	13
Pseudomonas sp.	-	-	14	14	19	-	-	-
Proteus sp.	14	-	-	-	-	-	-	12

CAZ: Ceftazidime (30 µg); GEN: Gentamicin (10 µg); CPR: Ciprofloxacin (5 µg); OFL: Ofloxacin (5 µg); AUG: Amoxycillin/Clavulanate (30 µg); NIT: Nitrofurantoin (30 µg); AMP: Ampicillin (10 µg); CRX: Cefuroxime (30 µg); -: No zone of inhibition

High level of resistance to antibiotics tested was observed among the bacterial isolates which are of significant interest. A possible explanation is the use of some of these antibiotics in food producing animals. Antibiotics have been reported to be useful in the prevention and control of infections caused by bacteria however, their indiscriminate use can have adverse results by promoting the selection and prevalence of drug resistant bacterial populations [21,22]. Factors responsible for the high antibiotic resistance observed may be due to the natural resistance of species to certain antibiotics [23], possible transfer of antibiotic resistance among species, and the use of sub-therapeutic antibiotic doses in animal feeds to improve animal productivity, which could also select for antibiotic resistant strains [22]. Piddock [24] reported possible ways in which antibiotics could pose a risk to human health which include selection of antibiotic resistant pathogens in animal. Food products can become contaminated during slaughter and /or food preparation. When contaminated food is ingested, it causes infection which requires antibiotic therapy and therapy becomes ineffective due to resistant strains. Selection of resistant non-pathogenic bacteria in animals which can be transferred to humans by consuming contaminated food products and resistant genes are subsequently transferred to other bacteria present in the gut is another way antibiotics may harmful to human health. Antibiotics may remain as residues in animal products such as meat and milk which can also be sources of resistant bacteria in the consumer of the food products [25]. The susceptibility results of the bacteria isolates shows the Gram negative bacteria were highly resistant compared to the Gram positive bacteria. The results correlates with that of Iroha, et al. [17] who reported Gram-negative organisms to be more resistant than the Gram-positive bacteria in the meat samples analyzed. This high resistance could be as a result of the intrinsic nature of gram-negative cell wall. The gram-negative micro-organisms isolated in our

Table 4: Distribution of antibiotic resistance among Gram-negative and Gram-positive bacteria isolates from Ugbogi market. Zone of inhibition (mm) of antibiotics used.

Bacterial isolates	GEN	CPR	OFL	CRX	CAZ	AMP	AUG	NIT	OFL*	ERY	CTR
Klebsiella sp.	21	-	-	18	13	-	-	-	-	-	-
Staphylococcus sp.	16	-	-	-	12	-	-	-	-	-	26
Staphylococcus sp.	14	-	-	-	-	-	-	-	-	-	-
Pseudomonas sp.	16	-	-	14	14	-	-	-	-	-	-
E.coli	14	-	-	-	-	-	-	-	-	-	-
Klebsiella sp.	14	-	-	-	-	-	-	12	-	-	-

CAZ: Ceftazidime (30 µg); GEN: Gentamicin (10 µg); CPR: Ciprofloxacin (5 µg); OFL: Ofloxacin (5 µg); AUG: Amoxycillin/clavulanate (30 µg); NIT: Nitrofurantoin (30 µg); AMP: Ampicillin (10 µg); CRX: Cefuroxime (30 µg); CTR: Ceftriaxone (30 µg); ERY: Erythromycin (5 µg); OFL*: Ofloxacin (300 µg); -: No zone of inhibition

Table 5: Distribution of antibiotic resistance among Gram-negative and Gram-positive bacteria isolates from Iguobazua market. Zone of inhibition (mm) of antibiotics used.

Bacterial Isolates	GEN	CPR	OFL	CRX	CAZ	AMP	AUG	NIT	OFL*	ERY	CTR
Pseudomonas sp.	14	-	-	-	-	-	-	15	-	-	-
Pseudomonas sp.	17	-	-	22	19	-	-	-	-	-	-
Staphylococcus sp.	-	-	-	3	-	-	-	-	-	-	13
Klebsiella sp.	31	-	-	-	-	-	-	13	-	-	-
Staphylococcus sp.	14	-	-	26	16	-	-	-	-	-	-
Klebsiella sp.	11	-	-	-	-	-	-	16	-	-	-
Proteus sp.	12	-	-	22	13	-	-	15	-	-	-
Klebsiella sp.	30	-	-	-	-	-	-	-	-	-	-
Staphylococcus sp.	14	-	-	-	16	-	-	-	-	-	28
Pseudomonas sp.	16	-	-	14	14	-	-	-	-	-	-
E.coli	14	-	-	-	-	--	-	-	-	-	-
E.coli	32	-	-	-	-	-	-	-	-	-	-
Klebsiella sp.	14	-	-	-	13	-	-	17	-	-	-
E.coli	-	-	-	-	18	-	-	-	-	-	-
Klebsiella sp.	17	-	-	-	13	-	-	-	-	-	-
Pseudomonas sp.	15	-	-	-	-	-	-	13	-	-	-
Proteus sp.	13	-	12	16	-	-	-	-	-	-	-

CAZ: Ceftazidime (30 µg); GEN: Gentamicin (10 µg); CPR: Ciprofloxacin (5 µg); OFL: Ofloxacin (5 µg); AUG: Amoxycillin/clavulanate (30 µg); NIT: Nitrofurantoin (30 µg); AMP: Ampicillin (10 µg); CRX: Cefuroxime (30 µg); CTR: Ceftriaxone (30 µg); ERY: Erythromycin (5 µg); OFL*: Ofloxacin (300 µg); -: No zone of inhibition

Table 6: Distribution of antibiotic resistance among Gram-negativebacteria isolates from Okada market. Zone of inhibition (mm) of antibiotics used.

Bacterial isolates	GEN	CPR	OFL	CRX	CAZ	AMP	AUG	NIT
Klebsiella sp.	21	-	-	-	-	-	-	19
Klebsiella sp.	13	-	-	-	18	-	-	13
Klebsiella sp.	-	-	-	-	13	-	-	-
Klebsiella sp.	18	-	-	-	16	-	-	-
Klebsiella sp.	16	-	-	14	14	-	-	-
Klebsiella sp.	14	-	-	-	-	-	-	-
Proteus sp.	21	-	-	-	-	-	-	14
Proteus sp.	14	-	-	-	13	-	-	17

CAZ: Ceftazidime (30 µg); GEN: Gentamicin (10 µg); CPR: Ciprofloxacin (5 µg); OFL: Ofloxacin (5 µg); AUG: Amoxycillin/clavulanate (30 µg); NIT: Nitrofurantoin (30 µg); AMP: Ampicillin (10 µg); CRX: Cefuroxime (30 µg); CTR: Ceftriaxone (30 µg); ERY: Erythromycin (5 µg); OFL*: Ofloxacin (300 µg); -: No zone of inhibition

Table 7: Distribution of antibiotic resistance among Gram-negative bacteria isolates from Utese market. Zone of inhibition (mm) of antibiotics used.

Bacterial isolates	GEN	CPR	OFL	CRX	CAZ	AMP	AUG	NIT
Klebsiella sp.	17	-	-	-	13	-	-	-
Pseudomonas sp.	15	-	-	-	-	-	-	13
Proteus sp.	13	-	12	16	-	-	-	-
Klebsiella sp.	21	-	-	-	-	-	-	19
Klebsiella sp.	13	-	-	-	18	-	-	13
Klebsiella sp.	-	-	-	-	13	-	-	-
Klebsiella sp.	18	-	-	-	16	-	-	-
Klebsiella sp.	16	-	-	14	14	-	-	-
Klebsiella sp.	14	-	-	-	-	-	-	-

CAZ: Ceftazidime (30 µg); GEN: Gentamicin (10 µg); CPR: Ciprofloxacin (5 µg); OFL: Ofloxacin (5 µg); AUG: Amoxycillin/clavulanate (30 µg); NIT: Nitrofurantoin (30 µg); AMP: Ampicillin (10 µg); CRX: Cefuroxime (30 µg); CTR: Ceftriaxone (30 µg); ERY: Erythromycin (5 µg); OFL*: Ofloxacin (300 µg); -: No zone of inhibition

Table 8: Resistant Phenotype of isolates obtained from samples from the markets.

Bacterial isolates	Resistant Phenotype [Antibiotics which isolates showed no zone of inhibition]	No of antibiotics isolates are resistant to
E.coli	CPR, OFL, CRX, CAZ, AMP, AUG	6
Klebsiella sp.	GEN, CPR, OFL, CRX, AMP, AUG, NIT	7
E.coli	CPR, OFL, CRX, AMP, AUG, NIT	6
Klebsiella sp.	CPR, OFL, CRX, CAZ, AMP, AUG	6
Pseudomonas sp.	GEN, CPR, AMP, AUG, NIT	5
Proteus sp.	CPR, OFL, CRX, CAZ, AMP, AUG	6
Ugbogi market		
Klebsiella sp.	CPR, OFL, AMP, AUG, NIT	5
Staphylococcus sp.	CRX, ERY, AUG, OFL	4
Staphylococcus sp.	CAZ, CRX, CTR, ERY, AUG, OFL	6
Pseudomonas sp.	CPR, OFL, AMP, AUG, NIT	5
E. coli	CPR, OFL, CRX, CAZ, AMP, AUG, NIT	7
Klebsiella sp.	CPR, OFL, CRX, CAZ, AMP, AUG	6
Iguobazua market		
Pseudomonas sp.	CPR, OFL, CRX, CAZ, AMP, AUG	6
Pseudomonas sp.	CPR, OFL, AMP, AUG, NIT	5
Staphylococcus sp.	CAZ, GEN, ERY, AUG, OFL	5
Klebsiella sp.	CPR, OFL, CRX, CAZ, AMP, AUG	6
Staphylococcus sp.	CTR, ERY, AUG, OFL	4
Klebsiella sp.	CPR, OFL, CRX, CAZ, AMP, AUG	6
Proteus sp.	CPR, OFL, AMP, AUG	4
Klebsiella sp.	CPR, OFL, CRX, CAZ, AMP, AUG, NIT	7
Staphylococcus sp.	CRX, ERY, AUG, OFL	4
Pseudomonas sp.	CPR, OFL, AMP, AUG, NIT	5
E.coli	CPR, OFL, CRX, CAZ, AMP, AUG, NIT	7
E.coli	CPR, OFL, CRX, CAZ, AMP, AUG, NIT	7
Klebsiella sp.	CPR, OFL, CRX, AMP, AUG	5
E.coli	GEN, OFL, CPR, CRX, AMP, AUG, NIT	7
Klebsiella sp.	CPR, OFL, CRX, AMP, AUG, NIT	6
Pseudomonas sp.	CPR, OFL, CRX, CAZ, AMP, AUG	6
Proteus sp.	CPR, CAZ, AMP, AUG, NIT	5
Okada market		
Klebsiella sp.	CPR, OFL, CRX, CAZ, AMP, AUG	6
Klebsiella sp.	CPR, OFL, CRX, AMP, AUG	5
Klebsiella sp.	GEN, CPR, OFL, CRX, AMP, AUG, NIT	7
Klebsiella sp.	CPR, OFL, CRX, AMP, AUG, NIT	6
Klebsiella sp.	CPR, OFL, AMP, AUG, NIT	5
Klebsiella sp.	CPR, OFL, CRX, CAZ, AMP, AUG, NIT	7
Proteus sp.	CPR, OFL, CRX, CAZ, AMP, AUG	6
Proteus sp.	CPR, OFL, CRX, AMP, AUG	5
Utese market		
Klebsiella sp.	CPR, OFL, CRX, AMP, AUG, NIT	6
Pseudomonas sp.	CPR, OFL, CRX, CAZ, AMP, AUG	6
Proteus sp.	CPR, CAZ, AMP, AUG, NIT	5
Klebsiella sp.	CPR, OFL, CRX, CAZ,AMP, AUG	6
Klebsiella sp.	CPR, OFL, CRX, AMP, AUG	5
Klebsiella sp.	GEN, CPR, OFL, CRX, AMP, AUG, NIT	7
Klebsiella sp.	CPR, OFL, CRX, AMP, AUG, NIT	6
Klebsiella sp.	CPR, OFL, AMP, AUG, NIT	5
Klebsiella sp.	CPR, OFL, CRX, CAZ, AMP, AUG, NIT	7

study belong to the *enterobacteriaceae* family, a group of organisms that are always resistant to various classes of antibiotics which could be transferred to other species of bacteria. The resistance shown by the isolates could be as a result of various possible mechanisms which include mutation in chromosomal genes, mobile resistance genes etc [26].

Conclusion

To the best of our knowledge, this study presents the first detailed study to detect antibiotic resistant organisms in retail meat in the study location**.** This study has demonstrated the role of retail meat as a reservoir of antibiotic resistant bacteria that can be transferred to humans, thereby constituting a public health problem. Meat handlers and sellers should be educated on the adverse effect of lack of proper personal hygiene and sanitation. Quality control units should be established in other to set standards in retail meat processing in Nigerian markets. Hazard Analysis Critical Control Point (HACCP) concept should be applied to the processing and sales of retail beef. This will help in reducing contamination and spoilage of meat products. The prudent use of antibiotics should be encouraged in animals which is important to prevent problems associated with antibiotic resistance. There is a need to recognize all possible factors involved in antimicrobial resistance, regulate the use of antimicrobials and select the best ways to tackle the development of antimicrobial resistance.

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