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Antibiogram of food borne pathogenic bacteria isolated from raw pork and beef meat

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Abstract

Meat is an ideal medium of growth for many organisms because it is high in moisture, rich in nitrogenous compounds (amino acids, peptides, and proteins), minerals, and accessory growth factors. This study isolated foodborne bacteria pathogens from raw pork and beef meat sold in some selected slaughter markets (Alakahia, Rumuosi, and Rumuokoro). We subjected the meat samples to a total viable count (TVC) and a total coliform count (TCC). The highest TVC and TCC for pork meat were log10 7.29 CFU/g and log10 8.66 CFU/g, respectively. For beef meat, TVC and TCC were log10 7.45 CFU/g and log10 6.25 CFU/g, respectively. In this study, most log TVC and TCC counts exceeded the permissible level (log10 ≥6 CFU/g). The most common bacteria found were Escherichia coli, *Salmonella enterica*, *Staphylococcus aureus*, and *Proteus mirabilis*. These bacteria were found in 35.2% of pork meat, 23.6% of beef meat, 35.8% of pork meat, and 7.4% of beef meat, respectively. The rates of antibiotic resistance of the pathogens ranged from 10–87.8% for pork and 14.3–88.5% for beef samples. Ampiclox, amoxicillin, streptomycin, and gentamycin exhibited higher antibiotic resistance. We observed the lowest resistance for ciclopirox, olamine, and cephaloridine. Also, the Multiple Antibiotic Resistance (MAR) index of bacterial isolates ranged from 0.5 to 0.9. This study confirmed an elevated prevalence of *Escherichia coli*, *Salmonella enterica*, and *Staphylococcus aureus* in raw meat products and found the bacterial strains to be resistant to multiple commonly employed antibiotics. Hence, the occurrence and presence of multidrug-resistant foodborne bacterial isolates is an indication that fresh beef meat products may act as reservoirs of drug-resistant bacteria. Careful handling of meat products and effective use of antibiotics are essential to controlling and preventing the spread of these pathogens.

Keywords: Antimicrobial resistance; Food borne pathogens; Food borne infections; Meat; Beef; Pork

1. Introduction

Foodborne illnesses and infections are prominent global health issues that pose significant risks to human health and lives. Researchers have recognized more than 250 foodborne diseases [1]. Foodborne disease not only affects the physical health and well-being of individuals, but it also has a substantial impact on the social and economic productivity of nations [2]. According to the World Health Organization, foodborne infections are responsible for an estimated 600 million cases, over 420,000 deaths, and 33 million disability-adjusted life years (DALYs) each year [3].

The key factors contributing to the increasing frequency of food-borne infections, especially in Africa, are the low standards of hygiene observed among the population. Inadequate personal hygiene among personnel involved in food preparation, along with inappropriate meat handling practices at slaughterhouses, could potentially result in the transmission of microbial pathogens that cause foodborne illnesses [9]. The main source of infection in humans is the consumption of infected food, particularly raw or undercooked meat, mainly beef and pork [8].

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Meat and meat products are highly nutritious and appealing to humans because they are high in protein, fat, vital amino acids, minerals, vitamins, and other nutrients [4]. Meat is a valuable source of protein, vital fatty acids, minerals, and vitamins. However, it is highly perishable because of its ability to create an ideal environment for the growth of different microorganisms [5]. Bacterial contamination occurs when meat comes into contact with bacteria while processing animal carcasses at the abattoir. These pollutants can be either external, such as the skin or environment, or internal, such as the contents of the gastrointestinal system [6].

Furthermore, during bleeding, handling, and processing, external sources like blades, tools, clothing, hands, and the surrounding air can easily contaminate meat. Contaminated meat and meat products pose a range of biological, chemical, physical, and especially microbiological risks to food safety and can lead to various illnesses. The primary foodborne bacterial pathogens commonly found in meat include Salmonella spp., *Staphylococcus aureus, Escherichia coli*, *Campylobacter jejuni*, *Listeria monocytogenes*, *Clostridium perfringes*, *Yersinia enterocolitica*, and *Aeromonas hydrophila* [7]. The degree of microbial contamination and the composition of microbial flora are indicative of the level of cleanliness maintained in meat. Foodborne pathogens have the potential to exist in a range of food items, such as beef and pork. It is crucial to identify and detect these pathogens to guarantee food safety and reduce instances of foodborne infections [5]. Lastly, it is imperative to reduce the pollution of meat sold in marketplaces through effective sanitation and inspection standards. This study aims to assess the prevalence and resistance to antimicrobial agents for foodborne pathogens found in raw pork and beef meat sold in the selected slaughter markets in Port Harcourt, Rivers State, Nigeria.

2. Materials and Methods

2.1. Study area

The study areas for this research were Alakahia, Rumuosi, and Rumuokoro, all under the Obio/Akpor local government area. Obio-Akpor is one of the eight local government areas that formed the Rivers East senatorial district, with its headquarters at Rumuodumaya. Because of its location, Obio-Akpor is popularly known as the gateway local government area. It has a land area of approximately 311.71 km2 (120.35 sq mi) and shares boundaries with Emohua, Ikwerre, Etche, Oyigbo, Eleme, Okirika, Port Harcourt Local Government Area of Rivers State. It is accessible by road, sea, and air transportation.

2.2. Sample collection

We randomly collected a total of 30 samples of beef and pork meat from various retailers in Alakahia, Rumuosi, and Rumuokoro. The samples were collected in sterile polythene bags, packed in a carrier box containing ice packs, and transported to the Microbiology Laboratory of the University of Port Harcourt, Nigeria.

2.3. Enumeration and bacterial isolation

One gram of each sample was weighed out and homogenized into 9 ml of sterile distilled water. From the ten-fold dilutions of the homogenates, 0.1 ml of 10-2 10-3, and 10-4 dilutions were plated in culture on the Nutrient Agar and MacConkey's Agar by the pour plate method. The plates were then incubated at 37°C for 24–48 h. We used a colony counter to count the colonies after the incubation period. The count was expressed as colony-forming units (CFU). Pure isolates of bacterial species were stored at 4oC for use in identification examination.

2.3.1. Total Coliforms and Fecal Coliform Count

An aliquot of 0.1mL from the appropriate dilution was pipetted and spread on violet red bile agar. The inoculated plates were then incubated at 32°C for 18–24 h to determine total coliforms and at 44.5°C for 18–24h to determine faecal coliforms.

2.3.2. Enterobacteriaceae Count

To count the members of Enterobacteriaceae, 0.1 mL of the aliquot from appropriate dilution was spread plated on MacConkey agar (M 081 Hi-Media, Mumbai) supplemented with glucose and was incubated at 35 ° C for 24h. All reddish purple/pink colonies were counted as members of the Enterobacteriaceae [10].

2.4. Detection of foodborne bacteria

2.4.1. Total Staphylococcus spp. Count

Staphylococcus species were enumerated by pour plate method and grown on Mannitol Salt Agar (MSA). 0.1 mL aliquot from the appropriate dilution was inoculated into pre-dried MSA plates. The inoculated plates were incubated at 37°C for 24 h. After incubation, yellow colonies were counted and recorded as Staphylococcus counts using the colony counter [10].

2.4.2. Detection of Escherichia coli

Escherichia coli species were isolated using MacConkey agar (Hi-Media, Mumbai, India). 0.1 mL of the sample was spread into MacConkey agar plates and incubated at 37 °C for 24 h. The colonies were confirmed by streaking 2-3 colonies onto MacConkey agar and colonies were further confirmed by Gram's staining and by biochemical tests [11].

2.4.3. Detection of Salmonella spp

A 25 g of meat sample (minced by stomacher) was transferred to 225 mL of Buffered Peptone Water (BPW) and incubated at 37°C for 24 hrs. An aliquot of 0.1 mL from pre-enrichment was pipetted to 10 mL tetrathionate broth (supplement with iodine). A loopful sample from tetrathionate culture was streaked onto SS agar plates. The plates were incubated at 37 °C for 24 h. After 24 h of incubation, the formation of colonies with black centers or with gray colors on SS agar was considered as presumptive Salmonella spp.

2.5. Identification of bacterial isolates

The growing colonies were transferred to new specialized media for each bacterium to obtain for a pure culture. The isolated bacteria were cultured at a 37˚C for 24h and staining procedure applied by using Gram stain. The biochemical tests were conducted to identify the isolated bacterial species.

2.5.1. Antibiotics Susceptibility Test

Kirby-Bauer disk diffusion method was be used to determine the antibiotic resistant characteristics of the isolated organisms (Eman et al., 2019). Mueller- Hinton agar plates was prepared and poured in Petri dishes after sterilization by autoclaving at 121C for 15 min. This was used for testing the isolated microorganism for antibiotic susceptibility. The diameter of inhibition zones (mm) was recorded for all of the plates and then compared with the standard [12].

3. Result

3.1. Mean bacterial load counts of pork and beef meat per sampling site

A comparison of the mean total viable count (TVC) and total coliform count (TCC) of raw meat samples from pork and beef was carried out and results are presented in Table 1. The highest mean bacterial counts were recorded in pork meat whereas the lowest counts were recorded in the beef meat. However, Rumuokoro slaughter market had the highest bacterial count while Alakhia slaughter market had the lowest count for both pork and beef meat. Statistical analysis using ANOVA revealed a statistically significant difference ($p<0.05$) between the mean total bacterial counts of the pork and the beef meat.

3.2. Total number and percentage of positive samples for bacteria pathogens in raw pork

The bacteriological examination of raw pork from the sampled locations is shown in Table 2. The results revealed that, food-borne pathogens (*Staphylococcus aureus*, *Salmonella enterica*, Escherichia coli and *Proteus mirabilis*) were isolated from 25 positive samples (83.3%) out of 30 raw pork meat sampled from various slaughter market.

Table 1 Variation of total viable bacterial load of pork and beef meat from various slaughter

Slaughter market	Meat sample	No. of sample	Mean TVC ($Log_{10}CFU/g$)	Mean TCC ($Log_{10}CFU/g$)
Alakahia	Pork	10	7.29 ± 2.27^{c}	6.36 ± 0.21^{c}
	Beef	10	4.21 ± 1.80^{c}	3.11 ± 0.16^{b}
Rumuosi	Pork	10	7.60 ± 2.13^{b}	6.81 ± 0.26^{b}
	Beef	10	6.37 ± 1.10^{b}	5.32 ± 0.19^{a}
Rumuokoro	Pork	10	8.66 ± 1.37^{a}	7.30 ± 0.22^{b}
	Beef	10	7.45 ± 1.2^{a}	6.25 ± 1.30^{a}

*Values with different subscript are significantly different, SE=Standard error of the mean.

Table 2 Total number and percentage of positive samples for bacteria pathogens in raw pork

Location	Number of sample (n)	Number of positive samples	Positive percentage		
			%[1]	%[2]	%[3]
Alakahia	10	7	70	28	23.3
Rumuosi	10	9	90	36	30
Rumuokoro	10	9	90	36	30
Total (N)	30	25	83.3	100	83.3

1Percentage in relation to total number of samples in each row. 2 Percentage in relation to total number of positive samples (25). 3 Percentage in relation to total number of collected samples (30)

The highest number of positive samples were isolated from pork samples collected from Rumuokoro and Rumuosi slaughter markets with a record of 9 positive samples (36 % & 30 %) each. Pork samples from Alakahia slaughter had the lowest records of 7 positive samples (28 % & 23.3 %) (Table 2).

3.3. Total number and percentage of positive samples for bacteria pathogens in raw meat

The bacteriological examination of raw meat from the three slaughters is presented in Table 3. The results revealed that, food-borne pathogens were isolated from 20 samples (66.6 %) out of 30 raw beef sampled from the slaughters. The highest number of positive samples were isolated from raw beef collected from Rumuosi slaughter markets with a record of 9 positive samples (45% & 30%). Beef samples from Alakahia slaughter had the lowest records of 7 positive samples (20 % & 13.3%).

3.4. Microscopic and biochemical characterization of pathogenic bacteria isolated from raw pork and beef meat

Microscopic and biochemical characterization of pathogenic bacteria isolated from raw pork and meat samples are presented in Table 4. The results showed that Escherichia coli tested indole (+), methyl red (+), Voges Proskauer (-), simmon's citrate (+), urease (-), kligler's iron agar (Alkaline/Acid with H2S), oxidase (-) and catalase (+). Salmonella species showed indole (-), methyl red (+), voges Proskauer (-), citrate (+), urease (-), kligler's iron agar (Alkaline/Acid with H2S), and oxidase (-). *Staphylococcus aureus* revealed positive for motility (+) coagulase (+) and catalase (+). *Proteus mirabilis* tested motility (+), indole (+), methyl red (+), Voges Proskauer (-), citrate (+), urease (+), kligler's iron agar (-), oxidase (-) and catalase (+).

Table 3 Total number and percentage of positive samples for bacteria pathogens in raw beef

Location	Number of sample (n)	Number of positive samples	Positive percentage		
			%[1]	%[2]	%[3]
Alakahia	10	4	40	20	13.3
Rumuosi	10	9	90	45	30
Rumuokoro	10	7	70	35	23.3
Total (N)	30	20	66.6	100	66.6

Table 4 Microscopic and Biochemical Characterization of Pathogenic Bacteria Isolated from Raw Pork and Beef

Gram reaction and microscopic characteristics	Biochemical reaction										Presumptive identity
	Motility	Indole	Methyl red	Voges- Proskauer	Simmon citrate	Urease	kligler's iron agar	Oxidase	Coagulase	Catalase	
Gram negative short rod	+	+	-	-	-	-	-	-	-	+	*Escherichia coli*
Gram negative slender rod	+	-	+	-	+	-	+	-	-	+	*Salmonella enterica*
Gram positive cocci in clusters	-	-	+	+	+	+	-	-	+	+	*Staphylococcus aureus*
Gram negative short straight rod	+	-	+	-	+	+	-	-	-	+	*Proteus mirabilis*

Keys: **+**= positive, **-** = Negative

3.5. Prevalence of foodborne bacteria in raw pork and beef meat

3.5.1. Prevalence of foodborne bacteria in raw pork

Table 5 Prevalence of foodborne bacteria in raw pork meat

Food borne pathogen	Slaughter market			Total (N (%))
	Alakahia (n (%))	Rumuosi (n (%))	Rumuokoro (n (%))	
Staphylococcus aureus	10 (40)	15(35.7)	18(32.7)	43 (35.2)
Salmonella enterica	05(20)	09(21.4)	14(25.5)	28(23.0)
E. coli	07(28)	15(35.7)	19(34.5)	41(33.6)
Proteus mirabilis	03(12)	03(7.1)	4(7.3)	10 (8.2)
Total (%)	25(20.5)	42(34.4)	55(45.1)	122 (100)

The prevalence of foodborne bacteria in raw pork is shown in Table 5. *Staphylococcus aureus* recorded the highest (35.2 %), followed by *E. coli* (33.6 %). The lowest number of foodborne pathogens was recorded for *Proteus mirabilis* (8.2%).

However, the highest level of bacterial prevalence was noted in raw pork meat collected from Rumuokoro slaughter market (45.1 %), followed by Rumuosi (34.5 %). The lowest bacterial prevalence was observed in Alakahia (20.5 %).

3.5.2. Prevalence of foodborne bacteria in raw beef meat

The prevalence of foodborne bacteria in raw beef meat is illustrated in Table 6. Escherichia coli recorded the highest (35.8 %), followed by S. aureus (29.5 %). The highest level of bacterial prevalence was noted in beef meat collected from Rumuokoro slaughter (28.9 %) while Alakahia slaughter had the lowest prevalence (18.9 %).

3.6. Antimicrobial susceptibility profiles of bacterial pathogens

3.6.1. Antimicrobial susceptibility profile of bacterial pathogens isolated from raw pork

Antimicrobial susceptibility profile of bacterial pathogens isolated from raw pork meat is presented in Table 7. Susceptibility pattern of the 43 isolates of *Staphylococcus aureus* showed high rate of susceptibility to gentamycin (90.7%), ciclopirox olamine (81.4%), erythromycin (73.9%), and cephaloridine (83.7%). High rate of resistance was observed for levofloxacin (73.9%), ampiclox (62.8%), amoxicillin (65.1%), and streptomycin (60.5%).

Twenty-eight (28) isolates of *Salmonella enterica*, showed high susceptibility to ciclopirox olamine (78.6%), gentamycin (67.9%), rifampicin (75%) and cephaloridine (67.9%). In addition, they showed high resistance for erythromycin (60.7%), ampiclox (82.1%), amoxicillin (64.3%) and streptomycin (60.7%).

Forty-one (41) isolates of *E. coli* showed high rate of susceptibility to ciclopirox olamine (77.8%), rifampicin (61.0%). and cephaloridine (73.2%). High resistance was recorded for levofloxacin (61.0%), erythromycin (78%), ampiclox (85.4%), amoxicillin (82.9) and streptomycin (87.8%). Ten (10) isolates of *Proteus mirabilis*, showed high susceptibility to Ciclopirox olamine (70%), gentamycin (60%), rifampicin (80%), neomycin (60%) and cephaloridine (70%). They showed high resistant to erythromycin (60%), ampiclox (60%), amoxicillin (60%) and streptomycin (60%).

Table 6 Prevalence of foodborne bacteria in raw beef meat

Food borne pathogen	**Slaughter market**			**Total (N (%))**
	Alakahia (n (%))	**Rumuosi (n (%))**	**Rumuokoro (n (%))**	
Staphylococcus aureus	06 (33.3)	09(32.1)	13(26.5)	28 (29.5)
Salmonella enterica	04(22.2)	07(25.0)	15(30.6)	26(27.4)
E. coli	05(27.8)	10(35.7)	19(38.8)	34(35.8)
Proteus mirabilis	03(16.7)	02(7.1)	02(4.1)	07 (7.4)
Total (%)	18(18.9)	28(29.5)	49(51.6)	95 (100)

Table 7 Antimicrobial susceptibility profile of bacterial pathogens isolated from raw pork

Bacterial isolates	**N**		**Antimicrobial susceptibility profiles n (%)**									
		Profile	**CPX**	**LV**	**CN**	**ER**	**APX**	**RD**	**NB**	**AMX**	**S**	**CH**
Staphylococcus aureus	43	S	35(81.4)	23(53.4)	39(90.7)	17(73.9)	11(25.6)	27(62.8)	28(65.1)	15(11.6)	12(27.9)	36(83.7)
		I	0(0)	3(7.0)	0(0)	2(4.7)	5(11.6)	0(0)	1(2.3)	0(0)	5(11.6)	1(2.3)
		R	8 (18.6)	17(73.9)	4(17.4)	24(55.8)	27(62.8)	16(37.2)	14(32.6)	28(65.1)	26(60.5)	6(14.0)
Salmonella enterica	28	S	22(78.6)	12(42.9)	19(67.9)	10(35.7)	5(27.8)	21(75)	13(46.4)	8(28.6)	10(35.7)	19(67.9)
		I	0(0)	2(7.1)	2(7.1)	1(13.6)	0(0)	0(0)	1(13.6)	2(7.1)	1(13.6)	2(7.1)

		R	6(21.4)	11(39.3)	9(3.2)	17(60.7)	23(82.1)	7(25)	14(50.0)	18(64.3)	17(60.7)	7(25)
E. coli	41	S	20(77.8)	16(39.0)	23(56.1)	8(19.5)	5(12.2)	25(61.0)	18(43.9)	7(17.1)	5(12.2)	30(73.2)
		I	4(0)	0(0)	5(12.2)	2(4.9)	1(2.4)	0(0)	0(0)	0(0)	0(0)	0(0)
		R	17(41.5)	25(61.0)	13(31.7)	32(78.0)	35(85.4)	16(39.0)	23(56.1)	34(82.9)	36(87.8)	11(26.8)
Proteus mirabilis	10	S	7(70)	3(30)	6(60)	4(40)	2(20)	8(80)	6(60)	3(30)	3(30)	7(70)
		I	1(10)	2(20)	0(0)	0(0)	2(20)	1(10)	0(0)	1(10)	0(0)	1(10)
		R	3(30)	5(50)	4(40)	6(60)	5(60)	1(10)	4(40)	6(60)	7(70)	2(20)

S= Susceptibility, I= Intermediate, R= Resistance, CPX=Ciclopirox olamine, LV= Levofloxacin, CN= Gentamycin, ER= Erythromycin, APX= Ampiclox, RD= Rifampicin, NB=Neomycin B, AMX= Amoxicillin, S=streptomycin, CH= Cephaloridine

3.6.2. Antimicrobial susceptibility profile of bacterial pathogens isolated from raw beef

Antimicrobial susceptibility profile of bacterial pathogens isolated from raw beef meat is shown in Table 8. Twenty-eight (28) isolates of *Staphylococcus aureus* showed high susceptibility to ciclopirox olamine (78.6%), levofloxacin (67.9%), rifampicin (82.1%), neomycin (64.3%), and cephaloridine (85.7%). High rate of resistance was observed for ampiclox (71.4%) and amoxicillin (82.1%). Twenty-six (26) isolates of *Salmonella enterica* had high susceptibility to ciclopirox olamine (69.2%), rifampicin (76.9%), neomycin (84.6%) and cephaloridine (80.8%). They showed high resistance to ampiclox (88.5%) and amoxicillin (65.4%).

Thirty-four (34) isolates of *E. coli* showed high susceptibility to ciclopirox olamine (61.8%), gentamycin (70.6%), rifampicin (79.4%) and cephaloridine (85.3%). In addition, they showed high resistance for ampiclox (73.5%), amoxicillin (85.3%) and streptomycin (67.6%).

Seven (7) isolates of *Proteus mirabilis*, showed high susceptibility to ciclopirox olamine (71.4%), rifampicin (85.7%), neomycin (85.7%) and cephaloridine (85.7%). High resistance was recorded for ampiclox (71.4%) and amoxicillin (71.4%).

Table 8 Antimicrobial susceptibility profile of bacterial pathogens isolated from raw beef meat

Bacterial isolates	**N**		**Antimicrobial susceptibility profiles n (%)**									
		Profile	**CPX**	**LV**	**CN**	**ER**	**APX**	**RD**	**NB**	**AMX**	**S**	**CH**
Staphylococcus aureus	28	S	22(78.6)	19(67.9)	16(57.1)	11(39.3)	8(28.6)	23(82.1)	18(64.3)	5(17.9)	12(42.9)	24(85.7)
		I	0(0)	2(7.1)	3(10.7)	1(3.6)	0(0)	0(0)	1(3.6)	0(0)	1(3.6)	1(3.6)
		R	6 (21.4)	7(25.0)	9(32.1)	14(50)	20(71.4)	5(17.9)	9(32.1)	23(82.1)	15(53.6)	3(10.7)
Salmonella enterica	26	S	18(69.2)	10(38.5)	10(38.5)	13(50)	3(11.5)	20(76.9)	22(84.6)	8(30.8)	12(46.2)	21(80.8)
		I	2(7.7)	2(7.7)	0(0)	1(3.8)	0(0)	0(0)	0(0)	1(3.8)	0(0)	0(0)
		R	6(23.1)	14(53.8)	9(34.6)	12(46.2)	23(88.5)	6(23.1)	4(15.4)	17(65.4)	14(53.8)	6(23.1)
E. coli	34	S	21(61.8)	13(38.2)	24(70.6)	20(58.8)	8(23.5)	27(79.4)	21(43.9)	5(14.7)	8(23.5)	29(85.3)
		I	0(0)	2(5.9)	0(0)	1(2.9)	1(2.9)	0(0)	2(5.9)	0(0)	3(8.8)	0(0)
		R	13(38.2)	19(55.9)	12(35.3)	13(38.2)	25(73.5)	7(39.0)	13(38.2)	29(85.3)	23(67.6)	5(14.7)
Proteus mirabilis	07	S	5(71.4)	4(47.1)	4(47.1)	3(42.9)	2(28.6)	6(85.7)	6(85.7)	1(14.3)	3(42.9)	6(85.7)
		I	0(0)	1(14.3)	0(0)	0(0)	0(0)	0(0)	0(0)	1(14.3)	0(0)	0(0)
		R	2(28.6)	2(28.6)	3(42.9)	4(47.1)	5(71.4)	1(14.3)	1(14.3)	5(71.4)	4(47.1)	1(14.3)

S= Susceptibility, I= Intermediate, R= Resistance, CPX=Ciclopirox olamine, LV= Levofloxacin, CN= Gentamycin, ER= Erythromycin, APX= Ampiclox, RD= Rifampicin, NB=Neomycin B, AMX= Amoxicillin, S=streptomycin, CH= Cephaloridine

3.7. Antibiotic resistant pattern of the bacterial isolates

3.7.1. Antibiotic resistant pattern of the bacterial isolates from raw pork

The comparison of the resistance pattern of the isolates from raw pork to the antibiotics used in this study is presented in Figure 1. The result revealed that isolates of *E. coli* were resistant to most of the antibiotics followed by *Salmonella enterica*, while others have moderate resistance. However, the isolates showed highest and lowest resistance to ampiclox and ciclopirox olamine respectively.

3.7.2. Antibiotic resistant pattern of the bacterial isolates from raw beef

The comparison of the resistance pattern of the isolates from raw beef to the antibiotics used in this study is presented in Figure 2. The result revealed *E. coli* were mostly resistant to most of the antibiotics followed by *Salmonella enterica* while others have moderate resistance.

3.8. Multiple antibiotics resistance index of the bacterial isolates from pork and beef

Multiple Antibiotics Resistance Index (MARI) of isolates from raw pork is shown in Table 4.9. Escherichia coli from raw pork had the highest MAR index of 0.9 followed by S. aureus and P. mirabilis with equal MAR index of 0.7 each.

High MAR index was recorded for isolates from raw beef (Table 4.9). Escherichia coli from raw beef had the highest MAR index of 0.9. The lowest MAR was recorded for P. mirabilis with an index value of 0.5.

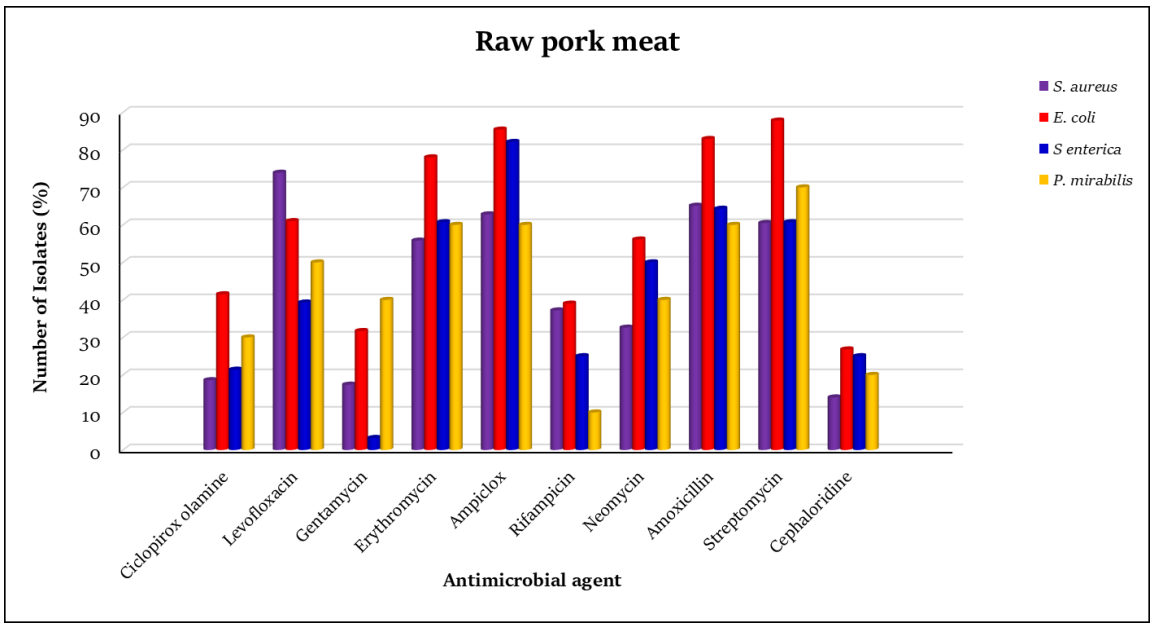

Figure 1 Comparison of resistance of the bacterial isolates from raw pork to different antibiotics

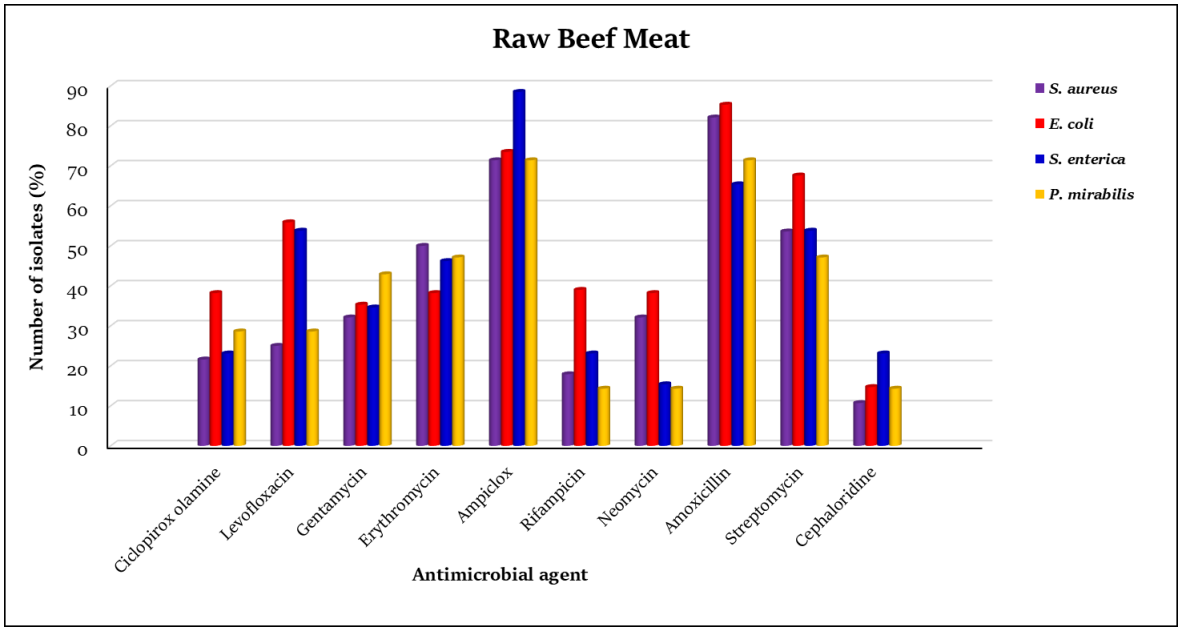

Figure 2 Comparison of resistance of the bacterial isolates from raw beef meat to different antibiotics

Table 9 Multiple antibiotics resistance index of isolates

Sample	Bacterial Isolates	Resistance pattern	Number of antibiotics resistant to	MAR Index
Raw pork	*S. aureus*	LV, ER, APX, RD, NB, AMX, S	7	0.7
	E. coli	CPX, LV, CN, ER, APX, RD, NB AMX, S	9	0.9
	Salmonella enterica	LV, ER, APX, NB, AMX, S	6	0.6
	Proteus mirabilis	LV, CN, ER, APX, NB, AMX, S	7	0.7
Raw beef	*S. aureus*	CN, ER, APX, NB, AMX, S	6	0.6
	E. coli	CPX, LV, CN, ER, APX, RD, NB, AMX, S	9	0.9
	Salmonella enterica	LV, CN, ER, APX, AMX, S	6	0.6
	Proteus mirabilis	CN, ER, APX, AMX, S	5	0.5

Keys: CPX=Ciclopirox olamine, LV= Levofloxacin, CN= Gentamycin, ER= Erythromycin, APX= Ampiclox, RD= Rifampicin, NB=Neomycin B, AMX= Amoxicillin, S=streptomycin, CH= Cephaloridine

4. Discussion

4.1. Bacterial counts of pork and beef meat per sampling site

Microbial contamination of meat is inevitable because microorganisms are present in animals and their environments. Thus, the initial level of bacteria in meat is important because it directly affects spoilage and shelf life [13]. This study recorded the highest TVC and TCC in pork and beef meat (from Rumuokoro slaughter), indicating very poor hygienic practices at the slaughterhouses [14]. According to the Livestock Products Sanitary Control Act and Modernization of Swine Slaughter Inspection [15], the hygienic quality of pork meat is considered satisfactory when aerobic bacteria and *E. coli* counts are < log10 5.00 CFU/g and < log10 4.00 CFU/g, respectively. The bacterial count for pork and beef meat in this study exceeded the acceptable limit. This result was lower than those reported by Hong et al. [14], who recorded a satisfactory bacterial count of log10 2.00 CFU/g to log10 4.00 CFU/g from 20 pig slaughterhouses across Korea. Van-Ba et al. [16] previously reported that the average counts of aerobic bacteria and *E. coli* from pork meat in Korea were satisfactory. Lindblad et al. [17] and Bohaychuk et al. [18] also reported that pork from Sweden and Canada met the

criteria for aerobic bacteria and *E. coli* counts, respectively. These developed countries strictly manage risk factors in slaughter houses because a high initial microbial load, poor hygiene practices, or high temperatures (>15 oC) in the slaughtering lines can affect the distribution of microorganisms [19]. Jakubowska-Gawlik et al. [20] suggest that the implementation of food safety/HACCP, non-conformity control, site hygiene, and pest control can enhance the quality of carcasses. The high TVC and TCC in the Rumuokoro slaughter market may be attributed to the slaughter house's proximity to a road and crowded market areas, which frequently lack water supplies for hand washing, cleaning, and cutting knives and utensils. Furthermore, workers in slaughterhouses lack adequate knowledge of personal hygiene and sanitation procedures, resulting in bacterial contamination of the meat [14]. The presence of higher coliform counts in the meat sold at the slaughter markets might result from faecal contamination during slaughter operations, evisceration, as well as poor personal hygiene of the slaughterhouse workers [21].

4.2. Prevalence of foodborne bacteria in raw pork and beef meat

The World Health Organization has estimated that 1 in 10 people is exposed to the disease, and about 4 million and 20 thousand people die each year as a result of eating contaminated raw, undercooked, and unpasteurized foods [22]. This study isolated food-borne pathogens (*Staphylococcus aureus*, *Salmonella enterica*, Escherichia coli, and *Proteus mirabilis*) from 25 (83.3%) and 20 (66.6%) out of 30 raw pork and beef meat samples, respectively. Food-borne pathogenic microorganisms are considered the leading cause of disease and death in developing countries, causing heavy losses in health care. Several studies have reported that the most common foodborne pathogens associated with pork and meat are Salmonella spp., S. aureus, *E. coli*, and Proteus spp. [23–25].

In this study, the prevalence of the four foodborne bacteria isolated from raw pork samples was 35.2% for *Staphylococcus aureus*, 33.6% for *E. coli*, 23% for *Salmonella enterica*, and 8.2% for *Proteus mirabilis*. A similar study was carried out in slaughterhouses across South Korea by Hong et al. [13], who isolated 87.0% of *E. coli* and 11.5% of *Staphylococcus aureus* from pork meat. Muinde et al. [24] isolated *E. coli* (49.2%), Salmonella spp. (17.0%), and S. aureus (4.2%) from raw pork meat in Kenya. Furthermore, a prevalence of 58.1% in Salmonella spp. [26], 18% in *E. coli* [27], 11.5% in P. aeruginosa [28], and 5.6% in S. aureus spp. [29] has been reported in pork meat samples from retail markets. These bacterial species are considered a real danger to human health [30].

It was found that *Staphylococcus aureus* was present in 29.5% of the raw beef samples, *E. coli* was present in 35.8%, *Salmonella enterica* was present in 27.4%, and *Proteus mirabilis* was present in 7.4%. These results are lower than those of Suad and Wisam [22], who isolated Salmonella (58%), *E. coli* (29%), and *Staphylococcus aureus* (38%) from beef meat. Bantawa et al. [5] isolated S. aureus, *E. coli*, and Salmonella spp. from beef meat, with a higher prevalence of 68%, 54%, and 34%, respectively. Mir et al. [31] isolated a higher prevalence of *E. coli* (48%). The unhygienic processing and poor sanitation of meat shops may account for the differences in prevalence rates between this study and others. The study revealed that meat retailers lacked awareness of basic meat requirements and guidelines. Direct contact with raw meat has been proven to pose health hazards to humans, especially butchers, due to the transmission of *E. coli*, Salmonella, Shigella, and Vibrio spp. through the fecal-oral route. [5]. The prevalence rates of P. mirabilis (8.2% from pork and 7.4% from beef meat) in this study were far lower than the findings of Ronanki et al. [25], Naidu et al. [32], and Salih et al. [33]. *Proteus mirabilis* may be present due to unhygienic slaughter and processing, or contaminated water used during the slaughter of pigs and cattle [25]. The presence of the *E. coli* indicator organism in meat likely indicates faecal contamination and poor hygiene [34]. The presence of *E. coli*, as an indicator of hygiene and sanitary quality, suggests that consumers are at risk of food poisoning and the presence of other pathogenic flora.

The isolation and identification of S. aureus from pork and beef meat could be due to inadequate cleaning, unsatisfactory handling, and post-processing contamination from the polluted atmosphere around markets and shops. [5, 23]. Suad and Wisam [22] also reported that *Staphylococcus aureus* may be present in meat samples from butchers' skin and noses. Humans and animals commonly carry *Staphylococcus aureus* on their skin and mucous membranes, making it one of the most common causes of food poisoning. It is particularly dangerous at slaughterhouses because of its potential for transmission from animals to slaughter operators and vice versa [35]. The high prevalence of S. aureus in pig meat may be related to a lack of skinning of pigs during slaughter; therefore, it is important to minimize skin contamination during slaughter [36].

4.3. Antimicrobial and multi drug resistance by the bacterial pathogens

Misuse and overuse of antimicrobials lead to antimicrobial resistance (AMR), a major global health concern [37, 39]. The resistance of the bacterial pathogens to the various classes of antimicrobial agents ranged from 10–87.8% for pork and 14.3–88.5% for beef samples. Traditional antimicrobial agents like ampiclox, amoxicillin, streptomycin, and gentamycin exhibited higher resistance. Levofloxacin, erythromycin, neomycin, and rifampicin showed moderate resistance. The lowest resistance was observed for ciclopirox olamine, and cephaloridine. Among the bacterial

pathogens isolated from pork and beef in this study, *E. coli* had the highest resistance to the ten antibiotics used, followed by S. aureus and *Salmonella enterica*. The pathogen with the least resistance is *Proteus mirabilis*. The high performance of ciclopirox olamine, and cephaloridine could also be due to their molecular sizes, a factor that enhances their solubility in diluents, thus promoting their penetration power through the cell wall into the cytoplasm of the bacterial pathogens [37]. Ayandele et al. [38] conducted a similar study which revealed that *E. coli* exhibited the highest resistance to streptomycin and erythromycin, as well as the highest susceptibility to gentamycin. The imprudent use of antimicrobials in both the human and animal sectors has resulted in the selection of pathogens resistant to multiple drugs. We now widely acknowledge that the development and spread of AMR significantly outpaces the development of new antimicrobial drugs.

According to Van Boeckel et al. [41], antimicrobials used in animal production (especially in poultry and pigs) remain key contributors to AMR. We expect their uses to exponentially increase due to the expansion of intensive production systems to meet the increasing demand for animal-sourced foods (ASFs) and the surge in disease burdens [24, 40].

During animal slaughter and food processing, antimicrobial-resistant bacteria from the gastrointestinal tract of animals can contaminate meat, contaminate the environment with animal faeces, and subsequently spread to humans through handling, consuming contaminated food, or coming into contact with animal waste. This can lead to antimicrobial-resistant intestinal infections [24]. Human pathogens, which are normally specific to humans, can sometimes transfer resistant genes from animals. Consuming contaminated food of animal origin can lead to human bacterial infections; therefore, the presence of antibiotic-resistant strains in animal products like pork and beef has raised concerns about the potential compromise of human infection treatment [41, 42].

This study observed multiple antibiotic resistance (MAR), which is defined as resistance to three or more different classes of antimicrobials. We determined the multiple antibiotic resistance index using the formula: "MAR Index = a/b." Where (a) is the number of antibiotics to which the isolates showed resistance, (b) is the total number of anti-biotics used in each class of antimicrobial agent" [43]. The MAR index of ≥ 0.2 infers that the strain of such bacteria originates from an environment where these drugs are misused, as well as that the plasmids contain one or more resistance genes, each encoding a single antibiotic resistance phenotype [44, 45].

This study's MAR index revealed slight variations, with the lowest MAR index of 0.5 and the highest MAR index of 0.9. This study also revealed that the highest multidrug resistance occurred in *E. coli*, with a MAR index of 0.9. However, other isolates had a MAR index of ≥ 0.5. Akinware et al. [23] and Afunwa et al. [37] also did similar studies and found that *E. coli* had the highest multidrug resistance, with MAR indexes of 0.8 and 1.00, respectively. Microorganisms with MAR indices of ≥0.2 confirm the presence of multidrug-resistant genes originating from the environment where these drugs are frequently used and abused [44]. According to Riaz et al. [45], multiple antibiotic resistance (MAR) in bacteria is most commonly associated with the presence of plasmids that contain one or more resistance genes, each encoding a single antibiotic resistance phenotype. Antibiotic-resistant genes can transfer to other bacteria of the same or different species [46]. The MAR index serves as an effective, valid, and cost-effective method for tracking the source of antibiotic-resistant organisms [45]. Using the MAR index analysis is also simple, requires no specialized training or expensive equipment, and provides the needed data [37].

5. Conclusion

Meat has the potential to transmit various pathogens to consumers. Pork and beef meat samples from three slaughter markets in Port Harcourt, Nigeria, tested positive for foodborne bacteria, indicating a high risk of food safety concerns. Therefore, slaughterhouses, markets, and other meat rendering outlets should adopt routine microbiological examination. In addition, consumers must carefully handle and cook raw meat, regardless of market source, to avoid foodborne illness.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Tonjo, T., Manilal, A. & Seid, M. (2022). Bacteriological quality and antimicrobial susceptibility profiles of isolates of ready-to-eat raw minced meat from hotels and restaurants in Arba Minch, Ethiopia. PLoS One, 17(9): e0273790. doi: 10.1371/journal.pone.0273790.

[2] Nyenke P, Akani NP and Aleruchi O. (2024). Prevalence and antibiogram of Salmonella species isolated from marketed pork meats in Port Harcourt Metropolis. Journal of Advances in Microbiology Research 2024; 5(1): 80-87.

[3] Aladhadh M. A Review of Modern Methods for the Detection of Foodborne Pathogens. Microorganisms. 2023 Apr 24;11(5):1111. doi: 10.3390/microorganisms11051111.

[4] Abd El Tawab, A.A., Maarouf, A.A., El-Hofy, F.I. & El-Said, A.A. (2015). Bacteriological studies on some food borne bacteria isolated from Chicken meat and meat products in Kaliobia Governorate. Benha Veterinary Medical Journal, 29 (2): 2:47-59.

[5] Bantawa, K., Rai, K., Subba Limbu, D. and Khanal, H. (2018). Food-borne bacterial pathogens in marketed raw meat of Dharan, eastern Nepal. BMC Research Notes, 11(1):618. doi: 10.1186/s13104-018-3722-x.

[6] Olaniyan, S.E., Kwaga, J.K.P., Saidu, A.S., Usman U. (2022). Multiple Anti-microbial Resistance Profile and Molecular Detection of Some Virulence Genes of Listeria Monocytogenes Isolated from Fresh Raw Meat Retailed in Zaria, Northwestern Nigeria. Afro-Egyptian Journal of Infectious and Endemic Diseases, 2022;12(1):3-15

[7] Hoffmann S, Devleesschauwer B, Aspinall W, Cooke R, Corrigan T, Havelaar A, Angulo F, Gibb H, Kirk M, Lake R, Speybroeck N, Torgerson P, Hald T. Attribution of global foodborne disease to specific foods: Findings from a World Health Organization structured expert elicitation. PLoS One. 2017;12(9): e0183641. doi: 10.1371/journal.pone.0183641.

[8] Touglo, K., Sanni, Y., Amegan, L., Akolly, K., Nuto, Y., Halatoko, W., Sadji, A., Bidjada, B., Djeri, B., Karou, S. and Ameyapoh, Y. (2023) Evaluation of the Microbiological Quality of Poultry Imported into Togo and the Antibiotic Resistance of Salmonella spp. Isolated. Advances in Microbiology, 13, 499-516. doi: 10.4236/aim.2023.1310032.

[9] Akagha TN, Gugu TH, Enemor EC, Ejikeugwu PC, Ugwu BC and Ugwu, MC. Prevalence and Antibiogram of Salmonella Species and *Staphylococcus aureus* In Retail Meats Sold in Awka Metropolis, Southeast Nigeria. International Journal of Biological & Pharmaceutical Research. 2015; 6(12): 924-929.

[10] American Public Health Association (APHA) (2012). Standard Methods for the Examination of Water and Waste Water. 22nd Edition, American Public Health Association, American Water Works Association, Water Environment Federation.

[11] Tefera, A. and Jermen, A.M. (2021). Microbiological Quality of Meat and Swabs from Contact Surface in Butcher Shops in Debre Berhan, Ethiopia. Journal of Food Quality; Cairo Vol. 2021, (2021). DOI:10.1155/2021/7520882.

[12] CLSI-Clinical Laboratory Standards Institute (2019): Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI document M100-S24 Wayne, Pennsylvania, 19087, USA.

[13] Hong, S., Kang, H.J., Lee, H.Y., Jung, H.R., Moon, J.S., Yoon, S.S., Kim, H.Y. and Lee, Y.J. (2023). Prevalence and characteristics of foodborne pathogens from slaughtered pig carcasses in Korea. Frontiers in Vertinary Science. 10:1158196. doi: 10.3389/fvets.2023.1158196.

[14] Julqarnain, S.M., Bose, P., Rahman, M.Z., Khatun, M.M. and Islam, M.A. (2022). Bacteriological quality and prevalence of foodborne bacteria in broiler meat sold at live bird markets at Mymensingh City in Bangladesh. Journal of Advanced Veterinary and Animal Research, 9(3):405-411. doi: 10.5455/javar.2022.i608.

[15] Food Safety and Inspection Service (FSIS). Modernization of Swine Slaughter Inspection; Final Rule. (2019). Available online at: https://www.fsis.usda.gov/federal-register/rules/modernization-swine-slaughter-inspection (accessed September 18, 2023).

[16] Van-Ba, H., Seo, H.W., Seong, P.N., Kang, S.M., Cho, S.H. and Kim, Y.S. (2019). The fates of microbial populations on pig carcasses during slaughtering process, on retail cuts after slaughter, and intervention efficiency of lactic acid spraying. International Journal of Food Microbiology, 294:10–7. doi: 10.1016/j.ijfoodmicro.2019.01.015

[17] Lindblad, M., Lindmark, H., Lambertz, S.T, Lindqvist R. (2007). Microbiological baseline study of swine carcasses at swedish slaughterhouses. J Food Prot. (2007) 70:1790–7. doi: 10.4315/0362-028X-70.8.1790

[18] Bohaychuk VM, Gensler GE, Barrios PR. (2011). Microbiological baseline study of beef and pork carcasses from provincially inspected abattoirs in Alberta, Canada. Canadian Veterinary Journal, (2011) 52:1095–100.

[19] Manios, S.G., Grivokostopoulos, N.C., Bikouli, V.C., Doultsos, D.A., Zilelidou, E.A. and Gialitaki M.A. (2015). A 3-year hygiene and safety monitoring of a meat processing plant which uses raw materials of global origin. International Journal of Food Microbiology, 209:60–9. doi: 10.1016/j.ijfoodmicro.2014.12.028

[20] Jakubowska-Gawlik, K., Kolanowski, W., Murali, A.P. and Trafialek, J. (2022). A comparison of food safety conformity between cattle and pig slaughterhouses. Food Control, 140:109143. doi: 10.1016/j.foodcont.2022.109143.

[21] Okorie-Kanu OJ, Anyanwu MU, Ezenduka EV, Mgbeahuruike AC, Okorie-Kanu CO, Ugwuijem EE, Idogwu MN, Anyaoha CO, Majesty-Alukagberie OL, Vidal RO, Vidal M. Occurrence and antibiogram of Listeria species in raw pork, beef, and chicken meats marketed in Enugu State, Southeast Nigeria. Veterinary World, 2020 Feb;13(2):317-325. doi: 10.14202/vetworld.2020.317-325.

[22] Suad, A.A and Wisam, H. A. (2020). Characterization of Foodborne Pathogens and Enterotoxigenic *Staphylococcus aureus* Isolates with Detection of Antibiotic Resistance from Beef Meat. Systematic Reviews in Pharmacy, 11(11):464-47.

[23] Akinware, O., Ogidi, C.O., Akinyele, B.J. (2023). Antibiotic sensitivity patterns and molecular detection of Enterotoxin genes in *Staphylococcus aureus* isolated from frozen muscle foods. Croatian Journal of food science and technology, 15 (1): 96-104.

[24] Muinde, P., Maina, J., Momanyi, K., Yamo, V., Mwaniki, J. and Kiiru, J. (2023). Antimicrobial Resistant Pathogens Detected in Raw Pork and Poultry Meat in Retailing Outlets in Kenya. Antibiotics, 12:613. https://doi.org/10.3390/ antibiotics12030613.

[25] Ronanki, S.P., Ramya, P., Babu, A.J. and Sreedevi, B. (2023). Epidemiological surveillance, molecular characterisation and virulent gene expression of *Proteus mirabilis* and Proteus vulgaris from milk and meat samples. The Pharma Innovation Journal, 12(1): 2019-2032.

[26] Ngo, H.H.T.; Nguyen-Thanh, L.; Pham-Duc, P.; Dang-Xuan, S.; Le-Thi, H.; Denis-Robichaud, J.; Nguyen-Viet, H.; Le, T.T.; Grace, D.; Unger, F. Microbial contamination and associated risk factors in retailed pork from key value chains in Northern Vietnam. Int. Journal of Food Microbiology, 2021, 346, 109163.

[27] Scheinberg, J.A.; Dudley, E.G.; Campbell, J.; Roberts, B.; DiMarzio, M.; DebRoy, C.; Cutter, C.N. Prevalence and Phylogenetic Characterization of Escherichia coli and Hygiene Indicator Bacteria Isolated from Leafy Green Produce, Beef, and Pork Obtained from Farmers' Markets in Pennsylvania. Journal of Food Protection. 2017, 80, 237–244.

[28] McLellan, J.E.; Pitcher, J.I.; Ballard, S.A.; Grabsch, E.A.; Bell, J.M.; Barton, M.; Grayson, M.L. Superbugs in the supermarket? Assessing the rate of contamination with third-generation cephalosporin-resistant gram-negative bacteria in fresh Australian pork and chicken. Antimicrob. Resist. Infect. Control 2018, 7, 1–7.

[29] Rortana, C.; Nguyen-Viet, H.; Tum, S.; Unger, F.; Boqvist, S.; Dang-Xuan, S.; Koam, S.; Grace, D.; Osbjer, K.; Heng, T.; et al. Prevalence of Salmonella spp. and *Staphylococcus aureus* in Chicken Meat and Pork from Cambodian Markets. Pathogens 2021, 10, 556.

[30] Thangavel, G. and Thiruvengadam, S. (2019). Microorganisms Isolated from Stored Meat in India, with Potential Antimicrobial Activity against Food Pathogens. Current Pharmaceutical Biotechnology, 20(5): p. 401-409.

[31] Mir R, Salari S, Najimi M, Rashki A. Determination of frequency, multiple antibiotic resistance index and resistotype of Salmonella spp. in chicken meat collected from southeast of Iran. Veterinary Medicine and Science, 2022 Jan;8(1):229-236.

[32] Naidu S, Bodempudi B, Chinnam B, Nelapati S, Tummati S, Cherukuri N, et al. Study of Virulence Genes, Antibiotic and beta-Lactamase Profiles of *Proteus mirabilis* Isolated from Livestock and Livestock Products. International Journal of Livestock Research, 2020;10(12):201-209.

[33] Salih, S.S., Mohammed, S.J., Noori, I.M., Mohammed, L.M. and Soor, T.A.H. (2019). Prevalence and molecular characterization of Beta-lactamase resistance gene in multidrug resistance bacteria, Proteus spp. Kurdistan Journal of Applied Research, 9: 20-28.

[34] Kim, J. and Yim D.G. (2016). Assessment of the microbial level for livestock products in retail meat shops implementing HACCP system. Korean Journal of Food Science and Anim Resources, 36(5):594–600. https://doi.org/10.5851/kosfa.2016.36.5.594.

[35] Peton, V. and Le Loir, Y. (2014). *Staphylococcus aureus* in veterinary medicine. Infection, Genetics and Evolution, 21:602–15. doi: 10.1016/j.meegid.2013.08.011.

[36] Komodromos, D., Kotzamanidis, C., Giantzi, V., Angelidis, A.S., Zdragas, A. and Sergelidis, D (2022). Prevalence and biofilm-formation ability of *Staphylococcus aureus* isolated from livestock, carcasses, the environment, and workers of three abattoirs in Greece. Journal of the Hellenic Veterinary Medical Society, 73:4097–4104. doi: 10.12681/jhvms.26469.

[37] Afunwa, R., Ezeanyinka, J., Afunwa, E., Udeh, A., Oli, A. and Unachukwu, M. (2020) Multiple Antibiotic Resistant Index of Gram-Negative Bacteria from Bird Droppings in Two Commercial Poultries in Enugu, Nigeria. Open Journal of Medical Microbiology, 10, 171-181. doi: 10.4236/ojmm.2020.104015.

[38] Ayandele, A.A., Oladipo, E.K., Oyebisi, O. and Kaka MO (2020). Prevalence of Multi-Antibiotic Resistant Escherichia coli and Klebsiella species obtained from a Tertiary Medical Institution in Oyo State, Nigeria. Qatar Medical Journal, 2020(1):9. doi: 10.5339/qmj.2020.9.

[39] World Health Organization (WHO) (2022). Antimicrobial Resistance. Available online: https://www.who.int/westernpacific/health-topics/antimicrobial resistance (accessed on 15 September 2023).

[40] Davies, S.C.; Fowler, T.; Watson, J.; Livermore, D.M.; Walker, D. (2013). Annual Report of the Chief Medical Officer: Infection and the rise of antimicrobial resistance. Lancet 2013, 381, 1606–1609.

[41] Teale, C.J. and Moulin, G. (2012). Prudent Use Guidelines: A Review of Existing Veterinary Guidelines. Revue Scientifique et Technique (International Office of Epizootics), 31, 343-354.

[42] Catry, B., Cavaleri, M., Baptiste, K., Grave, K., Grein, K., Holm, A., Jukes, H., Liebana, E., Navas, A.L., Mackay, D. and Magiorakos, A.P. (2015) Use of Colistin-Containing Products within the European Union and European Economic Area (EU/EEA): Development of Resistance in Animals and Possible Impact on Human and Animal Health. International Journal of Antimicrobial Agents, 46, 297-306.

[43] Mapipa, Q., Digban, T.O., Nnolim, N.E. et al. (2021). Antibiogram profile and virulence signatures of Pseudomonas aeruginosa isolates recovered from selected agrestic hospital effluents. Scientific Reports, 11, 11800 (2021). https://doi.org/10.1038/s41598-021-91280-6.

[44] Ejiofor, S.O., Edeh, A.D., Ezeudu, C.E., Gugu, T.H. and Oli, A.N. (2016). Multi-Drug Resistant Acute Otitis Media amongst Children Attending Out-Patient Clinic in Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, South-East Nigeria. Advances in Microbiology, 6, 495-501. https://doi.org/10.4236/aim.2016.67049

[45] Riaz, S., Faisal, M. and Hasnain, S. (2011) Antibiotic Susceptibility Pattern and Multiple Antibiotic Resistance (MAR) Calculation of Extended Spectrum β-Lactamase (ESBL) Producing Escherichia coli and Klebsiella Species in Pakistan. African Journal of Biotechnology, 10, 6325-6331.

[46] Osundiya, O.O., Oladele, R.O. and Oduyebo, O.O. (2013). Multiple Antibiotic Resistance (MAR) Indices of Pseudomonas and Klebsiella species Isolates in Lagos University Teaching Hospital. African Journal of Clinical and Experimental Microbiology. 14(3):164–168.