(RESEARCH ARTICLE)

Bacteriological quality and antimicrobial resistance profile of foodborne bacteria isolated from grilled meat sold in port Harcourt Nigeria

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Abstract

Street-vended grilled meats have gained popularity due to their convenient availability, affordability, diverse selection, and nutritional benefits. However, vendors' substandard handling practices raise concerns about their safety. The objective of this study was to evaluate the bacteriological quality and antimicrobial resistance profile of foodborne pathogenic bacteria isolated from grilled pork and beef purchased from five areas in Port Harcourt, Nigeria (Aluu, Choba, Alakhia, Rumuosi, and Rumuekini). The grilled pork samples exhibited a maximum Total Viable Count (TVC) of $\log_{10}$ 7.40 CFU/g and a maximum Total Coliform Count (TCC) of $\log_{10}$ 5.43 CFU/g. The Total Viable Count (TVC) and Total Coliform Count (TCC) of grilled beef are log10 6.06 CFU/g and log10 5.88 CFU/g, respectively. These values exceeded the log10 4.00 CFU/g acceptable limit. The five pathogenic bacteria isolated from grilled pork and beef meats were *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica*, *Pseudomonas aeruginosa*, and *Streptococcus* species. The rates of prevalence were 45.6, 32.0, 10.9, 4.08, and 7.5% for pork meat, and 59.8, 19.6, 9.3, 4.1, and 7.2% for beef meat. Antibiotic resistance of the pathogens ranged from 2.9–86.6% for pork and 5.2–79.3% for beef samples. The bacterial isolates showed higher resistance to ampiclox, amoxicillin, streptomycin, and gentamycin. The lowest resistance was observed for ciclopirox olamine and cephaloridine. The bacterial pathogens demonstrated a variety of Multiple Antibiotic Resistance (MAR) indices, falling between 0.4 and 0.9. Therefore, we recommend proper hygienic conditions before and after meat preparation to prevent them from being potential sources of infection for the public.

Keywords: Grilled meat; Antimicrobial resistance; Bacteriological quality; Pork; Beef; Pathogens

1. Introduction

Almost one out of ten people in the world fall ill after consuming food contaminated with pathogens and contaminants (bacteria, viruses, parasites, toxins, and chemicals [1, 2]. Foodborne diseases are a global public health problem; apart from the toxic infections that they can cause very quickly after consumption, they can also cause long-term illnesses, such as cancer, kidney or liver failure, and brain or nervous disorders [3]. These diseases can be more serious in children, pregnant women, the elderly, or those with a weakened immune system [2].

Meat and meat products are the most palatable of highly nutritive value foods for human being as they are important sources for protein, fat, essential amino acids, minerals, vitamins and other nutrients [4, 5]. There are different types of meat from different types of animals, examples pork (pig), multon (goat) and beef (cow) [6]. Meat can be served as prepared meat product e.g corn beef, fried meat, cooked meat and grilled meat. Meat is perishable food and its composition is ideal for the growth of wide range of spoilage bacteria [7].

Grilled meat (suya) is produced by smoking the raw beef with the addition of some spices, salt, oil, groundnut cake and flavors [10]. The meat is first sliced into smaller pieces and the spices are rubbed onto it, it is later oven dried or over

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local source of heat. This allow the meat to get dried properly with the right taste before is good to the consumers [8]. Grilled meat is good at a specific joint or hawked when it is constantly kept warm over fire source. The hawked grilled meat is carried about in open basin from place to place thereby exposing it to dust and other effects of the environment so doing harmful organisms find their ways into the meat there by causing for poisoning [9, 10].

The extent of microbial contamination and composition of microbial flora reflect the standard hygiene of meat. Foodborne pathogens can be present in a variety of foods including beef and pork [11]. Some of the microorganism present in grilled meat that causes food poisoning when consumed are *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Clostridium botulinum*, *Clostridium perfringes*, *Bacillus cereus*, *Salmonella enterica*, *Streptococcus pyrogenes* [12]. Some of these microorganisms in meat cause off flavours which make the meat to be unfit for eating and also reduce the taste value [13]. They can also cause diarrhea, dysentery, various infections, food poisoning, gastroenteritis, or typhoid fever [14].

Food borne pathogens can be controlled and avoided with an improved global food safety control system. One possible improvement would be a rapid and accurate detection system for microbial spoilage. This technique should ideally also be non-destructive and give result in real time for application in highly automated food processing environment [15]. Hence, it is critical to detect them in order to ensure a safe food and prevent foodborne illnesses [16]. This study aimed to assess the bacteriological quality of marketed ready to eat grilled pork and beef with a special emphasis on isolation of pathogenic bacteria.

2. Materials and Methods

2.1. Study area

The study area for this research is Choba and its environs (Alakahia, Rumuosi, Rumuekini, and Aluu axis). Choba is a suburb in Rivers State. It is located near Rumualogu and Mbuosi villages.

2.2. Sample collection

A total of 10 samples of suya meat and pork meat each, were randomly collected from different parts of Aluu, Choba, Alakahia, Rumuosi, and Rumuekini from different vendors. We collected the samples in sterile polythene bags, packed them in a carrier box containing ice packs, and transported them to the Microbiology Technology Laboratory of the University of Port Harcourt, Nigeria. The control samples were home-made grilled pork and beef carefully prepared under hygienic conditions.

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 was 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 hours.

After the end of the incubation period, colonies were counted using a colony counter. The counts were expressed as colony-forming units (CFU). Pure isolates of bacterial species were stored at 4 °C for use in identification examination.

2.3.1. Total coliforms and fecal coliform count

An aliquot of 0.1 mL from 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 18–24 h to determine fecal coliform

2.3.2. Enterobacteriaceae count

To count the members of Enterobacteriaceae, 0.1 mL of the aliquot from the appropriate dilution was spread and plated on MacConkey agar (M 081 Hi-Media, Mumbai) supplemented with glucose and incubated at 35 °C for 24 h. All reddish purple or pink colonies were counted as members of the Enterobacteriaceae [17].

2.4. Identification of bacterial isolates

The growing colonies were transferred to new specialized media for each bacterium to obtain a pure culture. The isolated bacteria were cultured at 37 oC for 24h and a staining procedure was applied using Gram stain. The biochemical tests were conducted to identify the isolated bacterial species. All bacterial isolates were biochemically tested following

standard procedures by using IMViC test including indole (I), methyl red (M), voges-proskauer (Vi), and citrate utilization test (C). In addition to that Kligler's iron agar (KIA), urease, coagulase, catalase, and oxidase tests were also used for identification of bacteria.

2.5. Detection of foodborne pathogenic bacteria

2.5.1. Total Staphylococcus spp. Count

Staphylococcus species were enumerated by the pour plate method and grown on Mannitol Salt Agar (MSA). An aliquot of 0.1 mL 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 S*taphylococcus* counts using the colony counter [17].

2.5.2. 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 [14].

2.5.3. Detection of Salmonella spp

A 25 gram of meat sample (minced by stomacher) was transferred to 225 mL of buffered peptone water (BPW) and incubated at 37 °C for 24 h. An aliquot of 0.1 mL from pre-enrichment was pipetted to 10 mL of 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 hours of incubation, the formation of colonies with black centers or with grey colours on SS agar was considered as presumptive *Salmonella* spp.

2.6. Determination of the Antibiotics Susceptibility Pattern of the Isolates

2.6.1. Antibiotic disc

Antibiotic discs used include Ciclopirox olamine (30 μg), Levofloxacin (30 μg), Gentamycin (30 μg), Erythromycin (30 μg), Ampiclox (30 μg), Rifampicin (30 μg), Neomycin (30 μg), Amoxicillin (30 μg), streptomycin (30 μg), and cephaloridine (30 μg).

2.6.2. Disc diffusion method

A 20-ml volume of Mueller-Hinton agar was prepared and dispensed aseptically into 90-mm Petri dishes. A loopful of each isolate was inoculated into 100 ml of nutrient broth and cultured overnight. From 100 μl of each isolate (equivalent to 0.5 ml), MacFarland standard was aseptically seeded into the Mueller Hinton agar plate. This was allowed to dry. The antibiotic disc was aseptically placed on the surface of the Muller-Hinton agar and allowed to pre-diffuse for 30 minutes. The set-up was done in triplicate with a control containing no antibiotic disc. It was incubated for 24 hours at 37 oC, and thereafter, the inhibition zone diameters were measured [18]. The values obtained were interpreted according to the Clinical and Laboratory Standards Institute [19].

2.6.3. Multiple Antibiotic Resistance (MAR) Index

The multiple antimicrobial resistant (MAR) index was calculated as the ratio of the number of antibiotics to which an organism is resistant to the total number of antibiotics to which the organism is exposed. MARI is calculated as a/b, where a is the number of antibiotics the isolate was resistant to and b is the total number of antibiotics used Bacteria having Ma MARindex ≥ 0.2 originate from a high-risk source of contamination where several antibiotics are used [20, 21].

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3. Results

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

The mean values of the total viable count (TVC) and total coliform count (TCC) of pork and grilled beef in four sampling locations in Port Harcourt are presented in Table 1. The mean TVC value of grilled pork was highest in Aluu (log10 7.40 CFU/g), followed by Choba (log10 6.29 CFU/g). Rumuekini had the lowest mean TVC for pork meat (log10 4.66 CFU/g). Similarly, samples collected from Aluu had the highest mean TCC for pork (log10 5.43 CFU/g), while samples from Rumuekini had the lowest (log10 3.22 CFU/g). However, the TVC and TCC of the control for grilled pork were log10 1.2

CFU/g and 0.0 CFU/g, respectively, while the TVC and TCC of the control for grilled beef were log10 1.0 CFU/g and log10 0.0 CFU/g, respectively. The results also showed that beef samples collected from Choba had the mean TVC (log10 6.06 CFU/g) and TCC (log10 3.22 CFU/g) of grilled beef, while Rumuekini had the lowest TVC (log10 5.18 CFU/g) and TCC (log10 3.10 CFU/g). According to statistical analysis, the mean counts for five sampling locations did not differ significantly (p > 0.05).

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

The bacteriological examination of grilled pork from the sampled locations is shown in Table 2. The results showed that 33 positive samples (66%) out of 50 grilled pork samples from various sites contained food-borne pathogens (*Salmonella enterica*, *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus* spp., and *Pseudomonas aeruginosa*). Pork samples collected from Aluu yielded the highest number of positive isolates, with 8 positive samples (24.2% and 16%). Pork samples from Choba and Rumuosi had equal records of 7 positive samples (21.2% and 14%) each. Pork samples collected from Rumuekini recorded the least number of positive samples, at 15.2% and 10% respectively.

Table 1 Mean total counts of grilled pork and beef

Location	Grilled pork (n=50)		Grilled beef (n=50)	
	Mean TVC (Log$_{10}$cfu/g)	Mean TCC (Log$_{10}$cfu/g)	Mean TVC (Log$_{10}$cfu/g)	Mean TCC (Log$_{10}$cfu/g)
Aluu (n=10)	7.40±0.11[c]	5.43±1.03[b]	5.96±0.06[b]	5.13±0.62[b]
Choba (n=10)	6.29±0.24 [b]	4.18±0.26[a]	6.06±0.21[b]	5.88±0.48[b]
Alakhia (n=10)	5.76±0.07[a]	4.31±0.76[a]	5.21±0.08[a]	4.25±0.06[a]
Rumuosi (n=10)	5.90±0.19[a]	5.07±0.92[b]	5.85±0.33[b]	4.39±0.07[a]
Rumuekini (n=10)	4.66±0.13[a]	3.22±0.11[a]	5.18±0.26[a]	3.10±0.19[a]
Control (n=10)	1.2±0.00	0.00±0.00	1.00±0.00	0.00±0.00
P- value	0.0311	0.415	0.443	0.521

Duncan Multiple Ratio Test. Mean values with different superscripts in same column are significantly different at p≤ (0.05). TVC= Total viable count, TCC=Total coliform count; count in log10 CFU per gram±standard error (SE)

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

Location	Number of sample (n)	Number of positive samples	Positive percentage		
			%[1]	%[2]	%[3]
Aluu	10	8	80	24.2	16
Choba	10	7	70	21.2	14
Alakahia	10	6	60	18.2	12
Rumuosi	10	7	70	21.2	14
Rumuekini	10	5	50	15.2	10
Total (N)	50	33	66	100	66

1Percentage in relation to total number of samples in each row. 2 Percentage in relation to total number of positive samples (33). 3 Percentage in relation to total number of collected samples (50).

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

The bacteriological examination of grilled beef from the sampled locations is shown in Table 3. Out of 50 grilled beef samples from various sites, 27 positive samples (54%) revealed food-borne pathogens. Grilled beef from Aluu and Choba yielded the highest number of positive samples, with 7 positive samples each (25.9% and 14%). The least positive sample of 3 (11.1 % and 6 %) was recorded for beef samples collected from Rumuekini.

3.4. Biochemical test

The results of the biochemical tests are presented in Table 4. The results revealed that *Salmonella* species showed indole (-), methyl red (+), voges Proskauer (-), simmon's citrate (+), urease (-), kligler's iron agar (Alkaline/Acid with H2S), and oxidase (-). *Escherichia coli* showed indole (+), methyl red (+), Voges Proskauer (-), simmon's citrate (+), urease (-), kligler's iron agar (Alkaline/Acid with H2S), oxidase (-) and catalase (+). The result of biochemical tests for S. aureus revealed positive for both coagulase and catalase. *Pseudomonas aeruginosa* showed indole (-), methyl red (-), Voges Proskauer (-), simmon's citrate (+), urease (-), kligler's iron agar (-), oxidase (+) and catalase (+). *Streptococcus* spp. showed indole (-), methyl red (+), Voges Proskauer (-), simmon's citrate (+), urease (-), kligler's iron agar (+), oxidase (-) and catalase (-).

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

Location	Number of sample (n)	Number of positive samples	Positive percentage		
			%[1]	%[2]	%[3]
Aluu	10	7	70	25.9	14
Choba	10	7	70	25.9	14
Alakahia	10	4	40	14.8	8
Rumuosi	10	6	60	22.2	12
Rumuekini	10	3	30	11.1	6
Total (N)	50	27	54	100	54

[1]Percentage in relation to total number of samples in each row. [2] Percentage in relation to total number of positive samples (33). [3] Percentage in relation to total number of collected samples (50).

Table 4 Gram stain and biochemical tests of three species of bacteria isolated from pork and meat

Gram staining	Indole	Methyl red	Voges-proskauer	Citrate	Urease	kligler's iron agar	Oxidase	Coagulase	Catalase	Bacteria species
-	-	+	-	+	-	+	-	-	+	*Salmonella enterica*
-	+	+	-	-	-	-	-	-	+	*Escherichia coli*
+	-	+	+	+	+	-	-	+	+	*Staphylococcus aureus*
-	-	-	-	+	-	+	+	-	+	*Pseudomonas aeruginosa*
+	-	+	-	+	-	+	-	-	-	*Streptococcus* spp.

3.5. Occurrence of foodborne bacteria in grilled pork and beef

3.5.1. Occurrence of foodborne bacteria in grilled pork

The occurrence of foodborne bacteria in grilled pork is presented in Table 5. *Staphylococcus aureus* recorded the highest (45.6%), followed by *E. coli* (32%), and Salmonella enterica (10.9%). Grilled pork from Choba had the highest level of bacterial occurrence (25.2%), followed by Aluu (24.5%), Rumuosi (22.4%), and Alakahia (14.3%). Rumuekini had the lowest bacterial occurrence at 13.6%.

3.5.2. Occurrence of foodborne bacteria in grilled beef

The occurrence of foodborne bacteria in grilled beef is presented in Table 6. *Staphylococcus aureus* recorded the highest (59.8%), followed by *E. coli* (19.6%) and *Salmonella enterica* (9.28%). Grilled pork from Aluu had the highest level of bacterial occurrence (28.9%), followed by Rumuosi (24.7%), Choba (22.7%), and Rumuekini (12.4%). Alakahia had the lowest bacterial occurrence at 11.3%.

Table 5 Occurrence of foodborne bacteria in grilled pork

Bacterial Pathogen	Sampling site (%)					Total (N/%)
	Aluu (n(/%))	Choba (n(/%))	Alakahia (n(/%))	Rumuosi (n(/%))	Rumuekini (n(/%))	
Staphylococcus aureus	18 (26.9)	15 (22.4)	11 (16.4)	13(19.4)	10 (14.9)	67 (45.6)
E. coli	09 (19.4)	13 (27.7)	07 (14.9)	12 (25.5)	06 (12.8)	47 (32.0)
Salmonella enterica	03 (18.8)	05 (31.3)	02 (12.5)	04 (25.0)	02 (12.5)	16 (10.9)
P. aeruginosa	02 (33.3)	02 (33.3)	00 (0.0)	01 (16.7)	01 (16.7)	6 (4.08)
Streptococcus spp.	04 (36.4)	02 (18.2)	01 (9.01)	03(27.3)	01 (9.01)	11 (7.50)
Total (%)	36 (24.5)	37 (25.2)	21 (14.3)	33 (22.4)	20 (13.6)	147 (100)

*n=number of pork sampled=10, *N=Total number of bacterial pathogens=147

Table 6 Occurrence of foodborne bacteria grilled beef per sampling site

Bacterial Pathogen	Sampling site (%)					Total (N/%)
`	Aluu (n(/%))	Choba (n (/%))	Alakahia (n (/%))	Rumuosi (n (/%))	Rumuekini (n (/%))	
S aureus	15 (25.9)	11 (19.0)	08 (13.8)	15 (25.9)	09 (15.5)	58 (59.8)
E. coli	07 (36.8)	05 (26.3)	02 (10.5)	04 (21.1)	01 (5.3)	19 (19.6)
S. enterica	02 (22.2)	03 (33.3)	01 (11.1)	02 (22.2)	01 (11.1)	09 (9.28)
P. aeruginosa	01 (25)	01 (25)	0 (0.0)	01 (25)	01 (25)	04 (4.12)
Streptococcus spp.	03 (42.9)	02 (28.6)	0 (0.0)	02(28.6)	0 (0.0)	07 (7.22)
Total (%)	28 (28.9)	22 (22.7)	11 (11.3)	24 (24.7)	12 (12.4)	97 (100)

*n=number of beef sampled=10, *N=Total number of pathogens=147

3.6. Antimicrobial susceptibility profiles of bacterial Pathogens

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

Antimicrobial susceptibility profile of bacterial pathogens isolated from grilled pork is illustrated in Table 7. Antimicrobial resistance patterns of the 67 isolates of *Staphylococcus aureus* showed high rate of susceptibility to gentamycin (91.0%), ciclopirox olamine (88.1%), rifampicin (71.6%) and levofloxacin (64.2%). High rate of resistance was observed for ampiclox (86.6%), streptomycin (82.1%), and erythromycin (79.1%). However, the lowest rate of resistance was observed for levofloxacin (2.9%) and ciclopirox olamine (4.5%).

Forty-seven (47) isolates of *E. coli* showed high rate of susceptibility to cephaloridine (83.0%), rifampicin (74.5%). and ciclopirox olamine (72.3%). High rate of resistance was recorded for gentamycin (87.2%), ampiclox (80.9%), and streptomycin (72.3%). Sixteen (16) isolates of *Salmonella enterica* showed high susceptibility to cephaloridine (87.5%), ciclopirox olamine (81.3%) gentamycin (77.8%) and erythromycin (77.8%). They also showed high resistant to ampiclox (77.8%) and levofloxacin (62.5%).

The isolates of *Pseudomonas aeruginosa*, showed high susceptibility to rifampicin (100%), neomycin (83.3%), cephaloridine (83.3%), and Ciclopirox olamine (83.3%). They showed resistance to gentamycin (66.7%) and amoxicillin (66.7%). Isolates of *Streptococcus* spp., showed susceptibility to ciclopirox olamine (81.8%) and cephaloridine (81.8%), and resistance to ampiclox (81.8%) and streptomycin (63.6%).

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

Bacterial isolates	N	Profile	Antimicrobial susceptibility profiles n (%)									
			CPX	LV	CN	ER	APX	RD	NB	AMX	S	CH
Staphylococcus aureus	67	S	59(88.1)	43(64.2)	61(91.0)	10(15.0)	6 (9.0)	48(71.6)	44(55.2)	29(43.3)	9(13.4)	28(41.8)
		I	5(7.5)	8(11.9)	2(3.0)	4(6.0)	3(4.5)	6(9.0)	7(6.9)	5(7.5)	3(4.5)	9(13.4)
		R	3 (4.5)	16(2.9)	4(6.0)	53(79.1)	58(86.6)	13(19.4)	16(23.9)	33(49.3)	55(82.1)	30(44.8)
E. coli	47	S	34(72.3)	23(69.0)	41(87.2)	20(42.6)	6(15.7)	35(74.5)	29(61.7)	7(14.9)	13(27.7)	39(83.0)
		I	2(4.3)	9(19.1)	2(4.3)	5(10.6)	3(21.1)	4(8.5)	5(10.6)	8(17.0)	0(0)	2(4.3)
		R	11(16.4)	15(31.9)	4(8.5)	22(46.8)	38(80.9)	8(17.0)	13(27.7)	32(68.1)	34(72.3)	6(12.8)
Salmonella enterica	16	S	13(81.3)	4(25)	11(77.8)	11(77.8)	5(1.6)	12(75)	10(62.5)	8(50.0)	9(56.3)	14(87.5)
		I	0(0)	2(12.5)	0(0)	1(6.3)	0(0)	0(0)	0(0)	3(1.9)	2(12.5)	0(0)
		R	3(6.4)	10(62.5)	5(22.2)	4(25)	11(77.8)	4(25)	6(37.5)	5(22.5)	5(31.3)	2(12.5)
P. aeruginosa	06	S	5(83.3)	4(66.7)	2(33.5)	4(66.7)	1(16.7)	6(100)	5(83.3)	1(16.7)	2(33.3)	5(83.3)
		I	0(0)	0(0)	0(0)	0(0)	2(33.3)	0(0)	0(0)	1(16.7)	1(16.7)	0(0)
		R	1(16.7)	2(33.3)	4(66.7)	2(33.3)	3(50)	0(0)	1(16.7)	4(66.7)	3(50)	1(16.7)
Streptococcus spp.	11	S	9(81.8)	5(45.5)	4(36.4)	4(36.4)	2(18.2)	8(72.7)	7(63.6)	4(28.6)	4(36.4)	9(81.8)
		I	0(0)	0(0)	2(18.2)	1(9.1)	0(0)	0(0)	2(18.2)	1(9.1)	0(0)	1(9.1)
		R	2(18.2)	6(54.5)	5(45.5)	6(54.5)	9(81.8)	3(27.3)	2(18.2)	6(54.5)	7(63.6)	1(9.1)

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 grilled beef

Antimicrobial susceptibility profile of bacterial pathogens isolated from grilled beef is shown in Table 8. Isolates of bacteria belonging to five different genera showed broad variations in their resistance and susceptibility profiles. Of the 58 isolates of *Staphylococcus aureus* tested against ten antibiotics, 93.1% of the isolates were found to be susceptible ciclopirox olamine followed by gentamycin (87.9%), and rifampicin (70.7%). With regard to the resistance profile, 79.3% of the isolates were found to be resistant to erythromycin followed by ampiclox (75.9%), and streptomycin (70.7%). Out of the 19 isolates of the *E. coli* isolated from beef meat, 89.1% isolates showed the highest susceptibility to gentamycin followed by ciclopirox olamine (84.2%) and cephaloridine (68.4%). Fourteen (14(73.7%)) of the *E.coli* isolates had highest resistance to amoxicillin followed by ampiclox (63.1%) and streptomycin (63.1%). Of the 9 isolates of *Salmonella enterica* tested, 8(88.9%) were susceptible to Ciclopirox olamine followed by gentamycin (77.8%). Rifampicin and cephaloridine had equal susceptibility by 66.7% isolates each. Five (5(55.6%) out of the 9 *Salmonella enterica* showed resistance to levofloxacin and ampiclox.

Three (3(75%)) out of the 4 isolates of *Pseudomonas aeruginosa* were susceptible to ciclopirox olamine, gentamycin, rifampicin, neomycin, and cephaloridine. They were also resistant to ampiclox, and streptomycin. Of the 7 isolates of *Streptococcus* spp., 5(71.4%) were susceptible to ciclopirox olamine, neomycin and cephaloridine while 5(71.4%) were resistant to ampiclox followed by levofloxacin (4(57.1%)), amoxicillin (57.1%) and erythromycin (42.9%).

Table 8 Antimicrobial susceptibility profile of bacterial pathogens isolated from grilled beef

Bacterial isolates	N	Profile	Antimicrobial susceptibility profiles n (%)									
			CPX	LV	CN	ER	APX	RD	NB	AMX	S	CH
Staphylococcus aureus	58	S	54(93.1)	23(39.7)	51(87.9)	12(20.7)	14(24.1)	41(70.7)	32(55.2)	10(17.2)	11(19.0)	37(63.8)
		I	1(1.7)	7(12.1)	3(5.2)	0(0.0)	9(15.5)	5(8.6)	4(6.9)	9(15.5)	6(10.3)	4(6.9)
		R	3 (5.2)	20(34.5)	4(6.9)	46(79.3)	44(75.9)	12(20.7)	22(37.9)	39(67.2)	41(70.7)	17(29.3)
E. coli	19	S	16(84.2)	12(63.1)	17(89.1)	10(52.6)	3(15.7)	11(57.9)	9(47.4)	4(21.1)	4(21.1)	13(68.4)
		I	0(0)	4(21.1)	0(0)	2(10.5)	4(21.1)	3(15.7)	5(26.3)	1(5.3)	3(15.7)	2(10.5)
		R	3(15.7)	3(15.7)	2(10.5)	7(36.8)	12(63.1)	5(26.3)	5(26.3)	14(73.7)	12(63.1)	4(21.1)
Salmonella enterica	09	S	8(88.9)	3(33.3)	7(77.8)	5(55.6)	2(22.2)	6(66.7)	5(55.6)	2(22.2)	3(33.3)	6(66.7)
		I	0(0)	1(11.1)	0(0)	1(11.1)	2(22.2)	0(0)	0(0)	2(22.2)	2(22.2)	1(11.1)
		R	1(11.1)	5(55.6)	2(22.2)	3(33.3)	5(55.6)	3(33.3)	4(44.4)	5(55.6)	4(44.4)	2(22.2)
P. aeruginosa	04	S	3(75)	2(50)	3(75)	4(100)	1(25)	3(75)	3(75)	1(25)	1(25)	3(75)
		I	0(0)	0(0)	0(0)	0(0)	1(25)	0(0)	0(0)	1(25)	0(0)	0(0)
		R	1(25)	2(50)	1(25)	0(0)	3(75)	1(25)	1(25)	2(50)	3(75)	1(25)
Streptococcus spp.	07	S	5(71.4)	3(28.6)	3(42.9)	3(42.9)	2(28.6)	4(57.1)	5(71.4)	2(28.6)	2(28.6)	5(71.4)
		I	1(14.30)	0(0)	2(28.6)	1(14.30)	0(0)	1(14.30)	0(0)	1(14.30)	2(28.6)	0(0)
		R	1(14.30)	4(57.1)	2(28.6)	3(42.9)	5(71.4)	2(28.6)	2(28.6)	4(57.1)	3(42.9)	2(28.6)

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 grilled pork

The comparison of the resistance pattern of the isolates from grilled pork to the antibiotics used in this study is presented in Figure 1. The result revealed that isolates of *Staphylococcus aureus* were mostly resistant to most of the antibiotics followed by *Pseudomonas aeruginosa* 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 grilled beef

The comparison of the resistance pattern of the isolates from grilled beef to the antibiotics used in this study is presented in Figure 2. The result revealed that isolates of *Pseudomonas aeruginosa* gave zero tolerance to rifampicin. Staphylococcus aureus was resistant to most of the antibiotics followed by *Streptococcus* spp. while others have moderate resistance. In addition, the isolates from grilled beef also showed highest and lowest resistance to ampiclox and ciclopirox olamine respectively.

3.8. Multiple Antibiotics Resistance Index of the Bacterial isolates from Pork and Grilled beef

Multiple Antibiotics Resistance Index (MARI) of isolates from grilled pork is shown in Table 9. *Salmonella* spp from grilled pork had the highest MAR index of 0.8 followed by *E. coli* and *Streptococcus* spp. with equal MAR index of 0.6 each. The lowest was observed found to be *S. aureus* (0.5).

High MAR index was recorded for isolates from grilled beef (Table 4.9). *Salmonella enterica*, *Pseudomonas aeruginosa*, and *Streptococcus* spp., had the highest MAR index of 0.9. The lowest MAR were recorded for *S. aureus* and *E. coli* with an index value of 0.7 each.
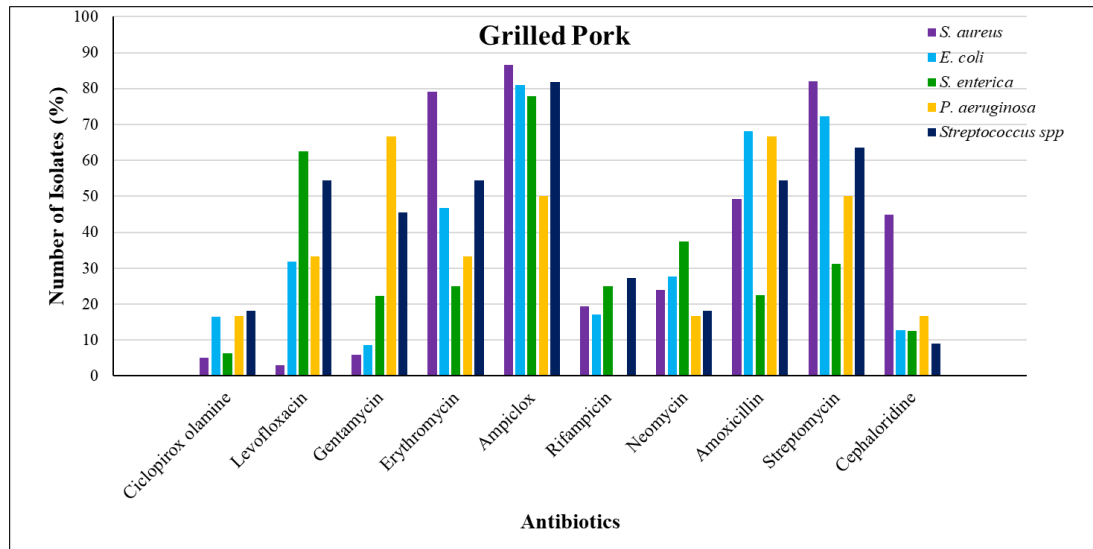
Figure 1 Comparison of resistance of the bacterial isolates from grilled pork to different antibiotics
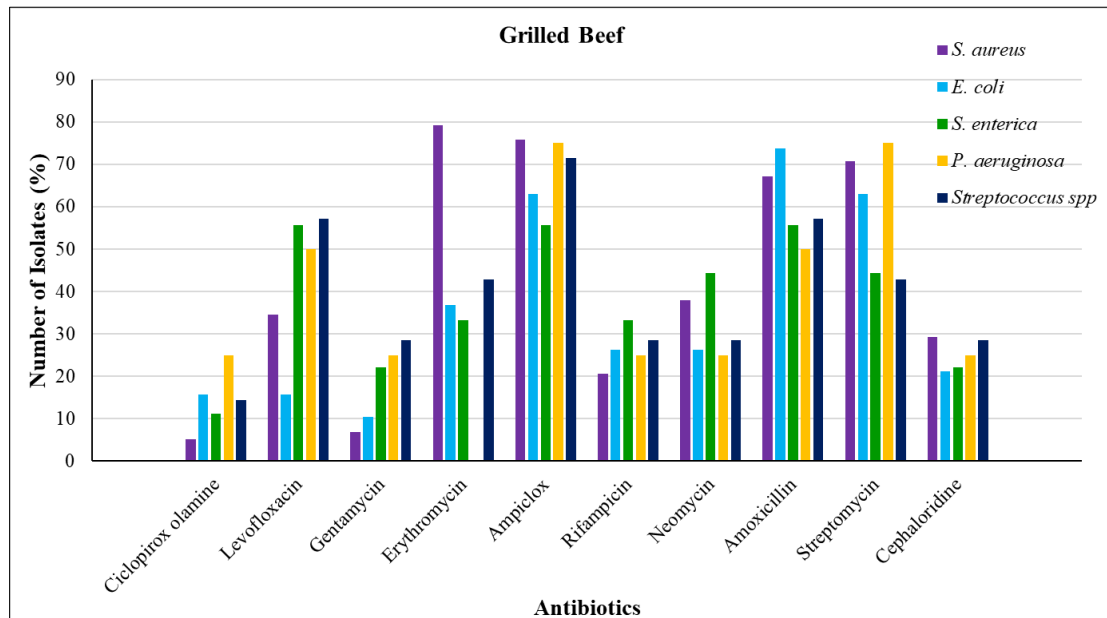
Figure 2 Comparison of resistance of the bacterial isolates from grilled beef to different antibiotics

Table 9 Multiple antibiotics resistance index of isolates

Sample	Bacterial Isolates	Resistance pattern	Number of antibiotics resistant to	MAR Index
Grilled pork	*S. aureus*	ER, APX, AMX, RD, NB	5	0.5
	E. coli	APX, AMX, S, LV, ER, NB	6	0.6
	Salmonella enterica	LV, APX, AMX, CN, ER, RD, NB, S	8	0.8
	P. aeruginosa	LV, APX, AMX, ER	4	0.4
	Streptococcus spp.	LV, APX, AMX, CN, ER, RD	6	0.6
Grilled beef	*S. aureus*	LV, ER, APX, RD, NB, AMX, CH	7	0.7
	E. coli	ER, APX, RD, NB, AMX, S, CH	7	0.7
	Salmonella enterica	LV, CN, ER, APX, RD, NB, AMX, S, CH	9	0.9
	P. aeruginosa	CPX, LV, CN, APX, RD, NB, AMX, S, CH	9	0.9
	Streptococcus spp.	LV, CN, ER, APX, RD, NB, AMX, S, CH	9	0.9

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 Grilled Pork and Beef Meat per Sampling Site

The quality of meat was assessed by counting total viable count (TVC) and total coliform count (TCC), which also indicate the food safety level of meat [22]. For pork meat, this study recorded highest TVC and TCC of log10 7.40 CFU/g and log10 5.43 CFU/g respectively (from Aluu). The highest TVC and TCC for beef meat were log10 6.06 CFU/g and log10 5.88 CFU/g respectively (from Choba). 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 [23,24]. The bacterial count for pork meat in this study exceeded the acceptable limits. This result was similar those reported by Anihouvi et al. [25], who recorded microbial count in the range of Log10 2.7 CFU/g and Log10 7.4 CFU/g, with 16.7% of pork samples exceeding the acceptable limit of <Log10 7.0 CFU/g recommended by the Health Protection Agency for this criterion. Lydia et al. [26], reported bacterial count in the range of Log10 3.9 CFU/g and Log10 8.2 CFU/g. Udoh et al. [27], reported microbial count of Log10 4.2 CFU/g - Log10 4.36 CFU/g from pork meat. Nwachukwu et al. [28], reported microbial count of Log 4.82 – 5.30 CFU/g from grilled beef sold in Yenagoa, Bayelsa state of Nigeria. Anihouvi et al. [25], reported that the possible source of contamination may be related to processors, processing methods, equipment used, raw materials, and processing/selling environment.

Pork and beef meat contain nutrients such as protein, lipid, fiber, carbohydrate, as well as moisture. These constituents make the meat product susceptible to microbial growth [27]. Most organisms utilize protein, a carbohydrate in the presence of moisture to multiply and thrive very well [3].

4.2. Prevalence of Foodborne Bacteria in Grilled Pork and Beef Meat

Pork and beef samples analyzed in this study were contaminated with pathogenic bacteria and were predominantly *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica*, *Pseudomonas aeruginosa* and *Streptococococcus* spp. *Staphylococcus aureus* was found with the highest percentage of frequency of occurrence in pork (45.6%) and beef (59.8%). Nwachukwu et al. [28], isolated *Staphylococcus aureus*, *E. coli*, *Salmonella* spp., *Klebsiella* spp., *Pseudomonas* spp., and *Shigella* spp. from grilled beef. Udoh et al. [27], Tinega et al. [29], and Yannick et al. [30], in their work also confirmed the presence of bacterial pathogens in pork with *Staphylococcus aureus* as the predominant organisms found with the highest percentage of frequency of occurrence. Alabi et al. [31], reported *Staphylococcus aureus* (15.8%), *Pseudomonas* spp. (42.1%), *Klebsiella* spp. (36.8%), and *Bacillus* spp. from grilled beef.

According to the findings of Owoseni et al. [32], the extensive distribution of meat products exacerbates the severity of contamination by foodborne pathogens. The isolation of these microorganisms from grilled pork and beef has significant public health implications due to their pathogenic nature. This finding raises concerns, particularly in the research area

where a substantial number of individuals consume these food products. The presence of these microbiological contaminants in the pork and beef samples may be related to the unclean quality of the slaughterhouses, which indicates that the pork and beef were poorly prepared and even the lengthy exposure to the surroundings. Additional factors that may contribute to the contamination of meat and the presence of these organisms include various processing stages, as well as the handling and distribution practices involved [30].

The high level of *Staphylococcus species* may be as a result of lack of personal hygiene of the processors, from dust and lack of good handling and manufacturing practices [30, 24]. Therefore, presence of *Staphylococcus aureus* in the roasted pork and beef is an indication of possible contamination from human sources to the meat from the skin, mouth or nose of the handler which can be introduces directly into the food by contact or other aerial-droplet mechanisms such as coughing or sneezing [30]. However, according to Udoh et al. [27], enterotoxin producing strains of *S. aureus* are leading cause of food intoxication as they can produce extremely potent gastrointestinal toxins. Bantawa et al. [16], reported that higher prevalence of *S. aureus* indicates that inadequate cleaning, unsatisfactory handling, and post-processing contamination from the polluted atmosphere around markets and shops. High prevalence of *S. aureus* in meat and handlers contain health hazards like toxin-mediated virulence and invasiveness to consumers [33].

Escherichia coli and *Enterobacter* species isolated in the study are enteric organisms. Their presence in the pork and beef is an indication generally traceable to fecal contamination either direct or indirect means [35]. They are normal flora of the intestine in human and animals and are widely distributed in the environment contaminating food and water. Moreover, their presences in foods are usually as a result of excessive human handling and possible contamination of pork itself during sales [34]. The pork that has been processed and kept for some days to be sold stands a chance to been contaminated especially when exposing such meat for consumers to see.

Meat containing the *E. coli* indicator organism is likely to be contaminated with feces and indicates poor hygiene [36]. As an indicator of hygiene and sanitary quality, the presence of *E. coli* suggests that consumers are at risk of being food poisoned and the presence of other pathogenic fora. Meat containing the *E. coli* indicator organism is likely to be contaminated with feces and indicates poor hygiene [37]. Notably is the fact that *Enterobacter* species are bacteria commonly known to cause gastroenteritis, meningitis, and infection in the bladder [38]. More so, an enterotoxigenic strain of *E. coli* is the most common cause of traveler's diarrhea and some strains of this pathogen can cause a wide variety of infections such as other forms of diarrhea and other gastrointestinal problems especially in a community setting [39]. Pork or other food products that contain *E. coli* in its infective dose can be a continuous source of infections leading to complications and death especially among children and immunocompromised individuals [40].

4.3. Antimicrobial and Multi drug Resistance by the Bacterial Pathogens

Antimicrobial Resistance (AMR) has become a big threat to global health. It has risen to dangerously high levels in all parts of the world, making it difficult to treat infectious diseases [41]. The rates of resistance of the bacterial pathogens to the various classes of antimicrobial agents ranged from 3–87.2% for grilled pork and 5.2-75.9% for grilled beef samples. Higher resistance was shown for frequently used antibiotics such as ampiclox, amoxicillin, streptomycin and gentamycin. Moderate resistance was observed for levofloxacin, erythromycin, neomycin and rifampicin. The lowest resistance was observed ciclopirox olamine and cephaloridine. Among the bacterial pathogens isolated from pork and beef in this study, *S. aureus* had the highest resistance to the ten antibiotics followed by *Streptococcus* spp. and *E. coli*. The pathogen with the least resistance is *S. enterica*. The high performance of ciclopirox olamine and cephaloridine could also be due to their molecular sizes a factor which enhances their solubility in diluents thus promoting their penetration power through cell wall into the cytoplasm of the bacterial pathogens [42].

Similar study carried out by Ayandele et al. [43], revealed that *E. coli* showed the highest resistance toward streptomycin and erythromycin, and susceptibility to gentamycin.

Antimicrobial resistance (AMR) is a major global health concern, caused by the misuse and overuse of antimicrobial agents [44, 45], which has led to microorganisms (including bacteria, fungi, viruses, and parasites) becoming resistant to the effects of these medications. The WHO defines AMR as the loss of susceptibility of these microorganisms to antimicrobial agents (such as antibiotics, antifungals, antivirals, and antiprotozoals) [46]. This imprudent use of antimicrobials in both the human and animal sector has resulted in the selection of pathogens resistant to multiple drugs. It is now widely acknowledged that the rate of AMR development and spreading far outstrips the rate at which new antimicrobial drugs are being developed.

In animals, antimicrobial use in animal production (especially in poultry and pigs) remains a key contributor to AMR [47]. The use is expected to increase exponentially due to the expansion of intensive production systems to meet the

increasing demand for animal sourced foods (ASFs), and the surge in disease burdens [47]. Over the next 20–40 years, the demand for ASFs will grow rapidly in Africa (meat consumption is forecast to grow by 30% by 2030) due to growth in the human population (from the current 1.2 billion to over 2.5 billion by 2050), increasing purchasing power and urbanization [48].

Antimicrobial-resistant bacteria that originate in the gastrointestinal tract of animals can contaminate meat during animal slaughter and food processing or contaminate the environment with animal feces, and thus be transferred to humans through handling or consuming contaminated food or coming into contact with animal waste. This can lead to antimicrobial-resistant intestinal infections [46].

Resistance bacteria of animal origin can be transmitted from animals to humans through the food supply, water or, through direct contact with animals. Sometimes resistant genes can be transferred from animals through human pathogens that are normally human-specific [48]. Contaminated food of animal origin is one source of human bacterial infections when consumed; therefore, the presence of antibiotic-resistant strains in the animal foods such as pork and beef has raised concerns that the treatment of human infections will be compromised [49].

Multiple antibiotic resistance (MAR), that is, resistant to 3 or more different classes of antimicrobials, was observed in this study. Multiple antibiotics resistance index was determined with the formula: "MAR Index = a/b. Where (a) is the number of antibiotics which the isolates showed resistance, (b) is the total number of anti- biotics used in each class of antimicrobial agent" [50]. MAR Index of ≥ 0.2 infers that the strain of such bacteria originates from an environment where there is an abuse of these drugs and also that the plasmids contain one or more resistance genes, each encoding a single antibiotic resistance phenotype [21, 51].

The MAR index from this study revealed slight variations with the lowest MAR index of 0.4 and the highest MAR index of 0.9. This study also revealed that the highest multidrug resistance was observed in isolates from grilled beef which occurred in *Salmonella enterica*, *P. aeruginosa* and *Streptococcus* species with equal MAR index of 0.9. However, other isolates had a MAR index of ≥ 0.4. A similar study carried out by Akinware et al. [52], Afunwa et al. [41], also revealed that the highest multidrug resistance occurred in their bacterial isolates with MAR index of 0.4-1.00. Organisms which have MAR indices of greater than ≥0.2 confirm the presence of multidrug-resistant genes originating from the environment where there is frequent use and abuse of these drugs [21]. According to Mir et al. [51], multiple antibiotic resistance (MAR) in bacteria is most commonly associated with the presence of plasmids which 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 [53]. MAR index is an effective, valid, and cost-effective method that is used in source tracking of antibiotic resistant organisms [43]. Using the MAR index analysis is also simple and does not require specialized training and expensive equipment, while also providing the needed data [41].

5. Conclusion

The bacteria isolated from grilled pork and beef meats were *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enterica*, *Pseudomonas aeruginosa*, and *Streptococcus* species. The antibiogram of these organisms demonstrated multidrug resistance to the majority of antibiotics. These organisms are known pathogens that cause foodborne illness in humans. The presence of these bacteria suggests improper handling of grilled meats, especially in highly unhygienic environments. Therefore, the rate of infections from these meats could be reduced and consumer safety could be guaranteed by appropriately sensitizing local vendors regarding proper animal husbandry, hygienic slaughter and storage of meat, and sanitation of utensils and equipment. We highly recommend reducing antibiotic exposure and encouraging the development of novel and more effective antibiotics.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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