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Phenotypic and Genotypic Characterisation of Antibiotic Resistance in *Escherichia coli*, *Klebsiella* spp., and *Listeria monocytogenes* Isolates from Raw Meat Sold in Nairobi

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Abstract

Worldwide, the increase in antimicrobial resistance (AMR) is a public health concern. Food-borne associated antibiotic-resistant pathogens can contaminate raw meat during slaughter, transportation, and at sale points. A cross-sectional study was conducted from March 2021 to December 2021 to determine antimicrobial susceptibility patterns and characterize the molecular basis of resistance in *E. coli*, *Klebsiella* spp., and *L. monocytogenes* contaminating raw meat collected from retail outlets in Nairobi. Isolation and identification of the strains were done using the standard culture methods and PCR. Antimicrobial susceptibilities of the recovered strains were determined using disk diffusion while the presence of antibiotic resistance gene determinants; bla_{TEM}, bla_{CTX-M}, bla_{OXA}, sul, and qnrS was done using PCR. Of 270 samples collected, 163 (60%) *Escherichia coli*, 19 (7%) *Klebsiella* spp., and *L. monocytogenes* 3 (1.1%) were recovered. Among *Escherichia coli*, high antibiotic resistance was found to Erythromycin 161 (98%) and ampicillin 88 (54%) while low resistance was found against imipenem 2 (1%). Similarly, high resistance was found among *Klebsiella* spp. to Erythromycin 19 (100%) and ampicillin 12 (63%) low resistance to ceftazidime 1 (5%), cefotaxime 1 (5%), aztreonam 1 (5%), and chloramphenicol 1 (5%). One isolate among the three *Listeria monocytogenes* strains isolated was resistant to Trimethoprim-sulfamethoxazole. No resistance was exhibited to gentamycin by all *Klebsiella* spp. The prevalence of multidrug-resistant (resistance to three or more classes of antibiotics) isolates was 95/182 (52.2%). The common resistance pattern observed was Erythromycin, ampicillin, tetracycline, and trimethoprim-sulfamethoxazole with a prevalence of 19 (20%). ESBL was confirmed in isolates that harbored: bla_{TEM} (65%), bla_{CTX-M} (44%), bla_{OXA} (33%) while sul and qnrS were detected

in 46.7% and 13.6% respectively. Circulation of antibiotic-resistant and MDR isolates found in this study could play a role in the dissemination of AMR among food-borne bacteria and suggest potential food safety and public health risk. Therefore, enhanced surveillance for antibiotic-resistant organisms in raw meat for early detection of emerging resistant bacteria species in the food chain is recommended.

Keywords

Raw Meat, *Escherichia coli, Listeria monocytogenes*, Multidrug Resistance, Extended Spectrum β-Lactamase (ESBL)

1. Introduction

Meat serves as an important source of proteins for humans. However, the recent emergence of antibiotic-resistant foodborne pathogens combined with indiscriminate use of antibiotics in food-producing animals is considered a worldwide public health concern [1]. Antibiotic-resistant pathogens can contaminate raw meat at an unhygienic slaughter, during transportation, processing, and sale point [2].

There is a risk of acquiring food-borne bacteria such as *E. coli*, *Klebsiella* spp., and *L. monocytogenes* strains when contaminated meat is consumed [3] [4]. *E. coli* and *Klebsiella* spp. easily acquire resistance genes from one another. The mobile genetic elements such as plasmids and transposons carry genes that encode resistance to antibiotics used and therefore can be transferred from one bacteria to another during contact [5], which limits the treatment options in humans and veterinary medicine. Reports have indicated high levels of antibiotic resistance in *E. coli*, *Klebsiella* spp., *and L. monocytogenes* among other bacteria isolated from retail meat [6] [7] [8] [9]. Notably, high antibiotic resistance was observed against ampicillin 71.4%, and tetracycline 47.6% in Ethiopia, a pattern similar to the findings in Ghana where antibiotic resistance against ampicillin was 57%, tetracycline 45%, sulfamethoxazole-trimethoprim 21% in *E. coli* isolates [10] [11]. Among *Klebsiella* spp. isolates from free-range chicken in South Africa high resistance was found against ampicillin 66.7%, nalidixic 61.8%, tetracycline 59.8%, and 50% trimethoprim [12]. The antibiotic resistance observed among foodborne isolates is high to the commonly used antimicrobials in both human and veterinary medicine. The frequency of resistance to different antimicrobials in *E. coli*, *Klebsiella* spp., *and L. monocytogenes* differ according to the source of isolates [7] [13]. A study by Viera, showed that there was a relationship between resistant isolates of *E. coli* from poultry and pigs with those from humans [14]. Indicating that many resistant isolates causing human infections may be derived from food sources. Compared to other Enterobacteriaceae *E. coli* is the common colonizer of the gastrointestinal tract of animals and humans and is known to widely cause bacteremia in humans [15]. According to Osail, *L. monocytogenes* pathogen has developed resistance to several antibiotics and is known to cause fatal infection in immune-compromised people with 30%

mortality in case of an outbreak. Meat contaminated with these pathogens has been associated with food-borne infections and outbreaks [3] [16].

There is an increase in Multidrug resistance among bacterial isolates from different food products which are considered a public health threat. Raw meat has been documented as an essential reservoir of MDR strains [17] [18] [19] [20] [21]. Previous studies have shown ESBLs as important MDR organisms and meat and meat products can serve as a route of transmission for MDR from animals to human beings [22].

A survey on antibiotic use in a farming community in Kenya found that over 70% of farmers obtained antibiotics directly including tetracycline, penicillin, sulphonamides gentamycin, and chloramphenicol without prescription [23] [24]. Due to the rise in economies in Kenya, there is an increased demand for meat and this has led to growth in intensive farming. As a quick way to produce meat, large-scale farmers overuse antibiotics for therapeutics, prevention, and growth promotion. Studies conducted in Kenya have shown the presence of antibiotic-resistant bacteria in meat. It is, therefore, necessary to provide enough knowledge on the antibiotic resistance status of raw meat isolates in Kenya, this information is key to improving antimicrobial stewardship and mitigating the emergence and spread of AMR.

2. Material and Methods

2.1. Study Design, Study Area

A cross-sectional study was conducted between March and December 2021 in Nairobi County, which is the largest city in Kenya. Nairobi residents are the highest consumers of meat with each person eating about two extra kilos of meat as compared to other counterparts in other towns within the country annually [25]. Economically, Nairobi County is subdivided into three main categories: upper-class estates like Karen, Kileleshwa, Muthaiga, and Westlands, middle-class residential areas such as Buruburu, Pangani, Lang'ata, and low-class residential such as the Kibera, Kawangware, and Kangemi informal settlements. Samples were collected from butcheries and open markets in Kibera, a slum, Lang'ata as a representative middle-income settlement, Karen representing a high-income settlement.

2.2. Sample Collection

Before sample collection, an informed consent was obtained from all persons agreeing to participate in the study. Using a purposive sampling technique, a total of 270 raw meat samples comprising; 97 raw beef, 85 raw chicken, 34 raw pork, and 54 raw goat portions of meat were purchased from retail outlets at the selected study sites and examined for the presence of *E. coli*, *Klebsiella* spp., and *L. monocytogenes*. Samples were placed into sterile zip lock bags and transported to the microbiology laboratory at Kenya Medical Research Institute-Centre for Microbiology Research for microbiological examination. Processing and analysis of the samples were carried out immediately upon arrival.

2.3. Bacterial Isolation and Characterization

Briefly, a 5 g sample was homogenized in 45 ml of buffered peptone water (Oxoid) and Listeria enrichment broth (Hi-media) for *E. coli*, *Klebsiella* spp., and *L. monocytogenes* respectively. The mixture was incubated aerobically for 18 - 24 hours at 37˚C. After enrichment, a loop-full of the incubated mixture from buffered peptone water was streaked on MacConkey agar (Oxoid), for *E. coli* and *Klebsiella* spp. isolation, and from Listeria enrichment broth in Hi-Crome Listeria Agar Base, Modified (Hi-media M1417) for *L. monocytogenes* isolation. Nalidixic, acriflavine, and cycloheximide were added to HiCrome Listeria Agar Base for selective isolation of *Listeria monocytogenes*. The streaked plates were incubated aerobically for 18 - 24 hours at 37˚C. Pure colonies obtained were then subjected to Gram staining and biochemical tests using methyl red, Voges-Proskauer, Lysin Indole motility, Triple sugar iron, urea, Citrate utilization for *E. coli*, and *Klebsiella* spp. identification. Pure colonies in HiCrome™ Listeria Agar Base with characteristic blue colonies with a yellow halo were preliminarily identified *as Listeria* spp. and were Gram-stained and subjected to Polymerase Chain Reaction for *L. monocytogenes* confirmation.

2.4. DNA Extraction and PCR for *L. monocytogenes* Confirmation and Resistance Genes Identification *in E. coli*, *Klebsiella* spp., and *L. monocytogenes*

2.4.1. DNA Extraction

The DNA was obtained by boiling bacterial suspension from an 18 - 24-hour culture at 95˚C for 12 minutes, then centrifuging it at 14,000 rpm for 5 minutes. After centrifugation the supernatant was obtained and stored at −20˚C until further or subsequent use [26].

2.4.2. PCR for *L. monocytogenes* Confirmation

Gene encoding listeriolysin O (*hly*A) was amplified by the oligonucleotide primer sequence 5'-CCT AAG ACG CCA ATC GAA-3 and 5'-AAG CGC TTG CAA CTG CTC-3' shown in Table 1 according to a method described by [13]. The PCR mixture (23 µl) consisted of: 5 × 4.0 µl FIREPol master mix, 1.0 µl each primer, 1.0 µl Betaine solution (SIGMA), 15 µl PCR water (Invitrogen), and 1 µl DNA template. The PCR mixture was subjected to the following thermal cycling conditions using Gene Amp (Applied Biosystems); Five minutes of 95˚C before 30 cycles of amplification at 95˚C for 30 mins, 53˚C for 45 s, 72˚C for 45 s with a final extension at 72˚C for 7 minutes. The reactions had a negative control (without DNA) and positive control *L. monocytogenes* (ATCC 19115).

Table 1. Primer sequence for Resistance genotype identification and confirmation of *Listeria monocytogenes*.

Genotype	Primer sequence	Annealing	Bp	reference
	Resistance genotype identification			
TEM	F5'-GCG GAA CCC CTA TTTG-3' R5'-TCT AAA GTA TAT AGA GTA AAC TTG GCT GAC-3'	55	851	[28]

Continued

SHV	F5'-ATT CTG CGC TTC TTT ACT CGC-3' R5'-TTT ATG GCG TTA CCT TTG ACC-3'	50	880	[17]
CTX-M	F5'-ATG TGC AGC ACC ACY AAR GTK ATG GC-3' R5'-TGG GTR AAR TAR GTS ACC AGA AYS AGC GC-3'	53	593	[29]
OXA	F5'-GGC ACC AGA TTC AAC TTT CAA G-3' R5'-GAC CCC AAG TTT CCT GTA AGT G-3'	60	820	[30]
sul	F5'-TGA GAT CAG ACG TAT TGC R5'-TTG AAG GTT CGA CAG CAC GT-3'	58	650	[31]
qnrS	F5'-GCA AGT TCA TTGAAC AGG GT-3' R5'-TCT AAA CCG TGA AGT TCG GCG	60	428	[32]
	***Listeria monocytogenes* identification**			
hlyA	F5'-CCT AAG ACG CCA ATC GAA-3' R5'-AAG CGC TTG CAA CTG CTC-3'	53	702	[13]

2.4.3. PCR for Resistance Genes Identification

Antimicrobial resistance-associated genes were detected by PCR using the primers listed in **Table 1** as described by [27]. The PCR mixture used was similar to the above-mentioned on *L. monocytogenes* identification except for the primers used. The PCR amplification conditions consisted of an initial denaturation at 95˚C for 5 minutes, 30 cycles of denaturation at 95˚C for 30 s, annealing at 55˚C (bla_{TEM}), 53˚C (bla_{CTX-M}) 60˚C (bla_{OXA}), 58˚C (sul), 60˚C (qnrS) for 1 min, 72˚C for 2 minutes with a final extension of 72˚C for 7 minutes. The reaction had a negative control (without DNA) and a positive control which was positive for the resistance genes identified. The amplicons were detected by electrophoresis in 1.5% agarose gel stained with SYBR green dye (life technologies) and kept at −20. 1 kb base pair molecular marker (Invitrogen) was used to determine the size of the PCR product.

2.5. Antimicrobial Susceptibility Test

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller Hinton Agar (Oxoid) according to the CLSI guidelines [33]. The antibiotics include; Ampicillin (AMP, 10 µg), AmoxicillinClavulanicacid (AMC, 110 µg), Cefotaxime (CTX, 30 µg), Ceftazidime (CAZ, 30 µg), Cefepime (FEP, 5 µg), Imipenem (IPM, 10 µg), Aztreonam (ATM, 30 µg), Ciprofloxacin (CIP, 5 µg), Chloramphenicol (C, 30 µg), Trimethoprimsulfamethoxazole (SXT, 25 µg), Gentamycin (CN, 10 µg), Erythromycin (E, 15 µg), tetracycline (TE, 30 µg) and vancomycin (V, 30 µg) *E. coli* ATCC 25922 was used as a positive control for Gram Negatives and *L. monocytogenes* ATCC 19115 was used as a control for *L. monocytogenes*. After 24 hours at 37˚C, the zones of inhibition were observed and measured, and compared to the CLSI Guidelines. Presumptive ESBL isolates were those which showed resistance to ampicillin and or ceftazidime, cefotaxime, cefepime, and cefpodoxime while those that were resistant to 3 or more antimicrobials belonging to different classes were identified as

MDR [34].

2.6. Antimicrobial Resistance Genotype Identification

Isolates that were resistant to ampicillin, were screened for bla_{TEM}, and those resistant to ampicillin and cefotaxime were screened for $bla_{CTX\text{-}M}$. Isolates that showed resistance to Imipenem were screened for bla_{OXA} and NDM, while those showing resistance to Trimethoprim-sulfamethoxazole were screened for sul, and those showing resistance to ciprofloxacin were screened for qnrS.

2.7. Data Management and Analysis

All data collected was entered into Epicollect5. Determination of proportions was used to summarize the generated data on the rates of bacterial isolation. The proportion of the positive was calculated by the number of positive samples divided by the total number of samples examined multiplied by 100. Whonet version 2020 was used to cluster antibiotic profiles and the MDR.

3. Results

3.1. Prevalence of *E. coli*, *Klebsiella* spp., and *L. monocytogenes*

Of the 270 collected samples, 185 (66%) were contaminated by at least one bacterial isolate. The predominant isolate was *E. coli* 163 (60%), followed by *Klebsiella* spp. 19 (7%) while the least was *L. monocytogenes* 3 (1.1%). Of all 87 isolates recovered from beef, 78 (89.6%) were *E. coli*, 7 (8%) *Klebsiella* spp., and 2 (2.4%) *L. monocytogenes*. Raw chicken meat yielded 44 isolates of which 39 (88.6%) were *E. coli*, and 5 (11%) were *Klebsiella* spp. with an absence of *L. monocytogenes*. Of the 33 isolates recovered from pork 33 (90.9%) were *E. coli*, 3 (9.1%) *Klebsiella* spp., and no *L. monocytogenes* were isolated. Out of a total of 21 isolates from raw goat meat 16 (76.2%) were *E. coli*, 4 (19%) *Klebsiella* spp., and 1 (4.8%) *L. monocytogenes*. Seventy-nine-point one percent of the isolates were from Kibera, 64.5% from Lang'ata, and 60% from Karen as shown in **Figure 1** and **Table 2**.

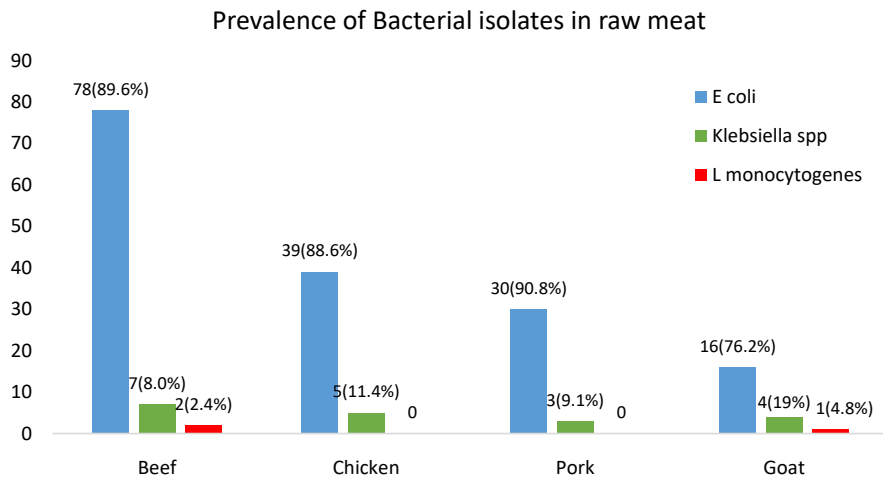

Figure 1. Bacterial prevalence in different meat types.

Table 2. Prevalence of *E. coli*, *Klebsiella* spp., and *L. monocytogenes* per sampling site.

Sampling site	Number of samples collected	*E. coli*+	*Klebsiella* spp.+	*L. monocytogenes*+	Total Number of isolates
Kibera	91	61 (67%)	8 (8.7%)	3 (3.3%)	72 (79.1%)
Lang'ata	93	55 (59%)	5 (5.4%)	0 (0)	60 (64.5%)
Karen	86	46 (53%)	6 (6.9%	0 (0)	52 (60.4%)
Total	**270**	**163 (60.3%)**	**19 (7.0%)**	**3 (1.1%)**	**185 (68.5%)**

3.2. Antimicrobial Resistance Profiles of *E. coli*, *Klebsiella* spp., and *L. monocytogenes*

In this study, a total of 185 isolates; 163 (60%) *E. coli*, 19 (7%) *Klebsiella* spp., and 3 (1.1%) *L. monocytogenes* isolates recovered were subjected to antimicrobial susceptibility tests to evaluate their resistance patterns, as shown in Table 2. High resistance levels were exhibited by *E. coli* against erythromycin (98%) and ampicillin (54%) followed by tetracycline (47%), trimethoprim-sulphamethoxazole (39%), and ciprofloxacin (19%) and low levels of resistance in other antibiotics tested (less than 11%). Similarly, *Klebsiella* spp. showed high levels of resistance to erythromycin (100%) and ampicillin (63%), moderate resistance to tetracycline (26%), and Trimethoprim-sulphamethoxazole (21%), and low levels of resistance to other antibiotics tested. *Klebsiella* spp. isolated were susceptible to gentamycin and aztreonam. All *Klebsiella* spp. isolates exhibiting resistance to amoxicillin, ceftazidime, ciprofloxacin, cefotaxime, cefepime, and Imipenem were from Karen. While those from Kibera and Lang'ata were susceptible to ceftazidime, cefotaxime, cefepime, aztreonam, Imipenem gentamycin, and ciprofloxacin. *E. coli* isolates from Kibera were 1 (99%) susceptible to gentamycin and Imipenem whereas those from Lang'ata were all susceptible to ceftazidime, gentamycin, and Imipenem as shown in Table 3. One *L. monocytogenes* isolate was resistant to trimethoprim-Sulfamethoxazole.

Table 3. Antimicrobial resistance profile of *E. coli* and *Klebsiella* spp. in raw meat samples.

Organism	Local Specimen code	Number of Isolates (N)	AMP n (R %)	AMC n (R %)	CAZ n (R %)	CTX n (R %)	FEP n (R %)	ATM n (R %)	IPM n (R %)	CN n (R %)	C n (R %)	SXT n (R %)	E n (R %)	CHL n (R %)	TCY n (R %)
E. coli	Beef	85	46 (54)	6 (7)	9 (10)	6 (7)	6 (7)	6 (7)	2 (2)	4 (4)	16 (19)	29 (34)	84 (100)	6 (7)	38 (45)
	Chicken	44	26 (59)	6 (13)	3 (6)	4 (9)	4 (9)	4 (9)	0 (0)	3 (6)	8 (18)	19 (43)	43 (97)	5 (11)	25 (56)
	Goat	33	17 (51)	4 (12)	4 (12)	5 (15)	4 (12)	3 (9)	0 (0)	1 (3)	6 (18)	9 (27)	32 (96)	3 (9)	10 (30)
	Pork	20	10 (50)	0 (0)	3 (15)	4 (20)	3 (15)	2 (10)	1 (5)	0 (0)	4 (20)	9 (45)	20 (100)	1 (5)	7 (35)
	All *E. coli*	**163**	**87 (53)**	**14 (8)**	**18 (11)**	**18 (11)**	**16 (9)**	**14 (8)**	**2 (1)**	**8 (4)**	**31 (19)**	**62 (38)**	**160 (98)**	**14 (8)**	**75 (46)**
Klebsiella spp.	Beef	7	4 (57)	1 (14)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (14)	1 (14)	7 (100)	1 (14)	2 (28)
	Chicken	5	3 (60)	1 (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (100)	0 (0)	1 (20)
	Goat	3	2 (66)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (33)	0 (0)	3 (100)	0 (0)	0 (0)
	Pork	4	3 (75)	0 (0)	1 (25)	1 (25)	1 (25)	1 (25)	1 (33)	0 (0)	1 (25)	3 (75)	4 (100)	0 (0)	2 (50)

Continued

	All *Klebsiella* spp.	19	12 (63)	2 (10)	1 (5)	1 (5)	1 (5)	1 (5)	1 (6)	0 (0)	3 (15)	4 (21)	19 (100)	1 (5)	5 (26)
All isolates		**182**	**99 (54)**	**16 (8)**	**19 (10)**	**19 (10)**	**17 (9)**	**15 (8)**	**3 (1)**	**8 (4)**	**34 (18)**	**66 (36)**	**179 (98)**	**15 (8)**	**80 (44)**

spp.: species, **n**: total number, ***E. coli***: *Escherichia coli*, **AMP**: ampicillin, **AMC**: Amoxicillin-Clavulanic, **CAZ**: ceftazidime, **CTX**: Cefotaxime, **FEP**: Cefepime, **ATM**: Aztreonam, **IPM**: Imipenem, **CN**: Gentamycin, **C**: Chloramphenicol, **SXT**: Trimethoprim-Sulfamethoxazole, **E**: Erythromycin, **CHL**: Chloramphenicol, **TCY**: Tetracycline.

3.3. Antimicrobial Resistance Patterns According to Meat Types and Sites

E. coli isolates from the chicken meat samples showed high ampicillin resistance (58%) followed by beef (53%), goat (50%), and 43% pork respectively. *E. coli* Isolates from chicken and goat meats were all susceptible to Imipenem. High levels of resistance were observed in *E. coli* isolates from pork against erythromycin (100%) and ampicillin (50%) and (100%) susceptibility to Augmentin and Imipenem. All *Klebsiella* spp. isolates from beef, chicken, and goat meat were susceptible to cefotaxime, ceftazidime, cefepime, aztreonam, Imipenem, and gentamycin. Resistance to chloramphenicol was observed only in isolates from beef while those from chicken, pork, and goat meat were all susceptible. One isolate from pork was notably resistant to Imipenem 33%. Despite the resistance observed there was no significant variation of antimicrobial resistance in different meat types against used antimicrobial agents. *E. coli* isolates from Karen were resistant to at least one antibiotic, whereas those from Kibera were resistant to other used antibiotics except gentamycin and Imipenem, and those from Lang'ata were susceptible to ceftazidime, cefotaxime, gentamycin, and Imipenem. All *Klebsiella* spp. isolates from the three sites were susceptible to Aztreonam, chloramphenicol, and gentamycin, as shown in Table 4.

Table 4. Percentage resistance patterns based on the sampling sites.

Organism	Location	Number of isolates (N)	AMP n (%R)	AMC n (%R)	CAZ n (%R)	CTX n (%R)	FEP n (%R)	ATM n (%R)	IPM n (%R)	GEN n (%R)	CIP n (%R)	SXT n (%R)	ERY n (%R)	CHL n (%R)	TCY n (%R)
E. coli	**Karen**	46	26 (56)	7 (15)	13 (28)	11 (23)	10 (21)	10 (22)	1 (2)	7 (15)	10 (22)	15 (33)	44 (97)	6 (13)	20 (44)
	Kibera	61	28 (45)	3 (4)	5 (8)	6 (9)	4 (6)	3 (5)	1 (1)	1 (1)	14 (22)	24 (39)	61 (100)	4 (6)	27 (44)
	Lang'ata	56	33 (58)	4 (7)	0 (0)	1 (1)	2 (3)	1 (1)	0 (0)	0 (0)	7 (12)	23 (41)	55 (98)	4 (6)	28 (50)
***Klebsiella* spp.**	**Karen**	6	5 (83)	1 (16)	1 (16)	1 (16)	1 (16)	1 (16)	1 (16)	0 (0)	3 (50)	1 (16)	6 (100)	0 (0)	1 (16)
	Kibera	8	5 (62)	1 (12)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (25)	8 (100)	0 (0)	2 (25)
	Lang'ata	5	2 (40)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (20)	5 (100)	1 (20)	2 (40)

spp.: species, **N**: total number of isolates tested **n**: total number of isolates showing resistance, ***E. coli***: *Escherichia coli*, **AMP**: ampicillin, **AMC**: Amoxicillin-Clavulanic, **CAZ**: ceftazidime, **CTX**: Cefotaxime, **FEP**: Cefepime, **ATM**: Aztreonam, **IPM**: Imipenem, **CN**: Gentamycin, **C**: Chloramphenicol, **SXT**: Trimethoprim-Sulfamethoxazole, **E**: Erythromycin, **CHL**: Chloramphenicol, **TCY**: Tetracycline.

3.4. Multi-Drug Resistance

Out of 182 isolates, 95 (52.5%) were MDR (Resistance to at least 3 or more anti-

biotic classes). Thirty-three to three classes of antibiotics, 35 isolates were resistant to four classes of antibiotics, 14 to five classes, 4 isolates were resistant to 9 classes of antibiotics, 3 to six classes of antibiotics, and not more than two isolates were resistant to 7, 8, 10, and 11 classes of antibiotics. Of all the isolates tested, those showing resistance to all antibiotics tested were from Karen. Among the isolates, the MDR from raw beef meat was higher at 47.4%, followed by chicken at 26.3%, goat at 17.9%, and pork at 9.5%. MDR isolates from Kibera were more 40% compared to Lang'ata at 32.65%, and Karen at 28.4%. The dominant MDR resistance was to ampicillin, erythromycin, tetracycline, and trimethoprim-sulfamethoxazole identically detected in 28.4% isolates as seen in Table 5, Figure 2, Figure 3.

Table 5. Antimicrobial resistance pattern of *E. coli* and *Klebsiella* spp.

Antibiotic Combination	Number of isolates	%
AMC AMP ATM CAZ CHL CIP CTX ERY FEP GEN SXT TCY	2	2.11
AMC AMP ATM CAZ CHL CTX ERY FEP GEN SXT TCY	2	2.11
AMP ATM CAZ CIP CTX ERY FEP IPM SXT TCY	1	1.05
AMP ATM CAZ CIP CTX ERY FEP GEN SXT TCY	2	2.11
AMP ATM CAZ CHL CIP CTX ERY FEP SXT TCY	1	1.05
AMP ATM CAZ CIP CTX ERY FEP SXT TCY	2	2.11
AMC AMP CIP CTX ERY FEP SXT	1	1.05
AMC AMP ATM CAZ CTX ERY FEP	1	1.05
AMP CHL CIP ERY SXT TCY	1	1.05
AMC AMP CIP ERY SXT TCY	1	1.05
AMC AMP ERY SXT TCY	3	3.16
AMC AMP ERY FEP TCY	1	1.05
AMP CHL ERY SXT TCY	2	2.11
AMP CIP ERY SXT TCY	4	4.21
AMP ERY GEN SXT TCY	1	1.05
AMP CHL CIP ERY TCY	2	2.11
AMP ATM CHL ERY TCY	1	1.05
ATM CAZ CTX ERY FEP	1	1.05
CIP ERY SXT TCY	1	1.05
CTX ERY SXT TCY	1	1.05
AMP CAZ CIP ERY	1	1.05
AMP ERY SXT TCY	27	28.40
AMC CIP ERY TCY	1	1.05
ATM ERY SXT TCY	1	1.05

Continued

ATM CAZ CTX FEP	1	1.05
AMP CTX ERY FEP	1	1.05
AMP CIP ERY	2	2.11
AMP ERY GEN	1	1.05
CAZ ERY TCY	1	1.05
CAZ CIP ERY	1	1.05
CHL ERY SXT	1	1.05
CHL ERY TCY	1	1.05
CHL CIP ERY	1	1.05
CIP ERY TCY	3	3.16
CIP ERY SXT	1	1.05
AMP CAZ ERY	1	1.05
AMP CHL ERY	1	1.05
CTX ERY FEP	1	1.05
ERY SXT TCY	5	5.26
AMC AMP ERY	3	3.16
AMP ERY TCY	5	5.26
AMP ERY SXT	3	3.16
ERY IPM TCY	1	1.05
Total	**95 (52.5%)**	100.00

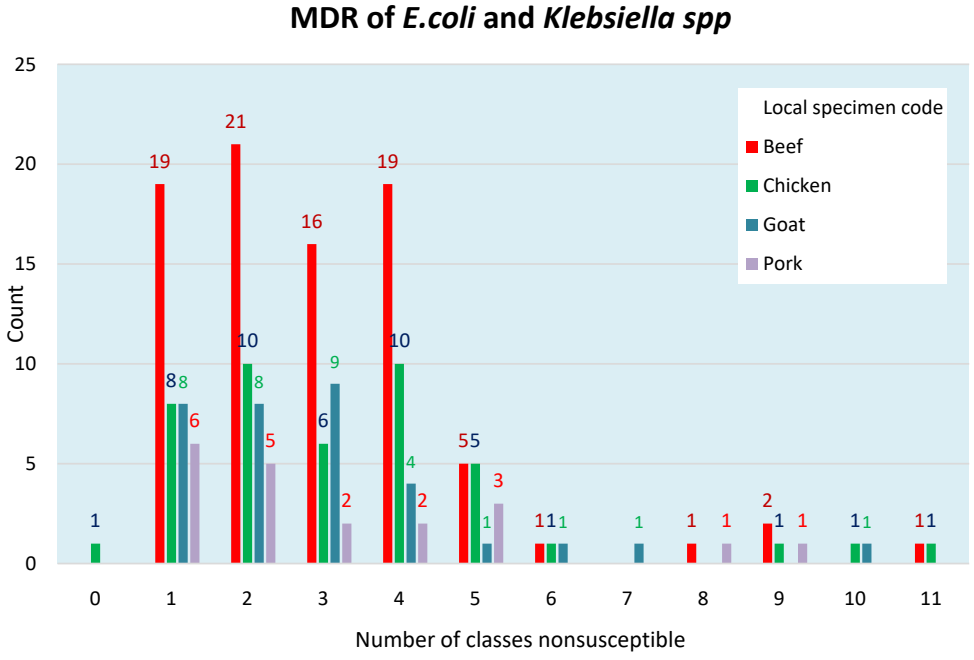

Figure 2. Multiple Drug Resistance of *E. coli* and *Klebsiella* spp. by meat type.

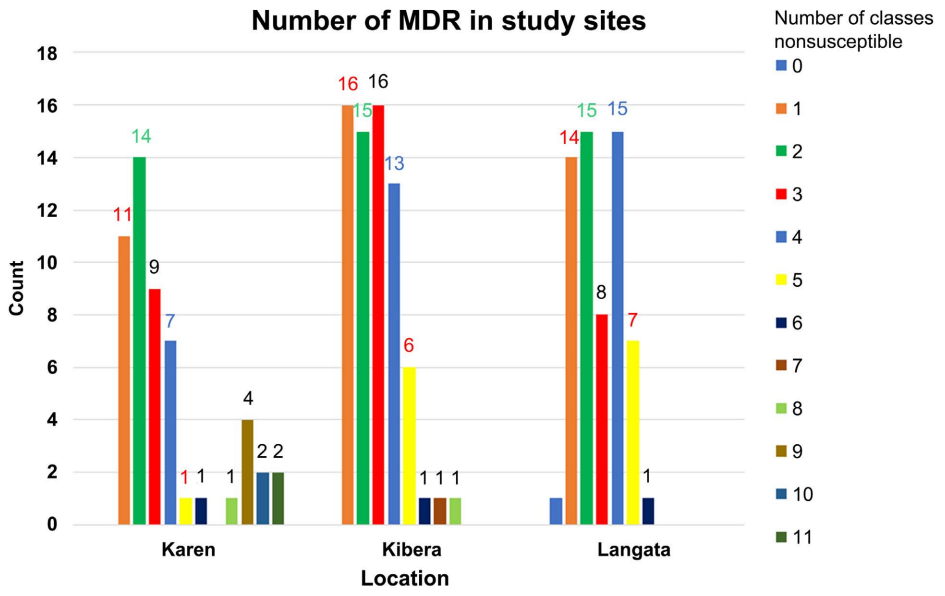

Figure 3. Multiple Drug Resistance in Karen, Kibera, and Lang'ata.

3.5. Antimicrobial Resistance Genotypes

Overall, the predominant ESBL genes were bla_{TEM} 17/26 (65%) followed by $bla_{CTX\text{-}M}$ 8/18 (44%), and bla_{OXA} 1/3 (33%). Other genotypes found were NDM 1/3 (33%), sul 14/30 (46.7%), and qnrS 3/22 (13.6%). It is noteworthy to mention that isolates tested for resistance genes from Karen carried at least one resistance gene. In Kibera, the most prevalent gene was bla_{TEM} 7 (26.9%), followed by sul 9 (20.4%), and no resistance gene was observed in OXA and NDM. Karen had more CTX-M 7 (38.8%), TEM 9 (34.66%), and sul (6.8%) respectively. Isolates from Lang'ata on the other hand had sul with a prevalence of 4.5% and TEM at 3.8%, CTX-M, OXA, and NDM were not found, sul was identified in one *L. monocytogenes* showing resistance to trimethoprim-sulphamethoxazole as shown in Table 6.

Table 6. Resistance genotypes.

Resistance genotypes	Total isolates tested (N)	Negative (N)	KIBERA n (%)	LANG'ATA n (%)	KAREN n (%)	Total n (%)
TEM	26	9	7 (26.9)	1 (3.8)	9 (34.6)	17 (65)
CTX-M	18	10	1 (5.5)	0 (0)	7 (38.8)	8 (44)
OXA	3	2	0 (0)	0 (0)	1 (33)	1 (33)
NDM	3	2	0 (0)	0 (0)	1 (33)	1 (33)
sul	30	20	9 (20.4)	2 (4.5)	3 (6.8)	14 (46.7)
qnrS	22	19	0 (0)	1 (4.5)	2 (9.1)	3 (13.6)

4. Discussion

4.1. Microbial Contamination of Raw Meat

Out of 270 samples obtained in the present study 185 (66%) were contaminated

by at least one bacterial isolate. Bacterial presence in raw meat has been widely reported in different parts of the world [1] [20] [21] [35] [36]. The predominant isolate was *E. coli* 163 (60%), followed by *Klebsiella* spp. 7% while the least one was *L. monocytogenes* 3 (1.1%). In a study conducted in South Africa, more *E. coli* (44%) were recovered when compared to *Klebsiella* spp. 32%. Another study isolated *E. coli* 38.7% and *Klebsiella* spp. 17.3% in beef and chicken samples [8] [37] which is in agreement with the current study. The high number of *E. coli* in the current study could be attributed to fecal contamination at slaughter or during processing. Since *E. coli* is widely recognized as an indicator organism for contamination in food and water, other Enterobacteriaceae could be present or can potentially contaminate the meat [11] [18]. In the current study, more isolates were recovered from Kibera compared to other study sites, the differences in proportions of contamination could be linked to the hygienic status of retail outlets which results in varying levels of contamination.

4.2. Phenotypic Antimicrobial Susceptibility Testing of *E. coli*, *Klebsiella* spp., and *L. monocytogenes*

Considerable resistance was exhibited by *E. coli* isolates against erythromycin 98% followed by ampicillin 54%, tetracycline 46%, and Trimethoprim-sulfamethoxazole 38%. Mgaya *et al.*, [38] found a similar antibiotic resistance pattern in *E. coli* isolates 91.9% tetracycline, 80.5% sulfamethoxazole-trimethoprim, 70.9% ampicillin, and 40.2% ciprofloxacin a pattern that has also been reported by [39]. Similarly, Momtaz *et al.*, [26] observed that tetracycline, sulfamethoxazole, chloramphenicol, and trimethoprim resistance was common. A study conducted in Kenya by Njoroge [35] found that *E. coli* isolates from goat meat were resistant to tetracycline 15%, chloramphenicol 4%, and 100% susceptibility to sulphamethoxazole-trimethoprim. Interestingly, trimethoprim resistance in the previous study was not observed while in the current study 27% resistance was observed. The difference in resistance could be linked to the indiscriminate use of antibiotics by farmers for the prevention and treatment of infections in animals. These drugs showing resistance in the current study are commonly used by farmers indiscriminately for medication and other prophylactic purposes as they are easily accessible and cheap [40]. In the current study, *E. coli* resistance to Imipenem was the least since the drug is not frequently used [18] Carbapenems possess a broad spectrum of activity and the greatest potency against bacteria. Because of this, they are often reserved for more severe infections or used as last-line agents.

Klebsiella spp. showed a similar resistance pattern to erythromycin 98%, ampicillin 54%, tetracycline 44%, trimethoprim-sulphamethoxazole 35%, and chloramphenicol 18%. Similar resistance patterns were observed against ampicillin 63%, tetracycline 14%, chloramphenicol 2%, and 100% susceptibility to gentamycin [41]. In a study in Nepal by Bhuvan [20] *Klebsiella* spp. isolates from raw chicken and raw buffalo showed a resistance of 41.6% and 12.0% to tetracycline respectively.

Isolated *L. monocytogenes* strains were highly susceptible to gentamycin (100%), chloramphenicol (100%), and Vancomycin (100%) except for one isolate which was resistant to trimethoprim-sulphamethoxazole (33%). The resistance observed in the current study is similar to other previous studies [42] [43]. The current study found susceptibility to chloramphenicol which contrasts with the findings of a study by Zhang [16]. Ampicillin is used for the treatment of listeriosis, and in the case of patients showing allergic reactions trimethoprim-sulphamethoxazole is often used [44]. Although the recovered isolates were not resistant to the first choice of treatment it is of concern that in the current study trimethoprim-sulphamethoxazole resistance was observed.

4.3. Multiple Drug Resistance

The current study found an overall rate of multiple drug resistance of 52.2%. The common resistance pattern observed was from Erythromycin 98%, tetracycline 44%, ampicillin 54%, and sulfamethoxazole-trimethoprim 36%, which unfortunately are the commonly used antibiotics in human and veterinary medicine [23]. These findings are similar to those reported by Adzitey *et al.* [45] who found that tetracycline, ampicillin, and erythromycin resistance patterns were common. In Canada [46] found that 32% of *E. coli* isolates from chicken were multidrug-resistant. Saud *et al.* observed 52.5% multidrug resistance in *E. coli* isolates from chicken and buffalo meat [20]. The common resistance pattern observed in this study shows there could be a link between antimicrobial use and the antimicrobial resistance observed. Multidrug-resistant isolates can be disseminated to humans during meat consumption if there is improper handling and cooking of meat. Multidrug-resistant organisms are a threat because it limits treatment options in both veterinary and human treatment. *E. coli* and *Klebsiella* spp. being indicator organisms of resistance can easily transfer resistance genes to other organisms. In the present study 1/3 (0.3%) isolate showed resistance to Trimethoprim-sulphamethoxazole only. Multidrug resistance in *L. monocytogenes* has been reported from raw meat to gentamycin, kanamycin, erythromycin, streptomycin, rifampin, and chloramphenicol [47], we found in the current study resistance to commonly used antibiotics. However, the study did not investigate the sources of contamination.

4.4. Molecular Detection of Resistance Genes

Large proportions 17 (65%) of the isolates possessed bla_{TEM}, followed by $bla_{CTX\text{-}M}$ resistance markers. Among the reservoirs for ESBL strains, food-producing animals are known to be important more so *E. coli* [6]. A similar high prevalence of bla_{TEM} was observed in India from foods of animal origin and human clinical samples [19]. According to a study by Smet, [48] possible sources of *E. coli* and *Klebsiella* spp. causing human infections have been reported to be animals colonized with ESBLs. Several studies have detected resistance genes among *E. coli* and *Klebsiella* spp. from hospital facilities, and human and clinical care, while

other studies have documented *E. coli* and *Klebsiella* spp. from raw meats that harbor resistant genes [49].

5. Conclusion

This study demonstrates the potential role of raw meat as a reservoir of antibiotic resistant bacteria that can be transferred to a human, therefore constituting a public health problem. Advocating for proper hygiene practices along the food chain and judicious use of antibiotics in animal husbandry is therefore important to help control the further emergence of antibiotic resistance. Good management practices should also be put in place for farmers who rear animals to prevent diseases that will call for antibiotic use.

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Ethical Consideration

The research was approved by Scientific Ethics and Review Unit (SERU), Kenya Medical Research Institute protocol number KEMRI/SERU/CMR/P00133/33991. Authentication to conduct the study was obtained from NACOSTI (NACOSTI/P/20/6611) and Nairobi Metropolitan Service.

Authors' Contribution

A.C who is the main author is behind the conceptualization of the study, drafting the proposal and the manuscript, she also did laboratory work throughout the study. Dr. J.M, Dr. A.N, and Dr. J.K took part in the supervision of the laboratory work and expert advice on proposal and manuscript writing.

Conflicts of Interest

The authors declare that they have no competing interests.

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