East African Medical Journal Vol. 98 No. 2 February 2021
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ANTIMICROBIAL RESISTANCE PROFILES OF SELECTED ENTEROBACTERIACEAE CONTAMINANTING RAW BEEF FROM RETAIL BUTCHERIES IN KAKAMEGA TOWN, KENYA

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ABSTRACT

Background: **Meat has been the main source of protein foodstuff globally since ancient times to date. However, it should not habour disease causing agents. Its high nutritive value has been found to hasten microbial growth.**
Objective: **To determine antimicrobial resistance profiles of selected Enterobacteriaceae isolates from raw beef samples.**
Design: **This was a cross-sectional study to determine antimicrobial resistance profiles of bacteria isolated from raw beef. Antibiofilm formation activity was determined. Statistical analysis techniques such as descriptive statistics and chi-square test of homogeneity were used for data analysis.**
Setting: **Samples were obtained from selected 54 retail butcheries in Kakamega town, analysis was done at Masinde Muliro University microbiology laboratory.**
Results: **Out of the 1296 samples collected, 548/1296(42.3%) were contaminated with *E. coli*, 80/1296(6.17%) with *Salmonella sp.* and 20/1296(1.54%) with *Shigella* spp. Among the 548 *E. coli* strains sensitivity to quinolones differed (nalidixic acid-486/548 and ciprofloxacin-535/548), and all the strains were sensitive to chloramphenicol and ceftriaxone, 72.6% of the *E. coli* isolates were sensitive to gentamicin, 21% to streptomycin, and 89% to kanamycin. About, 70 (87.5%) strains of *Salmonella* species isolated were sensitive to all the drugs though some [10 (12.5%)] were resistant to cotrimoxazole, ciprofloxacin and nalidixic acid. All the 20(100.0%) isolates of *Shigella sp*, were sensitive to all the drugs tested in this study.**
Conclusion: **Raw beef samples were found to be contaminated with enterobacteriaceae. *E. coli* was the main contaminant isolated, also major antimicrobial resistant isolate. The study recommends stringent hygiene measures on butcheries and personnel handling meat.**

INTRODUCTION

Meat has been the main source of protein since the Precambrian/ancient times to date globally (1). According to a study done by (1), retailed meat in butcheries is highly exposed thus contain high loads of microorganisms due to its high-water activity and nutrients content, also exposure to high level of oxygen. Poor management and improper handling methods greatly increase beef contamination with enterobacterioceae (2). Routine analysis of microbial contamination and resistance patterns is not carried out locally to curb this contamination.

The safety of food is the main parameter of food quality, which mainly entails the absence of contaminating microbes, chemicals or any materials that are harmful to human health or occurrence of these agents in small amounts within the permissible levels (2). Beef and other meat in slaughterhouses, wholesalers and retail butcheries can get microbial contamination through unhygienic practices such as use of contaminated display tables, knives,and water (3). Such contaminants also have been found to cause immense losses in the food industry with up to 50% being wasted globally (3). Due to the observed hygienic concerns on meat preparation and handling in Kenya, meat may be contaminated by pathogenic bacteria and heat-resistant toxins of bacterial origin (2,3). Misuse of antibiotics in animal husbandry especially the management of disease in cattle farming. The practice has given rise to antibiotic resistance against enteric pathogens., which is on a steady increase worldwide and is currently occurring with all major antimicrobial classes including quinolones, tetracyclines, β-lactam antibiotics, chloramphenicol, aminoglycosides, and sulfamethoxazole-trimethoprim (4). This has been compounded by lack of production of new groups of antibiotics with other modes of action in the last decade (5). This study, determined the antimicrobial susceptibility profiles of isolated enterobacteriaceae to the commonly used antibiotics in Kakamega Township.

MATERIALS AND METHODS

Study design: This was a cross-sectional study design.

Target Population: The study targeted all the 63 registered butcheries in Kakamega town where a sample of beef was collected from 54 randomly selected butcheries across the town. The choice of *Salmonella sp., Shigella sp*. and *E. coli* was because of the fact that they are generally associated with food poisoning, outbreaks as well as pandemics while the choice of beef was because it is the most widely used type of meat in the town (6).

Sample Size Determination and sampling technique: A sample size of 54 butcheries was determined based on the (7) sample size formula given as;

$$n = \frac{N}{(1+N(e)^2)}$$

Where n= desired sample size, N= Approximated target population size, and e= acceptable error level, taking 0.05 as alpha, N in this study is the total number of registered butcheries within Kakamega town. There were 63 registered butcheries in the town.

Therefore, $n = \frac{63}{(1+63(0.05)^2)}$

= 54 butcheries

The stratified sampling method was selected because it ensured that a sample representing the characteristics of the population and the study area was taken into consideration. Samples were collected randomly from random carcasses in butcheries in all zones. Approximately 250 grams of beef was bought for analysis from each butchery twice in a week. Sichirayi, in this study have been named Zone D. Township butcheries were divided zone A, Zone B, and Zone C based on psychographic profiles. The independent

variable is the zone while the contamination rate is dependent.

Zone A consisted of butcheries in the central business district, which appeared to be having/practicing high level of cleanliness. Zone B consisted of those in the downtown area where there was relative cleanliness, while Zone C consisted of butcheries in slum areas and other areas within the town with unhygienic surroundings. Samples were collected twice a week from the selected butcheries for a period of three months. Zones A and B had 11 registered butcheries each while zones C and D had 16 registered butcheries each. The Beef samples were collected in sterile polyethylene bags to prevent contamination during transit, labelled properly and placed in cool boxes with ice and transported to the of Department of Microbiology laboratory, Faculty of Health Sciences, Masinde Muliro University, Kakamega County, Kenya and were stored in a refrigerator until processed. In total, 1,296 (100.0%) samples were collected from the 54 (100.0%) butcheries within three months (Between the months of June to September 2016) in the study area. Out of the 1,296 samples collected in this study, 264 (20.4%) samples were from Township Zone A and B, respectively and 384 (29.6%) were from Township zone C and Zone D.

Isolation, detection of bacterial pathogens and antimicrobial susceptibility testing: Beef samples were sliced into small pieces of ten grams and crushed/macerated using a sterile mortar and pestle. They were then serial diluted using sterile 1ml distilled water to the power of negative 4 (10^{-4}). The dilution to the power of negative 2 were picked from each cluster and sub-cultured onto Plate count agar (Oxoid, Basingstoke, UK). About 20µl of the dilution was added to a sterile petri dish containing the plate count agar and spread evenly. The preparation was then incubated for 24 hours at 37° C. Isolation was done with distinct colonies being counted and recorded. However, exact number of colonies were counted by use of a Scan® 100 Manual colony counter. The counts obtained were expressed as colony forming unit per gram (C.F.U/g). The bacteriological quality of the meat was based on recommendation by Meat HACCP (Scotland) regulations 2002 No. 234(8) on acceptable and unacceptable total plate count on meat fit/unfit for human consumption.

Distinct colonies from plate count agar were streaked on both selective and differential media. MacConkey agar was used as selective media for *E. coli*, while Salmonella Shigella agar (SS) was used as selective media for both *Salmonella spp*, and *Shigella spp*. The isolates were further confirmed using biochemical techniques as described by (9). A set of MacFarland's standards (0.5, 1, 2, 4) were prepared using established procedures as described previously (10).

Disc diffusion method: It was done as per previously described by CLSI (10). MHA plates were inoculated using spread plate technique with 100µls of bacterial suspensions that were adjusted using the McFarland's standard. The single discs that were placed on MHA surface included; Amoxicillin-clavulanic acid (30 µg), Tetracycline (30 µg), Ampicillin (10 µg), sulphamethoxazole-trimethoprin (30 µg), Ceftazidime (30 µg), Kanamycin (30 µg), Streptomycin (10 µg), Gentamicin (10 µg), Ceftriaxone (30 µg), Cotrimoxazole(30), Nalidixic acid (30 µg), Chloramphenicol (30 µg) and Ciprofloxacin (5 µg). The inhibition zones were identified by a clear area of no bacterial growth around the antibiotic containing disks; the zones of inhibition were measured and interpreted according to criteria set by the (9). *E. coli* (ATCC 25922) *Shigella Sonnei* (ATCC 25931) were used as quality control standards. All tests were carried out in triplicates.

Data presentation: Antimicrobial susceptibility data was represented by disk diffusion diameter (zone of inhibition) and contamination was represented by the

number of contaminated samples. The results were analyzed using Excel spread sheet and presented in frequencies, percentages and tabular forms and mean values we represented as Mean ± SD. Relationship between variables was analyzed using chi square test at 95% confidence interval.

RESULTS

In total, 648 (50.0%) samples from the study area were contaminated. From the 648 (100%) contaminated samples, 548 (84.6%) were contaminated with *E. coli,* 80 (12.35%) with *Salmonella sp.* and 20 (3.1%) were contaminated with *Shigella species.* Zone D had the highest level of contamination followed by Zone C with zone A showing the least contamination. *E. coli* was the most contaminant of sampled beef in Kakamega followed by *Salmonella sp* and then *Shigella species* which increased gradually from zone A through zone D. Zone D region carried the greatest burden of pathogen contamination. Correlation analysis were performed using chi square test to determine the relationship between different sample collection sites (zones) verse contamination rates. The results revealed that there was significant association ($p \leq 0.05$) between contamination rates and the sample collection sites as shown in Table 1.

Table 1

Analysis of sample collection sites verse contamination rates

Sample collection area	**Contamination rates**			**p-values**
	Pathogens	**No. of isolates**	**CFU's/g**	
Township Zone A	*E. coli*	50	120 ± 20	
	Salmonella sp	5	112 ± 15	
	Shigella sp	1	109±2	
				0.05
Township Zone B	*E. coli*	100	150 ± 30	
	Salmonella sp	10	122 ± 5	
	Shigella sp	3	120 ± 7	
				0.05
Township Zone C	*E. coli*	198	215 ± 15	
	Salmonella sp	25	210 ± 4	
	Shigella sp	6	200 ± 5	
				0.012
Zone D	*E. coli*	200	256 ± 25	
	Salmonella sp	40	254 ± 6	
	Shigella sp	10	250 ± 7	
				0.001
Total		648		

Susceptibility patterns of the isolates

Different strains of *E. coli* exhibited different results in terms of antibiotic susceptibility test.. The degree of sensitivity (S, I, R) to various antibiotics was based on recommended standards by WHO.

Table 2

Susceptibility patterns of isolates from zone A

Isolates from Zone A							
Antibiotic	**Pathogen of interest**	**Average Zone of inhibition ± SEM (mm)**	**Sensitive**	**Average Zone of inhibition ± SEM (mm)**	**Intermediate**	**Average Zone of inhibition ± SEM (mm)**	**Resistant**
TE30	*E. coli*	≥15±3	30	12-14±2	15	≤11±1.5	5
	Salmonella spp.	≥15±3	5	12-14±2	0	≤11	0
	Shigella spp	≥15±3	1	12-14±2	0	≤11	0
NA30	*E. coli*	≥19±2.5	42	14-18	8	≤13	0
	Salmonella spp	≥19±2.5	4	14-18	0	≤13±1.5	1
	Shigella spp	≥19±2.5	1	14-18	0	≤13	0
C30	*E. coli*	≥19±2.5	50	13-17	0	≤12	0
	Salmonella spp	≥18±1.5	5	13-17	0	≤12	0
	Shigella spp	≥18±1.5	1	13-17	0	≤12	0
AMC30	*E. coli*	≥18±2.5	48	14-17	0	≤13±2	2
	Salmonella spp	≥18±2.5	5	14-17	0	≤13	0
	Shigella spp	≥18±2.5	1	14-17	0	≤13	0
CN10	*E. coli*	≥15±1.5	39	13-14±2	6	≤12±1.5	5
	Salmonella spp	≥15±1.5	5	13-14±2	0	≤12	0
	Shigella spp	≥15±1.5	1	13-14	0	≤12	0
K30	*E. coli*	≥18±2	50	14-17±1.5	0	≤13	0
	Salmonella spp	≥18±2	5	14-17±1.5	0	≤13	0
	Shigella spp	≥18±2	1	14-17	0	≤13	0
CRO30	*E. coli*	≥21±2	50	18-20±2	0	≤17	0
	Salmonella spp	≥21±2	5	18-20±2	0	≤17	0
	Shigella spp	≥21±2	0	18-20	0	≤17	0
SXT30	*E. coli*	≥16±1.5	50	11-15±2	0	≤10±2	0
	Salmonella spp	≥16±1.5	5	11-15±2	0	≤10	0
	Shigella spp	≥16±1.5	1	11-15	0	≤10	0
S10	*E. coli*	≥15±2	50	12-14±1.5	0	≤11±1	0
	Salmonella spp	≥15±2	5	12-14±1.5	0	≤11	0
	Shigella spp	≥15±2	1	12-14	0	≤11	0
AMP30	*E. coli*	≥17±2	50	14-16±4	0	≤13±2	0
	Salmonella spp	≥17±2	5	14-16±4	0	≤13	0
	Shigella spp	≥17±2	1	14-16	0	0	0
CIP5	*E. coli*	≥21±1.5	49	16-20	0	≤15	1
	Salmonella spp	≥21±1.5	4	16-20	0	≤15±2	1
	Shigella spp	≥21±1.5	1	16-20	0	≤15	0
CTR30	*E. coli*	≥22±2	48	20-22	0	≤19	2
	Salmonella spp	≥22±2	4	20-22	0	≤19±1	1
	Shigella spp	≥22±2	1	20-22	0	≤19	0

Key: TE-Tetracycline, TE30-The number indicates drug concentration, C- Chloramphenicol, NA- Nalidixic acid, AMC-amoxicillin-clavulanic acid, CTR- cotrimoxazole, CRO- Ceftriaxone, K-Kanamycin,SXT- sulphamethoxazole-trimethoprim, S-Streptomycin, CN- Gentamicin, AMP- Ampicillin, CIP-Ciprofloxacin Negative - N control (sterile distilled water) positive control CH; Chloramphenical ,IZD (sd) – Inhibition zone diameters (mm) (standard deviation) are given as the mean ± SD of triplicate experiments.

Table 3

Susceptibility patterns of isolates from Zone B

Isolates from Zone B							
Antibiotic	**Pathogen of interest**	**Average Zone of inhibition ± SEM (mm)**	**Sensitive**	**Average Zone of inhibition ± SEM (mm)**	**Intermediate**	**Average Zone of inhibition ± SEM (mm)**	**Resistant**
TE30	*E. coli*	≥15±3	71	12-14±2	24	≤11±1.5	15
	Salmonella spp	≥15±3	10	12-14±2	0	≤11	0
	Shigella spp	≥15±3	3	12-14	0	≤11	0
NA30	*E. coli*	≥19±2.5	90	14-18	8	≤13	2
	Salmonella spp	≥19±2.5	8	14-18	0	≤13±1.5	2
	Shigella spp	≥19±2.5	3	14-18	0	≤13	0
C30	*E. coli*	≥18±1.5	100	13-17	0	≤12	0
	Salmonella spp	≥18±1.5	10	13-17	0	≤12	0
	Shigella spp	≥18±1.5	3	13-17	0	≤12	0
AMC30	*E. coli*	≥18±2.5	97	14-17	0	≤13±2	3
	Salmonella spp	≥18±2.5	10	14-17	0	≤13	0
	Shigella spp	≥18±2.5	3	14-17	0	≤13	0
CN10	*E. coli*	≥15±1.5	82	13-14±2	10	≤12±1.5	8
	Salmonella spp	≥15±1.5	10	13-14±2	0	≤12	0
	Shigella spp	≥15±1.5	3	13-14	0	≤12	0
K30	*E. coli*	≥18±2	100	14-17±1.5	0	≤13	0
	Salmonella spp	≥18±2	10	14-17±1.5	0	≤13	0
	Shigella spp	≥18±2	3	14-17	0	≤13	0
CRO30	*E. coli*	≥21±2	100	18-20±2	0	≤17	0
	Salmonella spp	≥21±2	10	18-20±2	0	≤17	0
	Shigella spp	≥21±2	3	18-20	0	≤17	0
SXT30	*E. coli*	≥16±1.5	100	11-15±2	0	≤10±2	0
	Salmonella spp	≥16±1.5	10	11-15±2	0	≤10	0
	Shigella spp	≥16±1.5	3	11-15	0	≤10	0
S10	*E. coli*	≥15±2	100	12-14±1.5	0	≤11±1	0
	Salmonella spp	≥15±2	10	12-14±1.5	0	≤11	0
	Shigella spp	≥15±2	3	12-14	0	≤11	0
AMP30	*E. coli*	≥17±2	100	14-16±4	0	≤13±2	0
	Salmonella spp	≥17±2	10	14-16±4	0	≤13	0
	Shigella spp	≥17±2	3	14-16	0	≤13	0
CIP5	*E. coli*	≥21±1.5	98	16-20	0	≤15	2
	Salmonella spp	≥21±1.5	8	16-20	0	≤15±2	2
	Shigella spp	≥21±1.5	3	16-20	0	≤15	0
CTR30	*E. coli*	≥22±2	90	20-22	8	≤19	2
	Salmonella spp	≥22±2	7	20-22	0	≤19±1	3
	Shigella spp	≥22±2	3	20-22	0	≤19	0
P control		≥18±1.5	100	13-17	0	≤12	0
N control		0	0	0	0	0	0

Key: *TE-Tetracycline, TE30-The number indicates drug concentration, C- Chloramphenical, NA- Nalidixic acid, AMC-amoxicillin-clavulanic acid,S-Streptomycin, CTR- cotrimoxazole, CRO- Ceftriaxone,K-Kanamycin, SXT-sulphamethoxazole-trimethoprin, S-Streptomycin ,CN- Gentamicin, AMP- Ampicillin,CIP-Ciprofloxacin, Negative - N control (sterile distilled water), IZD (sd) – Inhibition zone diameters (mm)(standard deviation) are given as the mean ±SD of triplicate experiments. Positive control: CH: chloramphenicol (10 g/disc).*

Table 4

Susceptibility patterns of isolates from Zone C

Antibiotic	Pathogen of interest	Average Zone of inhibition ± SEM (mm)	Sensitive	Average Zone of inhibition ± SEM (mm)	Intermediate	Average Zone of inhibition ± SEM (mm)	Resistant
TE30	*E. coli*	≥15±3	77	12-14±2	41	≤11±1.5	80
	Salmonella spp	≥15±3	25	12-14±2	0	≤11	0
	Shigella sp	≥15±3	6	12-14	0	≤11	0
NA30	*E. coli*	≥19±2.5	178	14-18	16	≤13	4
	Salmonella spp	≥19±2.5	22	14-18	0	≤13±1.5	3
	Shigella spp	≥19±2.5	6	14-18	0	≤13	0
C30	*E. coli*	≥18±1.5	198	13-17	0	≤12	0
	Salmonella spp	≥18±1.5	25	13-17	0	≤12	0
	Shigella spp	≥18±1.5	6	13-17	0	≤12	0
AMC30	*E. coli*	≥18±2.5	188	14-17	4	≤13±2	6
	Salmonella spp.	≥18±2.5	25	14-17	0	≤13	0
	Shigella spp	≥18±2.5	6	14-17	0	≤13	0
CN10	*E. coli*	≥15±1.5	167	13-14±2	20	≤12±1.5	11
	Salmonella spp.	≥15±1.5	25	13-14±2	0	≤12	0
	Shigella spp	≥15±1.5	6	13-14	0	≤12	0
K30	*E. coli*	≥18±2	198	14-17±1.5	0	≤13	0
	Salmonella spp	≥18±2	25	14-17±1.5	0	≤13	0
	Shigella spp.	≥18±2	6	14-17	0	≤13	0
CRO30	*E. coli*	≥21±2	198	18-20±2	0	≤17	0
	Salmonella spp.	≥21±2	25	18-20±2	0	≤17	0
	Shigella spp.	≥21±2	6	18-20	0	≤17	0
SXT30	*E. coli*	≥16±1.5	198	11-15±2	0	≤10±2	0
	Salmonella spp.	≥16±1.5	25	11-15±2	0	≤10	0
	Shigella spp.	≥16±1.5	6	11-15	0	≤10	0
S10	*E. coli*	≥15±2	100	12-14±1.5	0	≤11±1	0
	Salmonella spp	≥15±2	10	12-14±1.5	0	≤11	0
	Shigella spp	≥15±2	3	12-14	0	≤11	0
AMP30	*E. coli*	≥17±2	198	14-16±4	0	≤13±2	0
	Salmonella spp.	≥17±2	25	14-16±4	0	≤13	0
	Shigella spp.	≥17±2	6	14-16	0	≤13	0
CIP5	*E. coli*	≥21±1.5	194	16-20	1	≤15	3
	Salmonella spp	≥21±1.5	22	16-20	0	≤15±2	3
	Shigella spp	≥21±1.5	6	16-20	0	≤15	0
CTR30	*E. coli*	≥22±2	180	20-22	14	≤19	4
	Salmonella spp.	≥22±2	22	20-22	0	≤19±1	3
	Shigella spp	≥22±2	6	20-22	0	≤19	0
P control		≥18±1.5	100	13-17	0	≤12	0
N control		0	0	0	0	0	0

Key: *TE-Tetracycline, TE30-The number indicates drug concentration C- Chloramphenical, NA- Nalidixic acid, AMC-amoxicillin-clavulanic acid, CTR- cotrimoxazole, CRO- Ceftriaxone, K-Kanamycin,SXT- sulphamethoxazole-trimethoprin, S-Streptomycin, CN- Gentamicin, AMP- Ampicillin,CIP-Ciprofloxacin. Negative - N control (sterile distilled water), IZD (sd) – Inhibition zone diameters (mm) (standard deviation) are given as the mean ±SD of triplicateexperiments. Positive control: CH: chloramphenicol (10 g/disc).*

Table 5

Susceptibility patterns of isolates from Zone D

Isolates from Zone D							
Antibiotic	**Pathogen of interest**	**Average Zone of inhibition ± SEM (mm)**	**Sensitive**	**Average Zone of inhibition ± SEM (mm)**	**Intermediate**	**Average Zone of inhibition ± SEM (mm)**	**Resistant**
TE30	*E. coli*	≥15±3	48	12-14±2	50	≤11±1.5	102
	Salmonella spp.	≥15±3	40	12-14±2	0	≤11	0
	Shigella spp.	≥15±3	10	12-14	0	≤11	0
NA30	*E. coli*	≥19±2.5	176	14-18	20	≤13	4
	Salmonella spp.	≥19±2.5	36	14-18	0	≤13±1.5	4
	Shigella spp.	≥19±2.5	10	14-18	0	≤13	0
C50	*E. coli*	≥18±1.5	200	13-17	0	≤12	0
	Salmonella spp.	≥18±1.5	40	13-17	0	≤12	0
	Shigella spp.	≥18±1.5	10	13-17	0	≤12	0
AMC30	*E. coli*	≥18±2.5	181	14-17	12	≤13±2	7
	Salmonella spp.	≥18±2.5	40	14-17	0	≤13	0
	Shigella spp.	≥18±2.5	10	14-17	0	≤13	0
CN10	*E. coli*	≥15±1.5	110	13-14±2	59	≤12±1.5	31
	Salmonella spp.	≥15±1.5	40	13-14±2	0	≤12	0
	Shigella spp.	≥15±1.5	10	13-14	0	≤12	0
K30	*E. coli*	≥18±2	200	14-17±1.5	0	≤13	0
	Salmonella spp.	≥18±2	40	14-17±1.5	0	≤13	0
	Shigella spp.	≥18±2	10	14-17	0	≤13	0
CRO30	*E. coli*	≥21±2	200	18-20±2	0	≤17	0
	Salmonella spp.	≥21±2	40	18-20±2	0	≤17	0
	Shigella spp.	≥21±2	10	18-20	0	≤17	0
SXT30	*E. coli*	≥16±1.5	200	11-15±2	0	≤10±2	0
	Salmonella spp.	≥16±1.5	40	11-15±2	0	≤10	0
	Shigella spp	≥16±1.5	10	11-15	0	≤10	0
S10	*E. coli*	≥15±2	100	12-14±1.5	0	≤11±1	0
	Salmonella spp	≥15±2	10	12-14±1.5	0	≤11	0
	Shigella spp	≥15±2	3	12-14	0	≤11	0
AMP30	*E. coli*	≥17±2	200	14-16±4	0	≤13±2	0
	Salmonella spp.	≥17±2	40	14-16±4	0	≤13	0
	Shigella spp.	≥17±2	10	14-16	0	≤13	0
CIP5	*E. coli*	≥21±1.5	194	16-20	1	≤15	4
	Salmonella spp.	≥21±1.5	36	16-20	0	≤15±2	4

	Shigella spp.	≥21±1.5	20	16-20	0	≤15	0
CTR30	*E. coli*	≥22±2	180	20-22	14	≤19	2
	Salmonella spp.	≥22±2	36	20-22	0	≤19±1	3
	Shigella spp.	≥22±2	10	20-22	0	≤19	0
P control		≥18±1.5	100	13-17	0	≤12	0
N control		0	0	0	0	0	0

Key: *TE-Tetracycline, TE30-The number indicates drug concentration, C- Chloramphenical, NA- Nalidixic acid, AMC-amoxicillin-clavulanic acid CTR- cotrimoxazole, CRO- Ceftriaxone,K-Kanamycin, SXT- sulphamethoxazole-trimethoprin,S-Streptomycin, CN- Gentamicin, AMP- Ampicillin,CIP-Ciprofloxacin, Negative - N control (sterile distilled water), IZD (sd) – Inhibition zone diameters (mm) (standard deviation) are given as the mean ±SD of triplicate experiments. Positive control: CH: chloramphenicol (10 g/disc).*

DISCUSSION

Currently, antimicrobial resistance is a big issue in the world as it is postulated now that soon we will be heading to an antimicrobial agent-free world as most antibiotics will not be active against most pathogens (5). Resistance to antibiotics among microorganism's results from misuse of chemotherapeutic drugs in animal and human use thus affecting meat industry too (10).

In this study, majority (548) of the *E. coli* strains were found to be sensitive to quinolones chloramphenicol and ceftriaxone. However, this finding does not concur with findings of studies done previously in Ethiopia, which did report that *E. coli* isolates from animal-derived food products were resistant to chloramphenicol (11- 12). The difference could be attributed to geographical locations of the study sites. Different regions have different antibiotic regiments based on antibiotic surveillance of each particular region. Other studies have indicated that *E. coli* isolated showed high resistance to erythromycin (100%), streptomycin (50%), tetracycline (75%), and ampicillin (50%) and high sensitivity to penicillin (100%), gentamicin (75%), chloramphenicol (75%), and amoxicillin (50%) as reported by (10) in Ethiopia.

These findings further contradict our findings from the current study. Multidrug resistance conveyed to pathogenic bacteria becomes a public health concern since resistance leads to treatment failure and the use of second-line therapy antibiotics is costly.

A similar study by (13) investigated the antibiotic resistance in diarrheagenic *Shigella and E. coli*) strains from isolates obtained from Vietnamese children. In their findings, *E. coli* strains were deduced to be resistant to cotrimoxazole, ampicillin, chloramphenicol, cefuroxime, cefotaxime, nalidixic acid, and ciprofloxacin. All diarrheagenic *E. coli* strains were resistant to imipenem. Ciprofloxacin resistance was limited to EPEC and ETEC *E. coli* strains. EAEC strains of *E. coli* were sensitive to ciprofloxacin. Multidrug resistance was observed in 89.5% of all *E. coli* strains.

In the current study, all the strains of *Salmonella* species isolated were sensitive to all the drugs except for ceftriazone, ciprofloxacin and nalidixic acid while all the isolates of *Shigella sp* were sensitive to all the drugs tested in this study. This implies that the peoples of Kakamega Township are at risk of contracting diseases associated with multidrug resistant Salmonella pathogens which may pose a problem to treat, if they consume partially or improperly cooked beeft sourced from the study sites. Similar findings were reported in Japan where some multidrug-resistant *Salmonella* isolates were isolated from raw chicken that was being retailed in Hiroshima, Japan (14). The isolates were resistant to ampicillin, streptomycin,

spectinomycin, kanamycin, tetracycline, and cotrimoxazole. In a prospective study by Nguyen *et al.,* (15) that surveyed 1,648 food items obtained from retail markets in Minneapolis, USA found that 69% of all beef and pork products were contaminated with antibiotic-resistant strains of bacteria.

Multidrug resistance and extended drug-resistant were noted among the bacterial isolates in the current study. The study found 12.5% of *Salmonella sp*. to be resistant to; Cotrimoxazole, ciprofloxacin and nalidixic acid. Some *E. coli* isolates (10.0%) were resistant to both tetracycline and penicillin. Generally, the bacterial strains isolated in this study could be having mechanisms that can inhibit their modes of action. Some of these mechanisms could include the presence of mobile genetic elements, such as phages, plasmids and transposons that possess genes responsible for their resistance (13). Other strains of *E. coli* (18.6%) were resistant to tetracycline, amoxicillin and cotrimoxazole. Some of the isolated *E. coli* (3.3%) were resistant to tetracycline, amoxicillin, gentamicin and amoxicillin-clavulanic acid. The findings of the current study on the resistance patterns of the isolated bacteria are consistent with the findings of (16) who did document that 60.3% of all the *E. coli* strains isolated from meat samples were resistant to tetracycline. He also found that 75% of all isolated *E. coli* strains isolated from the meat samples were resistant to at least one of the 12 antibiotics tested.

A study by (17) also did document that among all the isolated bacterium from food, *Escherichia coli* (45.60%) was the most common organism which is also associated with UTI infections and was highly resistant to cotrimoxazole (75.8%) and cefotaxime (78.27%). Also, (18) did document the same findings from foods, animals, and humans in Iceland. They found a high level of resistance to common antibiotics and genotypic relatedness of resistant *E. coli* species. But since these bacteria are present in both contaminated food and the environment, there could be a relationship in to the increasing number of clinical cases due to antimicrobial resistant pathogens. The use of antimicrobial agents in veterinary medicine has also been found to have a direct impact in the emergence, prevalence and it is an avenue of antimicrobial resistance in bacteria isolated from food-producing animals. The resulting antibiotic-resistant bacteria in beef have a direct influence on the antibiotic resistant bacteria to be found to infect beef consumers (19). Data analysis showed some significant association between sample collection sites and contamination rates. Zones C and D were located in slums hence explaining the high contamination rates. Zones A and B were located in well to do neighborhood in terms of social demographics but samples were contaminated to some extent.

CONCLUSION

The study findings demonstrated that beef could be a major source of antimicrobial resistance in Kakamega County. Beef investigated was highly contaminated with multidrug resistant enterobacteriaceae with *E. coli* as the main contaminant. Other contaminants were salmonella and shigella. Therefore, stringent hygiene measures on butcheries and personnel handling meat should be put in place and strictly adhered to.

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