MICROBIAL QUALITY OF RAW AND ROASTED BEEF

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ABSTRACT
Foodborne pathogens initiate infections that can affect consumers when contaminated food is ingested. Bacteria (Escherichia coli and Salmonella spp) are among the food industry's major foodborne pathogens that require control for consumer safety. The research investigated the prevalence of Escherichia coli and Salmonella spp in raw and roasted beef (Kebab) to ensure consumer safety. In addition, nine antibiotics were assessed for antibiotic response in Escherichia coli and Salmonella spp isolates. Forty-five (45) samples comprising 15 raw beef samples, 15 roasted beef samples from retailing points (zero storage) and 15 roasted beef samples from retailing points (stored for 1 h 30 min) were analysed. Escherichia coli and Salmonella spp were isolated and identified using colony characteristics of selective agar like LEMB agar and XLD (Xylose Lysine Desoxycholate) agar, respectively. From the results, Salmonella spp was only present in the raw samples (6 cfu/ml) but absent in the roasted samples, while Escherichia coli was present in both raw (14 cfu/ml) and roasted (1 cfu/ml) samples. The overall prevalence of Escherichia coli and Salmonella spp in the samples was 93.33% and 40.00%, respectively. For antibiotic resistance in Escherichia coli isolates, all antibiotics were non-reactive except for Teicoplanin (70.59%), Gentamicin (5.88%), and Suphamethoxazole (5.88%), which were reactive with an overall prevalence of 9.15%. The isolates were sensitive to all antibiotics and ranged from 100% to 23.53%, with the highest overall prevalence of 87.58%. Similarly, for antibiotic resistance, Salmonella spp isolates were non-reactive with Chloramphenicol, Ciprofloxacin, Ceftriaxone, Gentamicin, and Tetracycline, while the four remaining antibiotics had a percentage of 16.67% each. The overall prevalence of antibiotic resistance was 7.41%. Salmonella spp isolates were also sensitive to all antibiotics and ranged from 100% - 50%, with an overall prevalence of 83.33%. The presence of foodborne pathogens is a consumer risk that requires attention to ensure food safety and security. Excessive antibiotic use can increase resistant bacteria, making the antibiotics less effective for both animals and humans. The presence of antibiotic residues in food (roasted beef) can be associated with many health problems and allergies. It could negatively affect the health of consumers' liver, kidneys, reproductive system, and immune system.

Keywords: *Beef, contamination, resistance, sensitive, bacteria, antibiotics, safety.*

INTRODUCTION

A unique outstanding source of protein in human intake is meat (red meat, seafood, and poultry) (Komba *et al.,* 2012). The red meat class is primarily beef, mutton, chevon, and pork from cattle, sheep, goat, and pig. The meat market marks a vital contribution to individuals' safety through the supply of nutrients and sensory satisfaction. However, large consumption of red meat and meat products has been linked to heart disease and cancer, even though it is rich in protein (essential amino acids and collagen), vitamin B12, B6, K, zinc, and iron (Kerry *et al.*, 2011).

Street foods are mostly related to diarrheal diseases resulting from inappropriate usage of seasonings, the existence of pathogenic bacteria, contaminants from the environment, and neglect of good production as well hygienic practices (Tamberker *et al.*, 2008). The significant causes of food deterioration are natural decay, resulting from natural food enzymes, and contamination through microorganisms such as fungi and bacteria (Baxter *et al*., 2008). Among the most familiar street foods is Kebab, prepared from beef with spices, seasoning, and vegetables. According to the specific recipe, Kebab can consist of cut up or ground meat, sometimes with vegetables and other accompaniments.

The protein profile of meat was described as outstanding due to the availability of all essential amino acids needed for the body's building, repair, and maintenance (Ateba and Setona 2011). The protein and vitamins, particularly vitamin A and B12, are often not found in plant sources (Teshome *et al*., 2020). Over the world, beef is consumed by a more extensive section due to its nutrient content (Zhao *et al.,* 2001) and has been anciently preserved using salting, smoking, and drying techniques (Ratsimba *et al.,* 2017).

Microbial growth can lead to meat and meat products deterioration and foodborne poisoning leading to substantial economic losses due to the purchase of antibiotics and medical treatments for the sick (Komba *et al.,* 2012). Furthermore, unsafe food creates a vicious cycle of disease and malnutrition, particularly affecting infants, young children, the elderly, and the sick (WHO, 2009). Therefore, food security must be assured by establishing appropriate techniques at all phases of the food value chain. Therefore, the study examined the microbial and antibiotic safety of raw beef and roasted beef (Kebab) to ascertain consumer food (beef) safety and security.

MATERIALS AND METHODS

Sample Collection and Analysis

All beef samples (raw and roasted) were randomly selected and collected from the Nyankpala slaughterhouse and some vendors within the community. The samples were clustered into three as [raw, roasted (no storage), and roasted (stored for 1 and a half h)] and sent for analysis. Forty-five (45) samples were collected, with 15 samples for each cluster. Samples were collected into sterile zip lock polyethylene bags and placed in cold boxes containing ice cubes for laboratory analysis at the Spanish Laboratory of the UDS.

Microbial Determination of Samples

The total bacterial count was determined using the pour plate method. Sample beef surfaces swabbed with cotton were inoculated into 10 ml 0.1 % peptone water and homogenised for 2 minutes. A serial dilution from 10^{-1} to 10^{-4} was prepared by transferring 1 ml homogenised samples into 9 ml 0.1 % peptone water. Next, 0.1 ml of each homogenised serial diluted sample was pipetted into empty petri dishes, and about 12-15 ml of molten plate count agar (PCA) at 45 $\pm 1^{o}$C was poured on it. Samples were mixed thoroughly by rotating the petri dishes gently. The agar was allowed to solidify and further incubated at 35 ^{o}C for 24 h. After incubation, the colonies were counted to determine the colony-forming unit per centimetre square (cfu/cm^2). Identification and isolation of bacteria species were made according to Ansah *et al.* (2009).

Isolation of Salmonella spp

Salmonella spp identification was carried out using buffered peptone water as the pre-enrichment medium, Rappaport-Vassiliadis (RV) broth for enrichment, and Xylose Lysine Desoxycholate (XLD) agar for selective plating. One ml of the pre-enriched beef sample was pipetted into already prepared RV broth followed by incubation at 42 ^{o}C for 24 h. After 24 h, the culture was streaked on a Xylose Lysine

Desoxycholate (XLD) agar and then incubated for 24 h at 37 °C. Red colonies, with black centres on XLD agar, suspected to be *Salmonella* spp, were selected and cultured to obtain pure cultures for confirmatory tests.

Isolation of *Escherichia coli*

One ml of diluted beef sample was added to the sterilised bottles containing 9 ml of buffered peptone water and incubated aerobically at 37 °C for 24 h. After incubation, 0.5 ml from the incubated buffered peptone water was streaked on eosin methylene blue (EMB) agar under a lamina flow and incubated at 37 °C for 24 h. After 24 h, colonies with greenish metallic shine suspected as *Escherichia coli* were selected for culture and the confirmatory test.

Identified bacteria colonies were confirmed using gram staining according to Bauer *et al*. (1996) and biochemical tests such as catalase, oxidase, lysine agar, and triple sugar iron according to ASMP (2002).

Antibiotic Response Test for *Escherichia coli* and *Salmonella* spp Isolates

The antibiotic susceptibility was conducted using the standard disc diffusion method (Bauer *et al.,* 1996). The antibiotics used on the *Escherichia coli* and *Salmonella* spp isolates were Chloramphenicol, Ciprofloxacin, Ceftriaxone, Tetracycline, Suphamethoxazole, Amoxyxlline, Azithromycin, Gentamicin, and Teicoplanin. Pure colonies were inoculated in Trypticase Soy Broth (Oxoid Limited, Basingstoke, UK) and incubated at 37°C for 18 h. The turbidity was adjusted to 0.5 McFarland standard using sterile Trypticase Soy Broth and spread plated on Müller Hinton Agar (Oxoid, Basingstoke, UK). Four antibiotic disks were placed on the surface of the Müller Hinton Agar at a distance to avoid overlapping of inhibition zones and incubated at 37° C for 24 h. After incubation, the inhibition zones were measured, and the results were interpreted using the CLSI protocol as sensitive, intermediate, and resistant (CLSI 2008). The free inhibition zones were measured in ml for the antibiotics (NCCLS, 1997).

Statistical Analysis

The data obtained were collated in Microsoft excel spreadsheet and analysed using Statistical Package for the Social Sciences version 15 (SPSS, 2006). Finally, means were separated for significance using ANOVA.

RESULTS AND DISCUSSION

Microbial Quality of Samples

The mean total viable count (table 1) was used to establish samples safety. The mean total viable count for raw beef was higher than the roasted beef, indicating that beef must be cooked appropriately to improve the microbial quality of meat (beef) for consumption since microbial loads existed in the roasted samples. There were no significant statistical differences ($p<0.05$) between the roasted samples, while significant differences existed between the raw and roasted samples.

Table 1: Mean of total viable count of the beef samples

Sample	Mean (Log cfu/cm^2)
Raw	3.561[a]
Roasted (0hr)	2.936[b]
Roasted (1hr 30min)	2.892[b]
Sed	0.1714
P value	<.001

**Means with different superscripts are statistically different (P<0.05).*

The presence of the total viable counts was affirmed by McEvoy *et al*. (2000), that contamination of beef carcasses from various abattoirs could be related to the cleanliness of hides. In addition, equipment and tools such as knives used in flaying are reported to be responsible for cross contaminations from one carcass to another when equipment and tools are not sterilised (Adzitey *et al*., 2011). Therefore, best practices must be established to reduce contamination and cross-contamination of meat and meat products along the value chain from production, handling, processing, distribution, and storage.

Presence of *Escherichia coli* and *Salmonella* spp in Raw and Roasted Beef Samples

There were varying quantities of *Escherichia coli* and *Salmonella* spp present in the samples (figure 1). *Salmonella* spp was only present in

the raw sample (6 cfu/ml) but absent in the roasted samples, while *Escherichia coli* was present in both raw (14 cfu/ml) and roasted (1 cfu/ml) samples. The overall prevalence of *Escherichia coli* and *Salmonella* spp for samples was 93.33% and 40.00%, respectively. The high presence of *Escherichia coli* was affirmed by Soyiri *et al*. (2008), that the prevalence of coliforms and *Escherichia coli* could be as a result of meat contaminated with faecal matter from the surroundings and materials such as feed and water. The presence of *Escherichia coli* in animal source foods (meat) after heat treatment can be attributed to inappropriate and inefficient processing (undercooked) or recontamination from the equipment and workers (BCCDC, n.d.; FoodSafe, n.d.).

Similarly, Ulukanli *et al.* (2006) found *Escherichia coli* O157:H7 prevalence of 11.25% in beef doner kebab in Turkey. Differences in prevalence were seen among the different raw meat samples from the retailer shops. High prevalence was noted in beef (21.9%) than mutton (10.9%) and chevon (9.4%) from the slaughterhouses (McEvoy *et al*., 2004). Furthermore, Fantelli and Stephen (2001) ascertained that bovine could be a primary source of pathogens. A study conducted in Vietnam by Van *et al*. (2007) showed that the presence of *Salmonella* in retail beef and chicken samples had a prevalence of 62.0% and 53.3%, respectively. Furthermore, Phan *et al*. (2005) had 21.0% chicken and 48.6% beef.

Carcasses can be contaminated based on the slaughtering method, the environment, and handling techniques (Rather *et al*., 2017). Unfortunately, some workers lack knowledge on food safety and how diseases primarily linked with microbes such as *Escherichia coli* and *Salmonella* spp are transferred. Some workers may have little regard or consideration for the dangers of microbial or chemical contamination of carcasses and how to control them. For example, *Escherichia coli* O157 can survive during refrigeration and freezing and is tolerant of acid, salt, and dry conditions. Even at very low doses of consumption, can lead to death or untreatable severe illness. Some cases are left with permanent kidney or brain damage (FSS, 2017).

Antibiotic Response in *Escherichia coli* Isolates

Escherichia coli isolates reacted differently depending on the measured free inhibition zones and classifications and was interpreted using the CLSI protocol (table 2). For resistance, all antibiotics were non-reactive except for Teicoplanin (70.59%), Gentamicin (5.88%), and Suphamethoxazole (5.88%), which were reactive with an overall prevalence of 9.15%. In the intermediate

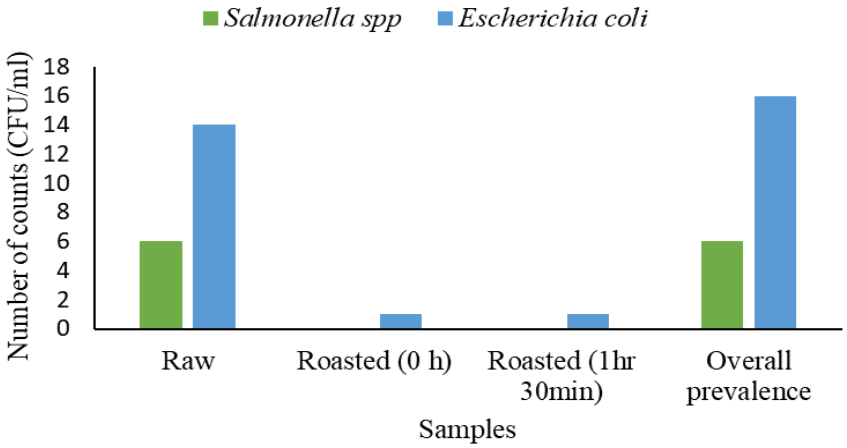

Figure 1: Number of Salmonella spp and Escherichia coli present in samples

zone, antibiotics Chloramphenicol, Ciprofloxacin, Ceftriaxone, and Suphamethoxazole were non-reactive in the *Escherichia coli* isolates but were 100% and 94.12% susceptible. The remaining antibiotics were reactive and ranged from 11.76% - 5.88%, with an overall prevalence of 3.27%. All antibiotics were reactive in the sensitive zone and ranged from 100% - 23.53%, with the highest overall prevalence of 87.58%. According to Hossain *et al*. (2008), samples of *Escherichia coli* from beef were highly sensitive to Gentamicin and Ciprofloxacin.

Table 2: Mean percentage reaction of antibiotic in *Escherichia coli* isolates

Antibiotics	R (%)	I (%)	S (%)
Amoxyclline	0.00	5.88	94.12
Azithromycin	0.00	5.88	94.12
Chloramphenicol	0.00	0.00	100.00
Ciprofloxacin	0.00	0.00	100.00
Ceftriaxone	0.00	0.00	100.00
Gentamicin	5.88	0.00	94.12
Tetracycline	0.00	11.76	88.24
Teicoplanin	70.59	5.88	23.53
Suphamethoxazole	5.88	0.00	94.12
Overall prevalence (%)	9.15	3.27	87.58

**R - resistance, I – intermediate, S - sensitive*

Microbial resistance to antibiotics is a public health concern as it can influence the development of resistance in the final consumer (livestock, man).

Antibiotic Response in *Salmonella* spp Isolates

The nine antibiotics reacted differently in the *Salmonella* spp isolates (table 3). For percentage resistance, the antibiotics Chloramphenicol, Ciprofloxacin, Ceftriaxone, Gentamicin, and Tetracycline were non-reactive, while the four remaining antibiotics had a percentage of 16.67 each. The overall prevalence of antibiotic resistance was 7.41%. In the intermediate zone, Amoxyclline, Azithromycin, Chloramphenicol, Ciprofloxacin, Ceftriaxone, and Suphamethoxazole were non-reactive while, Gentamicin (33.33%), Tetracycline (16.67%), and Teicoplanin (33.33%) were reactive with the *Salmonella* spp isolates. They had an overall prevalence of 9.26%. All antibiotics were sensitive to the *Salmonella* spp isolates, which ranged from 100% - 50%, with an overall prevalence of 83.33%. The antibiotic results were affirmed by Abunna *et al*. (2016), who stated that out of 30 meat (raw) samples tested, 21 (70%) were *Salmonella* spp positive.

Table 3: Mean percentage reaction of antibiotic in *Salmonella* spp isolates

Antibiotics	R (%)	I (%)	S (%)
Amoxyclline	16.67	0.00	83.33
Azithromycin	16.67	0.00	83.33
Chloramphenicol	0.00	0.00	100.00
Ciprofloxacin	0.00	0.00	100.00
Ceftriaxone	0.00	0.00	100.00
Gentamicin	0.00	33.33	66.67
Tetracycline	0.00	16.67	83.33
Teicoplanin	16.67	33.33	50.00
Suphamethoxazole	16.67	0.00	83.33
Overall prevalence	7.41	9.26	83.33

**R - resistance, I – intermediate, S - sensitive*

Getnet (2011) reported in Ethiopia that *Salmonella* spp isolates were highly susceptible to Ceftriaxone and Fluoroquinolones. Furthermore, some researchers reported that all *Salmonella* isolates were sensitive to Ciprofloxacin (Smith *et al*., (2009); Grob *et al*., (2011); Namboodiri *et al.,* (2011). Similarly, a study in Kenya indicated that all the eight (8) isolates of *Salmonella* from asymptomatic food handlers in Westland Nairobi were susceptible to Ciprofloxacin (Yegon *et al*., 2012). Antibiotic resistance in livestock (cattle) could lead to difficulties in successfully treating diseases.

CONCLUSION AND RECOMMENDATION

All samples had the presence of *Escherichia coli* and *Salmonella* spp except for the roasted samples where *Salmonella* spp were absent. The

Escherichia coli in all the samples was relatively higher than the *Salmonella* spp. Microbial loads and antibiotic resistance are significant sources of worry as they pose several hazards to consumers. Practising good personal hygiene, cleaning the surroundings, and adhering to good processing standards of operations such as effective and regular washing of the slabs at the slaughterhouse could help reduce the microbial loads in carcasses. Heat treatment (cooking) and preservation in the processing are required to reduce or eliminate microbial loads in processed beef. Without adequate and efficient cooking, these contaminations could lead to food poisoning and foodborne diseases. Particular attention should always be given to the handling and preparing meat and meat products for vulnerable groups, such as young children or the elderly.

Positive antibiotic response in meat (beef) could influence the consumers' resistance to treating ailments with antibiotics.

There is the need to sensitise farmers and herders on good agricultural practices (GAP) to curb the occurrence and recurring excessive incidences of antibiotic use in animal rearing for consumer safety. In addition to good agricultural practices, farmers and herders should keep records and intervals of antibiotic use on livestock.

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