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Prevalence of Enterobacteriaceae in Camel, Cattle, and Sheep Carcasses at Slaughterhouses and Butcher Shops

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Abstract: Enterobacteriaceae can contaminate meat during various processing stages, including slaughter, evisceration, and b utchering, potentially causing foodborne illnesses. The purpose of this study was to investigate the prevalence of Enterobacteriaceae in carcass samples obtained from slaughterhouses and meat cuts collected from butcher shops. A total of 120 samples of camel, cattle, and sheep meat were analyzed for microbial contamination and isolates were identified using the PCR test. Total viable count ranged from 4.91 to 5.37 Log_{10} CFU/g in slaughterhouses and butcher shops. *E. coli* dominated, with 84 out of the 120 samples (70%) contaminated, where contamination was highest in camel meat and lowest in sheep meat with 100% and 30% of contaminated samples, respectively. Salmonella was confirmed in 40% of camel, 47.5% of cattle, and 32.5% of sheep samples. In addition, twenty-five Enterobacteriaceae strains belonging to 19 different genera were detected in the meat samples. The highest occurrence was in the sheep samples with 15 different genera followed by the camels and the cattle samples with 14 different genera each. The presence of Enterobacteriaceae in camel, cattle, and sheep carcasses raises significant concerns regarding food safety. Adherence to good hygiene practices throughout animal slaughtering is crucial to minimize the risk of infection and transmission and ensure food safety.

Keywords: Enterobacteriaceae; camels; cattle; sheep; slaughterhouses; butcher shops; *Salmonella*; Al-Ahsa

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1. Introduction

Foodborne diseases pose a significant public health concern worldwide, leading to substantial morbidity and mortality (WHO, 2017) [1]. Every year, millions of people suffer from foodborne diseases that are globally important because of their high incidence and the costs that they impose on society. Meat, an excellent protein source for human beings, is a perishable food that is easily contaminated with microorganisms, resulting in potential economic losses and health hazards. The contamination of meat with Enterobacteriaceae is a significant public health concern with far-reaching consequences for both consumers and the food industry. These pathogens, including *Escherichia coli* (especially *E. coli* O157:H7), *Salmonella* spp., *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Klebsiella* spp., cause severe illnesses such as salmonellosis, hemolytic uremic syndrome, and hemorrhagic colitis [2]. In animals, Enterobacteriaceae are predominant in the gastrointestinal tract and can contaminate meat during slaughter [3]. Therefore, their prevalence in livestock warrants comprehensive investigation [4]. In most countries, including Saudi Arabia, camels, cattle, and sheep are primary meat sources, and the microbiological safety of meat is crucial, given the scale of their consumption. Slaughterhouses are critical points in the meat production process where many studies have shown the prevalence of *E. coli* and *Salmonella* spp. on carcasses is mainly due to improper slaughter practices and poor fed-animal hygiene [3]. Along the same lines, outlets have been documented as a source of Enterobacteriaceae prevalence in raw meats [5–8]. The contamination of Enterobacteriaceae can occur during

slaughtering and handling practices, causing health risks. The health risks that *E. coli* and *Salmonella* spp. pose are not uniform and depend on the specific strains involved. Some *E. coli* strains, such as enterohemorrhagic *E. coli* (EHEC), can cause severe bloody diarrhea, kidney failure, and even death, while *Salmonella* Enteritidis and *S. Typhimurium* are responsible for the majority of human salmonellosis cases [9].

According to the Centers for Disease Control and Prevention (CDCP), *Salmonella* spp. cause approximately 1.35 million infections, 26,500 hospitalizations, and 420 deaths in the United States annually, while in Europe, *Salmonella* spp. were reported as the second most frequent causative agent of foodborne illness and the second cause of bacterial inflammation of the small intestine in Germany [10,11]. Another area of concern is the potential for cross-contamination in slaughterhouses and butcher shops. Despite the ability to eliminate pathogens during cooking, there is a risk posed by cross-contamination with other foods, such as ready-to-eat food [12]. A study carried out by Brichta-Harhay et al. (2008) [3] found that the prevalence of *E. coli* O157:H7 on cattle carcasses ranged from 0% to 3.6%, while *Salmonella* spp. prevalence ranged from 0% to 1.8% on carcasses. In retail outlets, the presence of *E. coli* and *Salmonella* spp. can be influenced by cross-contamination, temperature control, and the overall quality of the meat [6]. Understanding the prevalence and load of Enterobacteriaceae contamination present on the hides and carcasses of animals during processing is a significant prerequisite for risk assessment and management. However, comprehensive research into the prevalence and diversity of genera of Enterobacteriaceae, particularly in camels, remains limited. This study, therefore, aims to elucidate the prevalence of Enterobacteriaceae in camels, cattle, and sheep during slaughtering and presentation in butcher shops in Al-Ahsa Governorate.

2. Materials and Methods

2.1. Sample Design

A random sampling technique was employed to ensure the unbiased selection of sampling units. This method facilitated the random selection of samples based on the number of animals slaughtered on sampling days. Data were gathered from two high-throughput municipality slaughterhouses (located in the north and the south) and six butcher shops located in Al-Ahsa governorate (Eastern province, Saudi Arabia) between August and October 2022. To comprehensively understand microbial diversity among consumed meat, this study focused on three distinct animal types, camels, cattle, and sheep. All animal intents to slaughter were subjected to comprehensive veterinary examination.

2.2. Sample Collection

A comprehensive collection of 120 samples from camels, cattle, and sheep was undertaken from two distinct municipal slaughterhouses and six butcher shops. From each animal variety, 40 samples (20 specimens from slaughterhouse, and the same from butcher shops) were analyzed. All animals were subjected to comprehensive veterinary inspection before and after the slaughtering phase. The samples at the slaughterhouses were obtained during slaughtering phase, after evisceration process, whereas in butcher shops, they were collected from the display refrigerators. Approximately 250 g of each carcass were aseptically taken from different parts of the carcass including the neck, chest, backchain, belly, and legs. The same weight was collected from butcher shops from different parts of the carcasses. All samples were placed in sterile plastic bags and stored in an ice box to avoid microbial development. The samples were then transferred to the laboratories within 4–8 h for microbiological analyses.

2.3. Microbiological Analyses

2.3.1. Enumeration of Total Viable Count

The Total Viable Count (TVC) of all samples was enumerated using standard microbiological techniques. Briefly, 25 g of sample was aseptically placed into sterile stomacher bag containing 225 mL of Buffered Peptone Water (BPW) (Oxoid, UK), and completely

homogenized using stomacher (Seward Medical Ltd., London, UK) at 200 rpm for 3 min. Serial decimal dilutions of the samples (up to 10^{-6}) were prepared. The samples were then cultured in plate count agar (PCA) (Oxoid, UK) in three replicates. The plates were incubated at 37 °C for 48 h, allowing bacterial growth. Only plates with 30–300 colonies were considered for TVC enumeration. The results were presented in $\log_{10}$ CFU/g.

2.3.2. Presumptive Testing for Enterobacteriaceae

All meat samples were analyzed for detection of Enterobacteriaceae. Aseptically, 25 g of sample was placed into sterile stomacher bag containing 225 mL of sterile 0.1% buffered peptone water (BPW) (Oxoid, UK and homogenized [13]. For *Salmonella* spp., pre-enrichment was carried out by incubating the mixture at 37 °C for 24 h. Aliquots of 100 µL were transferred into 100 mL. Tetrathionate Broth (TTB) (Oxoid, UK) tubes containing potassium iodide and iodine solution, as recommended by the manufacturer, were then incubated again at 37 °C for 24 h for enrichment. Aliquots of 1 mL from each final dilution were inoculated into Petri dishes containing different agars of MacConkey (MCA) (Oxoid, UK), Eosin Methylene Blue (EMB) (Oxoid, UK), and *Salmonella-Shigella* (SS) (Oxoid, UK), and incubated for a minimum of 24 h until visible colonies were observed. All suspected colonies were subcultured based on their phenotypic appearances as follows: colonies that appeared on MacConkey agar (MCA) as lactose and non-lactose fermenters were subculture separately using different MacConkey agar and Salmonella-Shigella (SS) agar, while colonies with dark centers and colonies with green metallic sheen were sub-cultured on *Salmonella-Shigella* (SS) agar and Eosin methylene blue (EMB) agar, respectively and subsequently screened on sorbitol MacConkey agar (SMAC) as described by Cox et al., (2010) [13].

2.3.3. Confirmation of Identification

Biochemical Testing

All bacterial isolates were subjected to preliminary standard biochemical test for identification. Presumptively identified members of Enterobacteriaceae were further screened using Analytical Profile Index API® 20E (BioMérieux®, Inc., Paris, France), following the manufacturer's instructions.

2.3.4. Molecular Testing

The identification of isolated bacterial strains was performed using Extract-N-Amp™ Tissue PCR Kit (XNAT2R, Sigma-Aldrich Pty Ltd., Darmstadt, Germany) according to the manufacturer's instruction [14]. For identification, 16SRNA sequences were used. For amplification, universal primers NS1 (forward 5′-AGA GTT TGA TCM TGG CTC AG-3′) and NS2 (reverse 5′-ACGGYTACCTTGTTACGACTT-3′) were used [15]. The polymerase chain reaction (PCR) was carried out using a Rotor-Gene 6000 thermocycler (Corbett Life Science, Qiagen, Australia) as the following procedure: 3 min initial denaturation at 95 °C, 35 cycles of denaturation (30 s at 95 °C), annealing (30 s at 55 °C), extension (1.5 min at 72 °C), and a final extension at 72 °C for 7 min. Each 25 µL PCR reaction mixture contained 12.5 µL of No-ROX Kit, 3.5 µL deionized water, 2 µL each of 10 µM forward and reverse primer, and 5 µL of the extracted DNA. The sequencing was performed by Macrogen Inc. (Seoul, Republic of Korea). All sequences were assembled using the Seqman program of DNASTAR 7.1 software (DNASTAR Inc., Madison, WI, USA).

2.4. Statistical Analysis

The data were analyzed using the Statistical Package for the Social Science (SPSS) version 26 (IBM Corporation, Armonk, NY, USA). Descriptive statistics were used to describe the frequency of Enterobacteriaceae along the camel, cattle, and sheep production chain. The statistical significance of the differences in counts and Enterobacteriaceae prevalence between different sources was determined. A p-value < 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Total Viable Count (TVC)

The total viable count of bacteria (TVC) in samples gathered from two slaughterhouses and six butcher shops was done using culture methods and listed in Table 1 and Figure 1. The mean TVC of camel samples ranged from $\log_{10}$ 3.3 to $\log_{10}$ 6.2 CFU/g in slaughterhouses and log10 2.8 to $\log_{10}$ 6.9 CFU/g in butcher shops. Ten of 20 camel samples collected from the slaughterhouses fell within the critical limits of the microbiological criteria for foodstuffs created by G.C.C Standardization Organization (GSO 1016:2015) [16]. while the remaining samples were within standard limits. Three out of the 20 butcher shops samples exceeded the limits with counts $\geq\log_{10}$ 6 CFU/g, 12 were within critical limits, and the remaining five were within standard limits. Similarly, the average TVC of cattle samples varied from $\log_{10}$ 3.8 to $\log_{10}$ 5.7 CFU/g in slaughterhouses and from $\log_{10}$ 4.2 to $\log_{10}$ 6.7 CFU/g in butcher shops. Half of the 20 cattle samples from slaughterhouses were within critical limits and the remaining were within standard limits while 3 cattle samples from butcher shops surpassed the standard limits and 8 were within standard limits.

Table 1. Prevalence of total viable bacteia and total coliforms in camel, cattle, and sheep samples from slaughterhouses and butcher shops.

Scheme.	Camels		Cattle		Sheep	
	TVC (Log_{10}. CFU/g) Mean (±SD)	Coliform (MPN/g) Mean (±SD)	TVC (Log_{10}. CFU/g) Mean (±SD)	Coliform (MPN/g) Mean (±SD)	TVC (Log_{10}. CFU/g) Mean (±SD)	Coliform (MPN/g) Mean (±SD)
Slaughterhouse (North)	4.2 (±0.61)	6.2×10^2 (±1.35)	4.58 (±0.34)	8×10^2 (±3.0)	3.9 (±0.48)	4.6×10^2 (±1.26)
Slaughterhouse (South)	4.6 (±0.61)	8.8×10^2 (±1.45)	4.45 (±0.56)	8.2×10^2 (±2.67)	4.5 (±0.30)	4.4×10^2 (±1.20)
Total mean	4.4 (±0.98)	7.5×10^2 (±0.98)	4.5 (±1.61)	8.1×10^2 (±1.68)	4.2 (±0.75)	4.5×10^2 (±0.87)
Butcher shops (A)	4.7 (±0.14)	6.6×10^2 (±0.80)	4.4 (±0.22)	7.2×10^2 (±1.69)	4.3 (±3.0)	2.7×10^2 (±0.73)
Butcher shops (B)	4.2 (±1.97)	4.7×10^2 (±1.03)	4.9 (±0.49)	6.8×10^2 (±0.91)	5.2 (±0.66)	7.6×10^2 (±3.13)
Butcher shops (C)	4.7 (±0.77)	1.1×10^3 (±0.23)	5.0 (±0.0.71)	8.7×10^2 (±0.85)	5.0 (±0.80)	8.6×10^2 (±0.20)
Butcher shops (D)	5.2 (±0.63)	7.4×10^2 (±1.69)	4.7 (±0.50)	6.8×10^2 (±1.19)	5.7 (±0.49)	7.2×10^2 (±1.69)
Butcher shops (E)	5.6 (±0.29)	9.4×10^2 (±1.75)	4.8 (±0.56)	72×10^2 (±1.69)	5.3 (±0.14)	6.3×10^2 (±0.23)
Total mean	4.9 (±0.76)	7.81×10^2 (±1.10)	4.8 (±0.49)	7.3×10^2 (±1.27)	5.1 (±1.02)	6.4×10^2 (±1.19)

All values are based on the number of samples in each sampling point, (10 replicates obtained from each slaughterhouse and 5 replicates obtained from each butcher shop

Sheep samples, in general, demonstrated lower bacterial contamination levels, with TVC means ranging from $\log_{10}$ 3.4 to $\log_{10}$ 6.6 CFU/g in slaughterhouses and from $\log_{10}$ 3 to $\log_{10}$ 6.9 CFU/g in butcher shops. Two sheep samples from slaughterhouses were within the critical limits with counts of $\log_{10}$ 5 CFU/g, and only one sample exceeded standard limits with $\log_{10}$ 6.6 CFU/g and 17 samples were within standard limits. With regard to butcher shop samples, 5 samples showed TVC values exceeding standard limits with $\geq\log_{10}$ 6 CFU/g, 9 samples were within the critical limits with $\log_{10}$ 5 CFU/g, while the remaining samples were within standard limits. These results are in alignment with findings reported in many researches [17,18].

The TVC levels found in this study were significantly higher in butcher shops than in slaughterhouses. This heightened contamination in butcher shops can be attributed to the

fact that different types of meat are often handled side by side in butcher shops, allowing bacteria to transfer from one product to another (cross-contamination). In addition, more hands touching meat products in butcher shops is a potential for contamination from humans, improper utensil sanitation is another source of contamination, and the long exposure time in dis-playing meats allows bacteria more time to multiply [19].

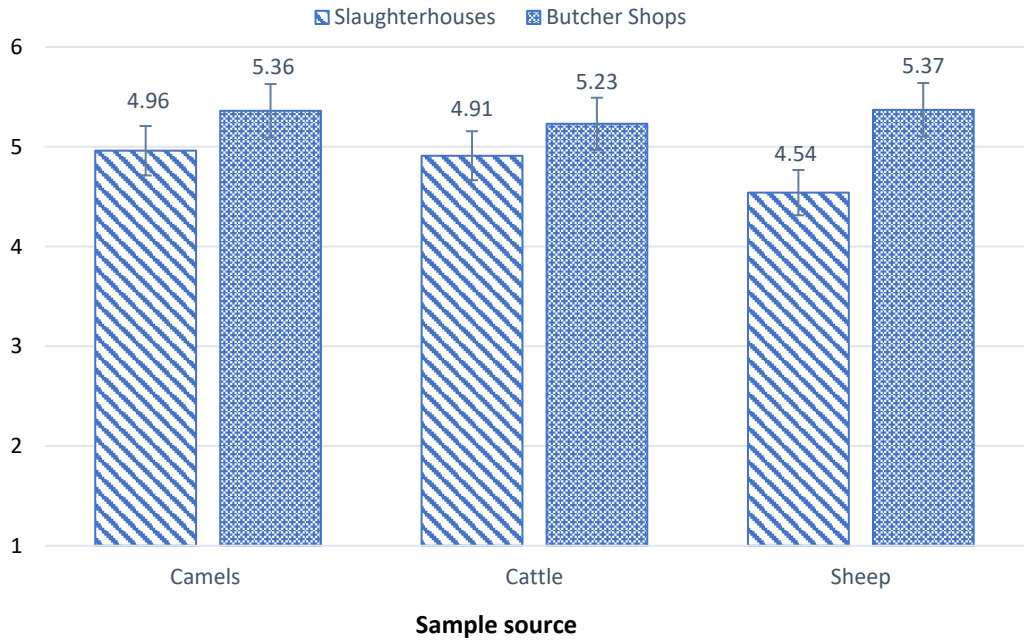

Figure 1. Contamination level of TVC in camel, cattle, and sheep samples collected from slaughterhouses and butcher shops.

3.2. Prevalence of Coliform and E. coli

Table 1 summarizes the presence of coliforms and *E. coli* in the evaluated samples. The assessment aimed to explore the overall hygiene quality and safety practices when handling carcasses in both slaughterhouses and butcher shops. In this study, coliforms were found in all of the 60 samples collected from slaughterhouses. The mean value of camel samples was 7.5×10^2 MPN/g. cattle samples 8.1×10^2 MPN/g and sheep samples was 4.5×10^2 MPN/g. The results from butcher shops were slightly lower than those found in butcher shops, except the sample of cattle that showed higher than those obtained from slaughterhouses. The high prevalence of coliforms indicates inadequate sanitary conditions and poor general hygiene during the slaughtering and handling practices of carcasses. In addition, coliforms can proliferate at temperatures from −2 to 37 °C [20], which allows bacteria to multiply during display in shops.

Detection of *E. coli* was carried out irrespective of whether the strain is pathogenic or non-pathogenic to estimate the level of hygiene. Among isolated Enterobacteriaceae genera, *E. coli* was the most predominant species as shown in Table 2. *E. coli*. was found in 84 (70%) out of the 120 camels, cattle, and sheep samples investigated, with 35.8% slaughterhouses and 34.1% butcher shops samples having levels of contamination higher than limits established in guidelines [21]. In detail, *E. coli* was detected in all camel carcasses (100%), 17 (85%) of cattle carcasses, and 6 (30%) of sheep carcasses obtained from slaughterhouses. While in butcher shops, the raw meat-cut samples contaminated with *E. coli* were 14 (70%) camel samples, 12 (60%) cattle samples, and 15 (75%) sheep samples. There were no significant differences ($p > 0.05$) in the occurrence of *E. coli* between the two slaughterhouses, nor between butcher shops.

Table 2. Presence of Enterobacteriaceae isolated from camels, cattle, and sheep in slaughterhouses and butcher shops (Identified using PCR-test).

No.	Enterobacteriaceae	Camels		Cattel		Sheep		
		Slaughterhouses (%)	Butcher Shops (%)	Slaughterhouses (%)	Butcher Shops (%)	Slaughterhouses (%)	Butcher Shops (%)	Total * (%)
1	*Escherichia coli*	20 (100)	14 (70)	17 (85)	12 (60)	6 (30)	15 (75)	84 (70)
2	*Salmonella* Paratyphi A	4 (20)	11 (55)	9 (45)	10 (50)	5 (25)	8 (40)	47 (39)
3	*Salmonella arizonae*	-	1 (5)	-	-	-	-	1 (0.8)
4	*Proteus maribilis*	4 (20)	3 (15)	3 (10)	1 (5)	-	3 (15)	14 (12)
5	*Proteus vulgaris*	1 (5)	-	-	-	-	1 (5)	1 (0.8)
6	*Citrobacter freundii*	-	1 (5)	1 (5)	-	-	3 (15)	5 (4.1)
7	*Citrobacter youngae*	-	-	1 (5)	-	2 (10)	-	3 (2.5)
8	*Raoultella ornithinolytica*	-	1 (5)		1 (5)	1 (5)	-	3 (2.5)
9	*Raoultella terrigena*	-	-	-	-	1 (5)	-	1 (0.8)
10	*Serratia odorifera*	-	1 (5)	-	1 (5)	-	1 (5)	3 (2.5)
11	*Serratia liquefacien*	-	-	1 (5)	-	-	-	1 (0.8)
12	*Leclercia adecarboxylate*	-	1 (5)	-	2 (10)	-	-	3 (2.5)
13	*Enterobacter cloacae*	-	1 (5)	-	-	1 (5)	-	2 (1.6)
14	*Klebsilla oxytoca*	-	1 (5)	-	1 (5)	-	-	2 (1.6)
15	*Kluyvera ascorbata*	-	1 (5)	-	-	1 (5)	-	2 (1.6)
16	*Pantoea ananatis*	-	-	2 (10)	-	-	-	2 (1.6)
17	*Enterobacter amnigenus*	-	1 (5)	-	-	-	-	1 (0.8)
18	*Enterobacter sakasakii*	-	-	-	1 (5)	-	-	1 (0.8)
19	*Hafnia alvei*	-	-	-	-	1 (5)	-	1 (0.8)
20	*Morganella morganii*	-	-	1 (5)	-	-	-	1 (0.8)
21	*Pasteurella multocidia*	-	1 (5)	-	-	-	-	1 (0.8)
22	*Pseudomonas oryzihabitans*	-	-	-	-	-	1 (5)	1 (0.8)
23	*Shigella* spp.	-	-	1 (5)	-	-	-	1 (0.8)
24	*Stenotrophomonas maltophilia*	-	-	-	-	-	1 (5)	1 (0.8)
25	*Yersinia pestis*	-	-	-	-	1 (5)	-	1 (0.8)

The value between two brackets represents the confirmed PCR test based on the number of each animal variety sample. * Based on the total no. of each animal variety sample (n = 20).

The presence of *E. coli* in food is a significant concern for public health and food safety. The bacteria of *E. coli* are typically found in the lower intestine of warm-blooded organisms. While many strains of *E. coli* are harmless, certain serotypes, including *E. coli* O157:H7, Shiga-toxin-producing *E. coli* (STEC), and enterohemorrhagic *E. coli* (EHEC), pose serious illnesses threats [22]. These pathogens carry several different virulence factors, controlled by genes located on chromosomes, plasmids, or phages [23]. Animals are considered the main source of *E. coli* found in fresh meat. This is mainly due to the abundance and natural presence of this bacterium in the digestive system of many animals [23]. The presence of *E. coli* in carcasses and raw meat typically indicates fecal contamination, which can occur during slaughtering, handling, packaging, or from cross-contamination with equipment, surfaces, or other foods [24]. This presents a significant risk to public health and can result in foodborne illnesses [25].

The high level of *E. coli* contamination in the samples tested indicates unhygienic practices, which is also an indication of the potential presence of unacceptable levels of other pathogens. It is worth mentioning that the samples of carcasses in this study were obtained after the evisceration phase. Thus, it is likely that the worker hands and utensils used during the slaughter process are some of the major causes of the *E. coli* prevalence in carcasses and meat cut samples. Similar results have been reported for abattoirs, and retail outlets in Lahore, Pakistan, where it was found that 63 (45%) out of 140 samples were contaminated with *E. coli* [5]. Previous studies have documented the prevalence of *E. coli* in meats. A study carried out in Nigeria revealed that the presence of *E. coli* in different types of meat including beef, pork, chicken, and mutton was 23.6%. Similarly, *E. coli* was the most frequently isolated bacterium with 45.4% contamination rate among all tested samples collected from pig slaughterhouse in South Africa [23]. The current study revealed a higher level of contamination in comparison with the study conducted in Ethiopia by Mohammed, Shimelis, Admasu, and Feyera (2014) [26] who found that the prevalence of *E. coli* in meat samples collected from abattoirs at 15.89%.

3.3. Detection of *Salmonella* spp.

The second most predominant isolated genera were *Salmonella* spp. Out of 120 different samples obtained from slaughterhouses and butcher shops, 48 (40%) tested positive for *Salmonella* spp. distributed on 16 (13.3%) camels, 19 (15.8%) cattle, and 13 (10.8%) sheep samples (Table 2). A total of 48 different isolates of *Salmonella* spp. were recovered from the positive sample, 47 (39%) of them identified as S. enterica serotype Paratyphi A and only 1 (0.8%) as *S. enterica* serotype Arizonae as illustrated in Table 2. The total prevalence of the most predominant bacteria in camel, cattle and sheep samples collected from slaughterhouses and butcher shops is shown in Figure 2. In general, the samples of butcher shops showed more contamination with Salmonella spp. (25%), compared with slaughterhouses (15%). S. Paratyphi A is causal agent for serious disease called paratyphoid fever, causing an esti-mated 5.4 million illnesses worldwide [27]. As per the GSO 1016:2015, FAO/WHO (2005) and Health Protection Agency (2009), *Salmonella* spp. must not be detected in meat and meat product intended to be consumed by humans [16].

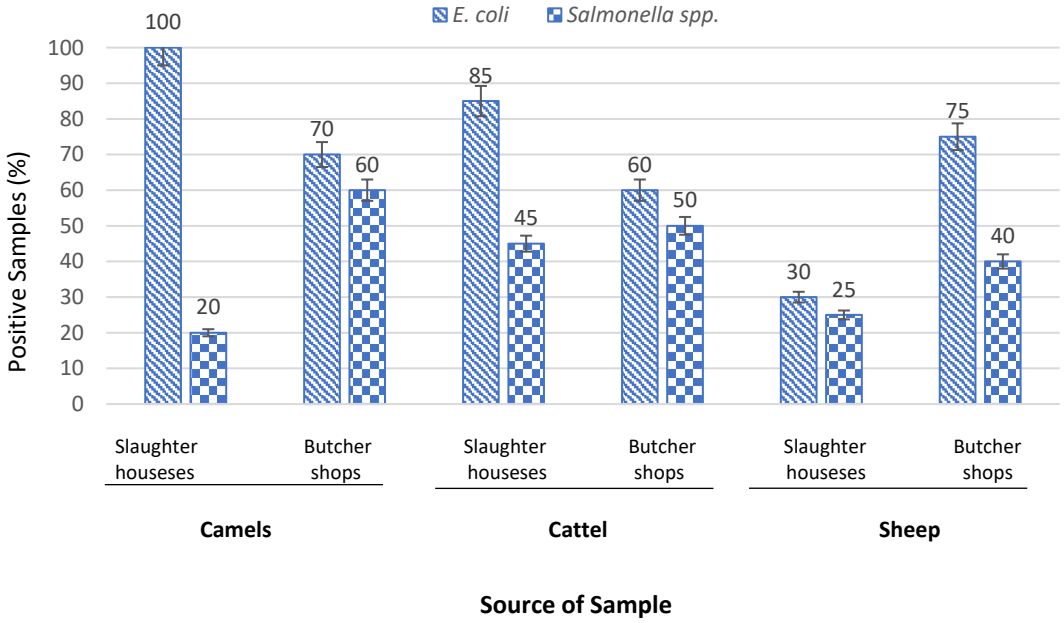

Figure 2. Prevalence of *E. coli* and *Salmonella* spp. In camel, cattle, and sheep samples collected from slaughterhouses and butcher shops.

Salmonella spp., a member of the Enterobacteriaceae family, are the second most common cause of global foodborne infections. Animal products, especially meat, are recognized as primary vectors transmitting *Salmonella* spp. to humans. In this study, the prevalence of *Salmonella* spp. in carcasses and meat cuts of camels, cattle, and sheep sourced from both slaughterhouses and butcher shops was investigated. According to our knowledge, a single research study was carried out by Mandour and Altabary (2014) [28] on the microbial quality of camel and mutton carcasses at Al-Ahsa abattoirs. The findings revealed 40% prevalence of *E. coli* in animal carcasses and meat cuts, aligning with several studies [29–31]. However, with respect to *Salmonella* spp. this study detects no *Salmonella* spp. [28].

The elevated levels of *Salmonella* contamination in the samples tested in this study underscore the suboptimal hygiene standards and practices during the slaughtering process. Additionally, exposing carcasses and meat cuts to high temperatures prior to refrigeration could markedly lead to acceleration of the growth of Salmonella and other food-borne microorganisms. The prevalence of *Salmonella* spp. in this study was higher than some previous studies [5,32,33]. However, some other research have reported more than 60% of prevalence in raw meat samples [34,35]. Based on the current results, *S. enterica* Paratyphi A, was the predominant serovar, it was found in 47 (39%) of the 120 meat samples studied,

while the serovar *S. arizonae* was found in only one sample. The distribution of *Salmonella* serovars in raw meat including beef, lambs and poultry can vary considerably among different regions of the world [36]. The difference could be influenced by local environmental factors. For instance, *S. enteritidis* was identified as the most prevalent contaminant in another study, with a rate of 37.5% contamination [31].

The rising incidence of *Salmonella*-induced foodborne illnesses underscores the urgency of addressing this public health concern. This study indicates that camel, cattle, and sheep carcasses and meat cuts obtained from slaughterhouses and butcher shops in Al-Ahsa are heavily contaminated with *Salmonella* spp., and this level of contamination in beef suggests poor sanitary conditions of raw meat handling where it is being produced. Such extensive contamination points to inadequate sanitation in meat production facilities. Potential sources of contamination include fecal matter near butchering sites, direct contact during skinning, and contaminated water used for rinsing meat [37]. Designing slaughtering lines to facilitate hygienic operations is critical. Moreover, effectively enforcing sanitary practices, including the regular disinfection of working tools, is crucial in mitigating the risk of microbiological contamination of carcasses.

3.4. Prevalence of Other Enterobacteriaceae Genera

Twenty-five different strains belonging to nineteen Enterobacteriaceae genera were isolated from camel, cattle, and sheep samples obtained from slaughterhouses and butcher shops, as illustrated in Table 2. Figure 3 shows the most predominant bacterial genera occurring in the samples, where *E. coli* was highest with 84 (70%) followed by *Salmonella* spp. with 48 (40%), *Proteus* spp. with 15 (12%), and *Citrobacter* spp. with 8 (7%) among all samples. Other genera including *Raoultella* spp. and *Serratia* spp. were also identified in four (3.2%) samples. *Klebsiella* spp., *Shigella* spp., and *Yersinia* spp. were also confirmed in meat samples at 1.6%, 0.83%, and 0.83% respectively. The occurrence of pathogens belonging to the Enterobacteriaceae family including *Salmonella* spp., *Shigella* spp., and *Yersinia* spp. in meat and meat products may pose significant risks to human health. These bacteria cause foodborne illnesses, leading to severe complications and even death, particularly in vulnerable populations like young children, the elderly, pregnant women, and immunocompromised individuals [38]. Despite the common practice in Saudi Arabia of cooking meat at high temperatures considered sufficient to eliminate any present pathogens, it does not guarantee a reduction in the risk associated with cross-contamination. This is particularly concerning in the context of other types of food, such as fruits and vegetables, which are often consumed raw. Thus, the potential for cross-contamination presents a significant risk to other foods, underscoring the need for attention and precautionary measures [7].

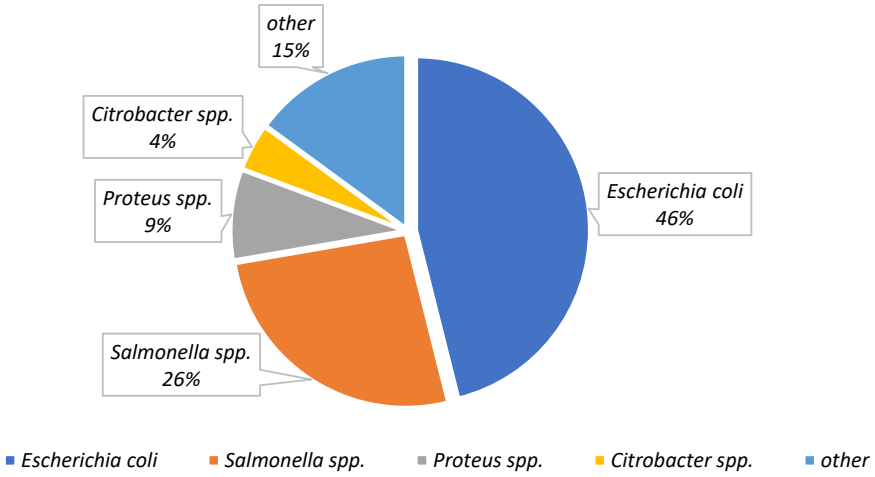

Figure 3. The most predominant bacterial genera detected in camel, cattle, and sheep carcasses and meat cuts. All values are based on the total samples (n = 120).

4. Conclusions

This study was carried out in two municipal slaughterhouses and six butcher shops in Al-Ahsa. The findings evidenced a considerable prevalence of well-known pathogens, including *E. coli*, *Salmonella* spp., *Shigella* spp., and *Klebsilla* spp. across different types of livestock including camels, cattle, and sheep in both slaughterhouses and butcher shops. The data indicate that animal carcasses exhibited high contamination levels of *E. coli* and *Salmonella* spp., with a rate of 70% and 40%, respectively. *E. coli* was predominant among all isolates with 70% presence in samples of carcasses and meat cuts. *E. coli* was found in 100% of camel carcasses, 58% of cattle carcasses, and 30% of sheep carcasses. While in butcher shops, *E. coli* was detected in 70%, 60%, and 75% in camel, cattle, and sheep meat samples, respectively. On the other hand, *Salmonella* was positive in 40% of camel 47.5% cattle and 32.5% sheep samples, collected from both slaughterhouses and butcher shops. Twenty-five Enterobacteriaceae species belonging to 19 bacterial genera were isolated and confirmed using PCR-test. The samples of sheep had the highest occurrence of Enterobacteriaceae with 15 different genera followed by camels and cattle samples with 14 different genera for both. In conclusion, the profound prevalence of Enterobacteriaceae among camel, cattle, and sheep carcasses collected from slaughterhouses and meat cuts obtained from butcher shops raises significant concerns regarding food safety. These findings underscore the need to enhance hygiene practices and implement stringent microbial monitoring procedures in both slaughterhouses and butcher shops. It highlights the potential risks to public health since these pathogens have historical of human foodborne illnesses outbreaks. More research is needed to identify the main reasons of the high occurrence of contamination and to help to design and implement an action plan to minimize or prevent foodborne illnesses. Designing slaughtering lines to facilitate hygienic operations is evidently critical. Moreover, the effective enforcement of sanitary practices, including the regular disinfection of working tools, plays a crucial role in mitigating the risk of microbiological contamination of carcasses.

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