Prevalence, molecular characterization and antimicrobial profiles of Enterohaemorrhagic E. coli O157 isolated from ruminants slaughtered in Al Ain, United Arab Emirates

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Abstract

**Background:** Shiga toxin-producing *Escherichia coli* (STEC) are associated with major food illness around the world. *E. coli* O157, has been widely reported as the most common STEC serogroup, and has emerged as an important enteric pathogen. Further, cattle have been identified as a major *E. coli* O157:H7 reservoir for human infection; however, the ecology of this organism in camels, sheep and goats is less understood. The current study aims to evaluate the prevalence of *E. coli* serotype O157 in feces of cattle, camels, sheep and goats slaughtered in United Arab Emirates (UAE) for meat consumption. This study was carried out on fecal samples of healthy cattle (*n* = 137), camels (*n* = 140), sheep (*n* = 141) and goats (*n* = 150) during the period of September 2017 to August 2018. We have used the traditional sensitive immunomagnetic separation technique (IMS) coupled with a culture plating method for detection of *E. coli* O157. Non-sorbitol fermenting colonies were assessed via the latex agglutination test and the positive cultures were subjected to PCR for detection of attaching and effacing genes (*eaeA*), hemolysin A (*hlyA*) and Shiga toxin-producing genes (*stx1* and *stx2*) and genes specific for *E. coli* O157:H7 (*rfb* O157, *uid A* and *flic H7*). All *E. coli* O157 isolates were analyzed for their susceptibility pattern toward 20 select antibiotics.

**Results:** *E. coli* O157 was present in the fecal samples of goats, camels and cattle at 2%, 3.3%, and 1.6%, respectively. In sheep we failed to detect any *E. coli* O157 strains. The most prevalent *E.coli* O157 gene identified across all species’ isolates was *stx2*, while *stx1* was not detected in any of the samples. After testing samples from camels, goats and cattle, Cefotaxime (100%), Chloramphenicol (100%), Ciprofloxacin (100%), Norfloxacin (100%) and Polymixin B (100%) showed susceptibility showed susceptibility to all *E.coli* O157 isolates.
Conclusion: This is the first study, to our knowledge, to report on the prevalence of *E. coli* O157 in the slaughter animals in UAE and clearly demonstrates the presence of these pathogens in slaughtered animals, which could possibly contaminate the meat products intended for human consumption.

Introduction

Access to safe food is a basic human right and yet remains a major health concern across the world with food-borne infections creating a major global public health issue. These infections have not only affected the health and well-being of consumers but have also negatively impacted economies in food export countries. As such, the frequency with which food items are being rejected at border crossings due to microbial and chemical contaminants has increased dramatically over the past few years resulting in huge economic loss and food wastage to both the importing and exporting countries [1, 2]. The lack of efficient food safety programs in Arabian countries, specifically, resulted in rejection of food products from entering into the international market during 2003 [3]. Raw meat and salads along with unpasteurized dairy products play an important role in contributing to food contamination risk, and increasing the burden of food-borne diseases [4]. Moreover, few Arab countries have carried out microbial contamination profiling regarding the food-borne outbreaks [3]. Nevertheless a few studies have reported on food-borne illness prevalence among Arabian countries with 1,926 cases reported in Lebanon in 2003, 779 cases in Libya in 2004, and 112,904 cases of acute gastroenteritis and diarrhea in Oman in 2002 [3].

Recently, enterohemorrhagic strains of *Escherichia coli* have emerged as significant enteric pathogens. During the early 1980s, various serotypes were implicated in human disease, however, *E. coli* O157 proved the most prevalent. This serotype is responsible for severe abdominal illness, specifically enterohemorrhagic colitis (HC) and hemolytic uremic
syndrome (HUS) and generally causes severe diarrhea [5, 6]. E. coli O157 belongs to the larger category of Shiga toxin-producing E. coli (STEC) which has the ability to produce Shiga toxin type 1 (Stx1), Shiga toxin type 2 (Stx2), or both toxins along with other variants [7]. There are various ways by which humans can become infected with E. coli O157, however, a large proportion of infections and human outbreaks have occurred following consumption of contaminated food products, such as meat and dairy products [8]. Although the prevalence of STEC infections is often lower, major outbreaks resulting in serious medical conditions have occurred throughout the world [9].

Although healthy cattle serve as the primary reservoir for STEC, they are also carried by sheep and other animals [10]. Several host and farm practices have been associated with increasing E. coli O157:H7 prevalence including animal age, season and herd type [11–14].

Currently, another major concern to human health is the rise in antimicrobial resistance due to the overuse of antibiotics in livestock production as well as human disease conditions in developing countries [15–18]. Various studies have been carried out worldwide to better understand the antimicrobial resistance of food-borne pathogens. Thus far, no studies have been conducted on the burden and drug sensitivity profile of E. coli O157 in United Arab Emirates (UAE). An abattoir or the slaughterhouse is a place approved by an authority for practicing hygienic slaughtering and processing of meat products for human consumption [19]. Due to an increase in population and urbanization, improper slaughterhouse practices can encourage the growth and spread of pathogenic organisms if the slaughtered animals carry pathogens in their feces. Thus, it is necessary to determine the prevalence and subsequently develop cost-effective and safe interventions to ensure control of E. coli O157 shedding at the farm level to prevent further food chain contamination [11]. Our study primarily focuses on evaluating the prevalence, concentration, molecular characterization, and antimicrobial resistance...
profiles of E. coli O157 in feces of healthy camels, cattle, sheep, and goats slaughtered for meat consumption. The prevalence and concentration of E. coli O157 have yet to be extensively reported on in these animals within UAE.

Results

Examination of camel, goat and cattle fecal samples collected from the slaughterhouse over a period of ten months revealed the prevalence of *E.coli* O157 from Al Ain, UAE. It was found that, the prevalence of *E. coli* serotype O157 in camels, goats and cattle were 4.3%, 2% and 1.46%, respectively; however, no *E. coli* O157 was detected in the sheep fecal samples as shown in Table 1. Furthermore, no significant differences were observed in *E.coli* O157 prevalence between animal genders or breeds (Data not shown). Being the first to report *E.coli* O157 prevalence in UAE, we carried out microbiological and molecular biological techniques to confirm the *E.coli* O157 strain.

The biochemical tests confirmed the presence of *E.coli* O157 strain. The strains were sorbitol and indole negative, yet positive for urease and citrate utilization. Bacterial enumeration was carried out by plating the samples and manually counting the colonies to quantify colony forming units (CFU). The concentrations of CFUs (CFU/mL) are presented in Table 2. Results indicate that the fecal concentrations of *E.coli* O157 are comparatively high enough to promote the spread of infection. We also sought to understand the seasonal prevalence of *E.coli* O157 and found that from the ten month study, isolates were only obtained from the samples in February, March and April in UAE (Table 2). According to the National Center of Meteorology, the temperature range during these three months was 37 °C – 45 °C. However, due to the low number of positive samples it was not possible to carry out the appropriate statistical analyses.

*PCR analysis*
Of the culture positive samples, we were unable to amplify all the samples using PCR confirmation tests and thus, were considered to be negative for *E. coli* O157. Out of the 12 positive samples in camels, five (42%) lacked the *fliC* gene; while the virulent gene, *hlyA*, was present in nine samples (75%). Alternatively, the *eaeA* gene was absent from only one sample and thus, was present in 11 of the 12 samples isolated from camels. Similarly, of the two positive samples one cattle sample contained the *flic* gene. Further, the *hlyA* gene was present in only one of the cattle samples, while the *eaeA* gene was present in both samples. In the case of goats, out of ten positive *E. coli* O157 samples all lacked (100%) the *flic* gene. Alternatively, the *hlyA* and *eaeA* genes were present in 6/10 (60%) goat samples. The most prevalent genes identified in all species' *E. coli* O157 isolates were *stx*₂; while *uidA* *stx*₁ was absent in all samples (Fig. 1, Table 3).

**Antimicrobial susceptibility of isolates**

After testing all samples isolated from goats, cattle and camels, Cefotaxime (100%), Chloramphenicol (100%), Ciprofloxacin (100%), Norfloxacin (100%) and Polymixin B (100%) showed susceptibility to all *E. coli* O157 isolates. Another antibiotic Kanamycin showed growth inhibition zones on five isolates out of 24 positive samples (80%). Other antibiotics showed irregular susceptibility and resistance patterns that cannot be considered to be effective (Table 4).

**Discussion**

Human *E. coli* O157:H7 infections primarily originate from animal food sources [20]. Specifically, domestic ruminants, including cattle, sheep and goats have been reported to be major natural reservoirs for *E. coli* O157 and contribute significantly to the epidemiology of human infections [21]. In the present study, we completed a survey on the occurrence and concentration of *E. coli* O157 in feces of meat animals from a
slaughterhouse in Al Ain, UAE. We successfully identified E. coli O157 in 1.5%, 2% and 4.3% of cattle, goat and camel samples, respectively, with concentration ranges from too few to count (TFTC) to $4 \times 10^4$ CFUs/mL. Hence, our observed E. coli O157:H7 prevalence in the UAE slaughterhouse is in agreement with previous studies carried out in various countries like Ethiopia, South Africa, United Kingdom and Ireland where the prevalence of E. coli O157:H7 at abattoir level was reported as 2.7, 2.8, 2.9, 3.2 and 3.0% [22, 23, 24, 25, 26]. Further, the highest prevalence of E. coli O157 (4.3%) was identified in camel feces compared to the other animals, which was similar to reports carried out in neighboring countries including Riyadh, Saudi Arabia, where the prevalence in camel feces was 2.4% [27]. Alternatively, our reported prevalence was found to be slightly lower compared to those reported in Ethiopia (8%) and Iran (6.4–9.6%) [28, 29]. Moreover, reports from Qatar showed that camel fecal samples contained E. coli O157 at a ten times higher prevalence than that of cattle [30]. Interestingly, in Iraq, Al-Gburi (2016) [31] reported the highest number of camels infected with E. coli O157 (19%), however, these isolates were found to be multi-drug resistant. Previous studies carried out in UAE camels were unable to isolate any E. coli O157. Furthermore, a study conducted by Moore et al. [32] on racing camel calves failed to detect E. coli O157 from UAE, with similar results obtained by El-Sayed et al. [33] when large herds of camels were investigated. However, this discrepancy with our results may be due to the absence of a reliable ‘‘gold standard’’ for detecting the presence of STEC O157 in samples as well as the inherent difficulty associated with direct comparison of results between the methods employed in this study and those obtained using other culture techniques. The results from these other studies were primarily based on characterization of very few colonies picked from the sample while lacking a specific screening and isolation technique. Alternatively, rather than using a direct culturing
method, we employed enriched buffered peptone water (BPW) followed by immunomagnetic separation of E. coli O157 (IMS) and culturing on cefixime-tellurite sorbitol MacConkey (CT-SMAC) agar to improve isolation efficiency of E. coli O157 from fecal samples [34, 35]. All isolates were confirmed as E. coli O157 using the latex agglutination test as well as via PCR techniques. Conversely, from sheep fecal samples, we were unable to identify E. coli O157 strains. Similar results were published by Alhelfi et al. [36] where no E. coli O157 was detected in either cattle or sheep fecal samples within the target area studied in UK. Failure to detect positive samples may be indicative of several factors, study design, sample size, geographical origin, age and sex of animals and/or abattoir conditions, animal husbandry as well as diet have been shown to impact prevalence rates within livestock [37–42]. In our study, save for the age and animal breeds, we did not have access to sufficient information regarding the local sheep population including husbandry and diet history. Hence, additional studies on larger herds, using a meta-analysis approach are required to confirm the absence of E.coli O157 in local sheep samples.

Although previous studies have reported cattle to be the most common E.coli O157 reservoir [43], in our study we only observed a prevalence of approximately 1.5%. Alternatively reports carried out in Riyadh, Saudi Arabia, determined that 10.7% of cattle feces samples contained E.coli O157:H7. Moreover, in comparison with other countries in Asia, cattle in Jordan had the highest prevalence of E.coli O157:H7 (12.22%) [44], whereas the lowest was reported in Taiwan (0.13%) [45]. Other studies from across the world have reported the prevalence rate of E.coli O157:H7 in cattle fecal samples to range from 2.4–24% [46–48].

We also examined whether there was seasonal variation in E. coli O157 prevalence, as seasonal variations in cattle and meat products have previously been reported to affect
human E.coli O157 infections [49-51]. Specifically, cattle feces have been reported to have low prevalence in winter and higher prevalence in spring reaching peak levels in summer [52]. The warmer summer months may provide more suitable environments outside of the host in soil, feed, and water for E. coli O157:H7, resulting in a continual source of infection or re-infection for cattle populations. Al Ain, UAE is located in a tropical dry area where the average temperature during different seasons can serve to enhance bacterial growth. In fact, studies have shown that the prevalence of E. coli O157 increased during summer months and declined in the winter as summer temperatures provided favorable conditions for bacteria to survive and potentially multiply [53, 54]. Our results showed that the highest prevalence occurred in spring and early summer months (February to April) which was likely due to the intense heat during summer months in UAE when daytime temperatures may reach as high as 50 °C which causes E. coli O157:H7 prevalence to decline due to its inability to persist in the extreme environment. These results are in agreement with previous studies carried out in Riyadh, Saudi Arabia, where the highest prevalence was reported in spring and early autumn months [27]. In fact we were unable to detect E. coli O157:H7 in any other months as was also reported in other parts of the world.

The concentration of E.coli O157 within the animal fecal samples were also quantified. In camels the CFU ranged from TFTC to $4 \times 10^4$ CFU/mL, whereas goat and cattle samples contained $4 \times 10^4$ CFU/mL and $2 \times 10^3$ CFU/mL bacteria, respectively. These results demonstrate that the animals containing E. coli O157 were considered super shedders and they maybe colonized with E. coli O157. These high levels of fecal E. coli O157 would cause the carcasses of these animals to be considered a high risk for contamination if the proper precautions were not taken. Previous work carried out in Scottish slaughterhouses
reported animals shedding > 10^4 CFU/g; these animals accounted for > 96% of the bacteria that were shed by all animals tested [55]. These findings underscore that the presence of high-shedding animals in a herd could prove to be more important than the prevalence of colonization in the entire cattle population [55].

Shiga toxins are associated with HC and HUS, while intimin is responsible for attaching/effacing (A/E) lesions on intestinal epithelial cells. As such enterohemolysins have been proposed as potential epidemiological markers for STEC strains [56]. Intimin, encoded by the eaeA gene, adheres to intestinal mucosa and causes formation of intestinal lesions [57, 58]. In addition, enterohemolysin, encoded by hlyA, leads to lysis of erythrocytes, which may contribute to the iron intake of bacterium residing in the intestine [59]. Previous studies have reported that E. coli O157:H7/H- strains isolated from the feces of slaughtered ruminants exhibited Stx2 gene prominence over the Stx1 gene [39, 42, 45, 60, 61]. In our study, Stx1 was absent in all the strains irrespective of the species. Various studies have found that Stx2 and eaeA are clinically important virulence genes; in fact carriage of these genes have been shown to be associated with the severity of human disease, especially for HUS [62, 63]. Hence, in this study E. coli O157 strains harboring the major virulence genes may be considered to be more virulent to humans than those without. Nevertheless, it has been reported that the production of major virulence genes was not essential for pathogenesis, as a number of sporadic cases of HUS were induced by Stx and eaeA-negative strains [64–66].

Antimicrobial resistance has been recognized as a global health issue for many decades. Food animals are considered to be key reservoirs of antibiotic resistant bacteria as certain antibiotic resistance genes identified in the bacteria of animal food products have also been identified in humans [67]. However, effectively treating E. coli O157:H7/H- infections is challenging due to differing opinions presented by various investigators [68–70]. As a
result, many studies have reported increasing incidence of multi-drug resistant E. coli O157:H7/H- strains isolated from the feces of slaughtered ruminants [42, 71-73].

Our results show susceptibility of all isolated strains for three antibiotics i.e., norfloxacin, chloramphenicol and polymyxin B. Further, cefotaxime and ciprofloxacin only elicited a significant effect on some of the E.coli O157 strains and only intermediate effects on others. This is in agreement with previous reports on antimicrobial resistance patterns of E. coli O157:H7 isolates from animal and human sources [74-76]. Further, previous studies on antibiotic resistance of bacteria in the feces of slaughtered ruminants have reported that isolates showed higher resistance or multiple resistance [42, 72, 73]. In fact, a study found that gentamycin resistance was the most common (56.0%), followed by ampicillin (48.0%), erythromycin (40.0%), amoxicillin (16.0%), tetracycline (12.0%), chloramphenicol (8.0%), nalidixic acid (8.0%) and streptomycin (4.0%) [72]. Although studies concerning antibiotic resistance of E. coli O157 strains isolated from ruminant feces samples are limited in the UAE geographical variations, testing of only a small proportion of a study population for susceptibility, variability in resistant genes within isolates for particular antimicrobials, as well as differences in preferred antibiotics and the origins of strains, may all impact observed resistance or even conclude higher resistance rates.

Recently we conducted a whole genome sequencing analysis of E.coli O157:H7 isolates from camel feces and deposited the results in GenBank [77]. These sequences allow for potential identification of 5,444,610 bp containing 5,399 coding sequences (CDSs) from these isolates. The data obtained from whole genome analysis will advance the current understanding regarding the evolution of these particular strains in relation to other isolates, thereby serving to improve our understanding of these specific pathogenic strains.

Conclusions
In the present study, the presence of the E. coli O157 serotype, its major virulence genes and associated antibiotic resistance was investigated in the strains isolated from feces of slaughtered ruminants including cattle, camel, sheep and goats in UAE. The presence of high concentrations of pathogens in some animals, along with seasonal variations, highlights the need for improved risk mitigation strategies to screen for high-shedding animals prior to slaughter. This study has established the presence of high-shedding animals at slaughterhouses in UAE, which provide an increased risk of contamination to both the food chain and the environment. These results also demonstrate that high concentration of shedding by few animals may play a more significant role in pathogen dissemination than does prevalence rat, as it can directly affect carcass contamination. Although the use of antibiotics in the treatment of E. coli O157 infections is controversial, monitoring of antibiotic resistance in the strains continues to be useful for epidemiological purposes. Moreover, further studies are needed to accurately determine the level of contamination in the carcasses and hides of animals in slaughterhouses for the presence of E.coli O157:H7. Within our study certain limitations arose, specifically related to the small sample size. The number of positive samples was insufficient to detect monthly variation in prevalence within statistically acceptable confidence limits, however, did allow for detection of minimum prevalence rate in the species studied. This work clearly supports the need for a platform to design regular screening programs in the food safety and processing units to ensure early and rapid detection of these food-borne pathogens. The need for suitable control measures for such animals cannot be underestimated, and further studies are needed to devise mitigation strategies that will reduce the risk of gross contamination of the food chain or environment. Studies are also needed to characterize the involvement of E. coli O157 isolates and other non-E.coli O157 pathogens in the contamination of meat food products.
Methods

Animal fecal samples

The study was conducted on healthy sheep, goats, camels and cattle slaughtered in the public abattoir in Al Ain, UAE during the study period (Fig. 2). Most animals were transported to the slaughterhouse and hence, this study considers these animals starting from the lairage. Samples were collected for a period of ten months, from September 2017 to July 2018. As per the project design, the samples were collected and tested on a biweekly basis. When samples were collected the breed, species, gender and fecal consistency of the animal were noted. All the animals, save for cattle, were local. The mature Holstein dairy cattle were culled from a local dairy farm. A total of 568 fecal samples were collected from healthy animals (sheep: 141, goats: 150, camels: 140, cattle: 137) from the slaughterhouse. Approximately, 10 cm of the recto-anal junction were cut immediately after the slaughter and the fecal samples were collected, refrigerated (4 °C), transported and examined in the laboratory. The microbial testing was performed within 3 h of collection.

Immunomagnetic separation technique

Approximately 10 g of fecal samples were diluted in 90 mL of BPW (Oxoid, Basingstoke, UK) and mixed for 30 sec. A 15 mL fecal mixture aliquot was stored for later use. Next, 1 mL of enriched fecal sample was mixed with 20 µL of magnetic beads coated with O157 antibody and IMS was performed according to the manufactures instructions (Oxoid, UK). The bead suspension (100 µL) was then plated onto CT-SMAC (Oxoid, UK). The plates were incubated at 37 °C for 24 h and presumptive E. coli O157 colonies were identified as E.coli O157 do not ferment sorbitol. These colonies were picked and inoculated into nutrient agar slants at 37 °C for 24 h and stored in the refrigerator for further biochemical
analysis. These isolates were further verified using conventional biochemical tests previously described by Harrigan [78]. The tests examined indole production and motility using sulfide, indole, motility (SIM) medium (Merck, Germany); citrate utilization with Simmons citrate agar (Merck, Germany); methyl red and vogues-Proskauer using MR-VP medium (Merck, Germany) and urease production using urea agar (Oxoid, UK) [79]

**Latex agglutination test**

Individual isolate colonies of non-sorbitol-fermenting colonies (white-gray color) on CT-SMAC medium were agglutinated with *E. coli O157* latex reagent (Oxoid, UK) according to manufacturer’s instruction. This was performed for confirmation of *E. coli O157*. Isolates showing visible agglutination following reaction with the test latex solution were again subcultured for virulent gene identification.

**Enumeration of *E. coli O157***

The latex positive samples were directly subjected to enumeration techniques. Briefly, 100 µL of enriched fecal samples were serially diluted on 900 µL of BPW to three dilutions (10⁻¹, 10⁻² and 10⁻³). The serially diluted samples were subjected to IMS for separation and counting of *E. coli O157* cells [80]. Finally 100 µL of separated bacteria-bead complexes were plated onto two Sorbitol MacConkey agar (SMAC) plates (50 µL/plate) and incubated at 37 °C for 18-24 h. After incubation, the CFUs were counted and recorded. Samples ranged from TFTC to too numerous to count (TNTC).

**DNA extraction**

After enumeration the positive samples from each animals from each collections were subjected to DNA extraction as previously described [81]. The pure culture samples were centrifuged at 10,000 rpm for 1 minute and the supernatant was discarded and re-suspended with sterile water. These tubes were incubated in a dry bath for 10 min, after
which they were transferred to ice for 5-10 min. Crude DNA supernatant was extracted by centrifugation. The DNA was later analyzed for the presence of virulence genes.

**Confirmation of E.coli O157 by multiplex PCR**

All biochemically confirmed *E. coli* isolates that were O157 agglutination-positive were tested for the presence of a gene in the *E. coli* O157 antigen gene locus, *rfbEO157:H7*, which codes for GDP perosamine synthetase (*rfbO157*), *uidA* and the H7 flagellar protein (*flicH7*) by multiplex PCR analysis as previously described by Al-Ajmi et al. [82]. Briefly, 1 µL of crude DNA extract was amplified in 25 µL mixtures containing 10 µL PCR master mix (Thermo Scientific), 5 µL nuclease free water and 1 µL of each forward and reverse primer (Table 5) for *rfbE*, *FlicH7* and *uid*. PCR reactions were carried out in a thermal cycler (Bio-Rad, USA). The cycling program was as follows: 1 min at 94 °C, 30 cycles of 30 s at 94 °C, 1 min at 55 °C, 1 min at 72 °C and a 5 min extension at 72 °C, with a negative control. The thermal cycling program was as follows: initial denaturation at 95 °C for 2 min, 30 cycles of denaturation (30 s, 94 °C), annealing (15 s, 58 °C), extension (1 min 68 °C) and a final extension (10 min, 68 °C).

An additional multiplex PCR analysis was carried out using *eaeA*, *hlyA*, *Stx1*, *Stx2* primers to quantify virulent genes [83]. The primers used are provided in Table 5. Briefly, 1 µL of crude DNA extract was amplified in 20 µL mixtures containing 10 µL of PCR master mix, 5 µL nuclease free water and 1 µL of each forward and reverse primer. The cycling program was as follows: 1 min at 94 °C, 30 cycles of 30 s at 94 °C, 1 min at 55 °C, 1 min at 72 °C and a 5 min extension at 72 °C, with a negative control without the DNA template. PCR products were separated on 2% agarose gels containing ethidium bromide and DNA bands were visualized on an ultraviolet light box using a gel image capture system and software (Bio-Rad, USA).
**Antimicrobial testing**

The Kirby-Bauer disk diffusion method was performed to test nine antimicrobials, which are commonly used for human infection treatments. Inoculums from each *E.coli* O157 strain were grown in 5 mL Mueller-Hinton (MH) broth (Himedia, India) and incubated at 37 °C and assessed for turbidity to a McFarland 0.5 standard according to the Clinical and Laboratory Standards Institute [84] standards. All test discs were obtained from Oxoid, UK. The standard reference strain of *E. coli* ATCC 25922, sensitive to all tested antimicrobial agents, was used as the control strain. MH agar plates were swabbed with sterile cotton swabs, and antimicrobial paper disks of Amoxicillin (25μg), Ampicillin (10μg), Bacitracin (10μg), Cefotaxime (30μg), Cefoxitin (30μg), Ceftazidime (10μg), Cefuroxime Sodium (5μg), Chloramphenicol (30μg), Ciprofloxacin (30μg), Cloxacillin (5μg), Doxycycline (30μg), Gentamycin (10μg), Kanamycin (30μg), Nalidixic acid (30μg), Nitrofurantoin (300μg), Norofloxacin (10μg), Polymyxin B (30μg), Streptomycin (10μg), Tetracycline (30μg), Vancomycin (30μg) were impregnated on surface of inoculated MH agar plates. These plates were incubated at 37 °C for 16-18 h and the diameters for each zone of microbial growth inhibition around the antimicrobial disk was measured. The minimum inhibitory concentration (MIC) was determined according to the criteria established by CLSI sensitive, intermediate, or resistance to the tested antimicrobial agent [84].

**Declarations**

*Ethics approval:*

This study was conducted on a slaughter house. In addition, the research proposal was approved to conduct the experiment and was permitted by UAE University.

*Consent for publication:*

Not Applicable
Availability of data and materials:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests:

The authors declare that they have no competing interests.

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Author’s contributions:

Conceived and designed the study: DA, SR. conducted the experiments and analyzed the results: DA, SR, SB. Drafted the manuscript: DA, SB. All authors reviewed the manuscript. All authors read and approved the final manuscript.

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**Tables**

Table 1. Number of *E. coli* O157 positive samples and *E. coli* O157 concentration isolated from animal feces. TFTC- Too Few to count
| Species | No. of animals | *E. coli* O157 Positive samples | Percentage (%) | *E. coli* O157 concentration (CFU/mL) |
|---------|----------------|---------------------------------|----------------|-------------------------------------|
| Goat    | 150            | 3                               | 2              | $4 \times 10^4$                      |
| Camel   | 140            | 6                               | 4.3            | TFTC- $4 \times 10^4$                |
| Sheep   | 141            | 0                               | 0              | 0                                    |
| Cattle  | 137            | 2                               | 1.46           | $2 \times 10^3$                     |

Table 2.
| Animals | Months and number of animals positive for *E.coli* O157 | Total number of animals tested |
|---------|-------------------------------------------------------|------------------------------|
| Sep 17  | Oct 17 | Nov 17 | Dec 17 | Jan 18 | Feb 18 | Mar 18 | Apr 18 | May 18 | Jun 18 | July 18 |
| Camels  | 17 (0)  | 08 (0) | 10 (0) | 10 (0) | 12 (0) | 25 (2) | 4 (1)  | 11 (3) | 23 (0) | 10 (0) | 10 (0) | 14 (0) |
| Goats   | 15 (0)  | 15 (0) | 15 (0) | 15 (0) | 15 (2) | 15 (1) | 10 (0) | 10 (0) | 15 (0) | 10 (0) | 15 (0) | 15 (0) |
| Cattle  | 15 (0)  | 11 (0) | 15 (0) | 10 (0) | 10 (1) | 15 (0) | 5 (1)  | 21 (0) | 10 (0) | 15 (0) | 14 (0) | 14 (0) |
| Sheep   | 11 (0)  | 11 (0) | 11 (0) | 11 (0) | 14 (0) | 12 (0) | 16 (0) | 12 (0) | 13 (0) | 15 (0) | 15 (0) | 13 (0) |

**Table 3. Distribution of virulent genes detected by PCR**

| Sl No. | Species | *rfbE* | *FlicH7* | *hlyA* | *uidA* | *eaeA* | *stx2* | s |
|--------|---------|--------|----------|--------|--------|--------|--------|--|
| 1      | Camel   | +      | +        | -      | -      | +      | +      | - |
| 2      | Camel   | +      | +        | +      | -      | +      | +      | - |
| 3      | Camel   | +      | -        | -      | -      | +      | +      | - |
| 4      | Camel   | +      | +        | -      | -      | -      | -      | - |
| 5      | Camel   | +      | +        | +      | -      | +      | +      | - |
| 6      | Camel   | +      | -        | +      | -      | +      | +      | - |
| 7      | Camel   | +      | -        | +      | -      | +      | +      | - |
| 8      | Camel   | +      | +        | +      | -      | +      | +      | - |
| 9      | Camel   | +      | +        | +      | -      | +      | +      | - |
| 10     | Camel   | +      | +        | +      | -      | +      | +      | - |
|   | Camel | Cattle | Goat |
|---|-------|--------|------|
| 11| +     | +      | +    |
| 12| +     | +      | +    |
| 13| +     | +      | +    |
| 14| +     | +      | +    |
| 15| +     | +      | +    |
| 16| +     | +      | +    |
| 17| +     | +      | +    |
| 18| +     | +      | +    |
| 19| +     | +      | +    |
| 20| +     | +      | +    |
| 21| +     | +      | +    |
| 22| +     | +      | +    |
| 23| +     | +      | +    |
| 24| +     | +      | +    |
Table 4. Antimicrobials used, their symbols and corresponding zones of inhibition for gram-negative enteric bacteria

| Antimicrobial used         | Symbols | Diameter of zone of inhibition (mm) |
|----------------------------|---------|------------------------------------|
|                            |         | Resistant | Intermediate | Susceptible   |
| Amoxicillin (25 μg)        | AML     | ≤13        | 14-16        | ≤17            |
| Ampicillin (10 μg)         | AMP     | ≤13        | 14-17        | ≤18            |
| Bacitracin (10 μg)         | B       | ≤14        | 15-16        | ≤17            |
| Cefotaxime (30 μg)         | CTX     | ≤14        | 15-22        | ≤23            |
| Cefoxitin (30 μg)          | FOX     | ≤14        | 15-17        | ≤18            |
| Ceftazidime (10 μg)        | CAZ     | ≤12        | 13-17        | ≤18            |
| Antibiotic                     | Symbol | Sensitivity |
|-------------------------------|--------|-------------|
| Cefuroxime Sodium (5 µg)      | CXM    | ≤14         |
| Chloramphenicol (30 µg)       | DA     | ≤14         |
| Ciprofloxacin (30 µg)         | CIP    | ≤15         |
| Cloxacillin (5 µg)            | OB     | ≤13         |
| Doxycycline (30 µg)           | DO     | ≤13         |
| Gentamycin (10 µg)            | GEN    | ≤12         |
| Kanamycin (30 µg)             | K      | ≤13         |
| Nalidixic acid (30 µg)        | NAL    | ≤13         |
| Nitrofurantoin (300 µg)       | F      | ≤13         |
| Norofloxacin (10 µg)          | NOR    | ≤12         |
| Polymyxin B (300 Unit)        | Pb     | ≤8          |
| Streptomycin (10 µg)          | STR    | ≤11         |
| Tetracycline (30 µg)          | TET    | ≤12         |
| Vancomycin (30 µg)            | VAN    | ≤14         |

Sensitivity ranges:
- ≤14: 14 or less
- 15-22: 15 to 22
- ≤23: 23 or less
- ≤21: 21 or less
- ≤20: 20 or less
- <=17: 17 or less
- ≤16: 16 or less
- ≤15: 15 or less
Table 5. Primers used for confirming *E.coli* O157:H7 and virulent genes are given below
| Target gene | Primer Name | Primer Sequence (5′-3′) | Amplification size (bp) |
|-------------|-------------|--------------------------|------------------------|
| rfbE        | O157AF      | AAGATTGCCTGAGCTTTT       | 497                    |
|             | O157AR      | CATGGCATCGTGTTTGAGCAT    |                       |
| flic        | FLICH7 F    | GCGCTGCTAGTTATCGAGC      | 625                    |
|             | FLICH7 R    | CAACGGTGACTTTTATCGCCATTCC|                       |
| uidA        | PT-2        | GCGAAAACTGAGATTG          | 252                    |
|             | PT-3        | TGATGCTCCATAACTTCTG       |                       |
| hlyA        | hlyA F      | GATCATCAAGCGTACGG          | 534                    |
|             | hlyA R      | TATGACCGAGCTGGTAAGCT      |                       |
| Stx2        | Stx2 F      | GGCACCTGTCTGAAACTGCTCC    | 255                    |
|             | Stx2 R      | TCGCCAGTTATCTGACATTCTG    |                       |
| Stx1        | Stx1 F      | AAATCGCCATTGGTGAATAC      | 180                    |
|             | Stx1 R      | AGAACGCCACTGAGATCATC      |                       |

Figures
Figure 1

Amplification products for rfbO157, flicH7, hlyA, eaeA and stx1, isolated from
camels, goats and cattle M = 100 bp DNA marker; 1–12 PCR products from camel, 13-14 from cattle and 15-24 goat.

Figure 2

Study area where samples were collected in Al Ain, UAE