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Original Article

Prevalence, virulence genes, and antimicrobial profiles of *Escherichia coli* O157:H7 isolated from healthy cattle in Tunisia

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Abstract

Introduction: Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is associated with intestinal infection in humans and is considered an important cause of food-borne diseases. The aim of the study was to assess the incidence of *E. coli* O157:H7 in fecal samples of healthy cattle collected in slaughterhouses (n = 160) and from five farms (n = 100).

Methodology: *E. coli* isolates were detected on MacConkey agar. A total of 236 *E. coli* isolates were recovered from fecal samples of healthy cattle. We used sorbitol MacConkey medium to detect non-sorbitol fermenting colonies. These bacteria were examined for the presence of O157:H7 antigen by latex agglutination. The isolation of *E. coli* O157:H7 has been confirmed with PCR amplification of *rfbEO157* and *fliCH7* specific genes for serogroup O157 and with multiplex PCR of *stx1, stx2*, *eaeA,* and *ehxA*. All isolates were examined for their susceptibility to 21 antibiotics by the disc diffusion method.

Results: Of the 236 *E. coli* isolates, 4.2% (10/236) were positive for STEC O157:H7. Shiga toxin gene (*stx2*) and *ehxA* were present in 70% of isolates, *stx1* and *eae* were confirmed in 60% of the isolates. Other virulence factors screened (*fimH*, *sfa/focDE*, *cdt3*, *traT*, *iutA,* and *hlyA*) were present among the 10 isolates. All *E. coli* O157:H7 isolates were sensitive to amoxicillin/clavulanic acid, cefotaxime, cefepime, aztreonam, colistin, and sulfamethoxazole/trimethoprim. All isolates belong to the phylo-group E.

Conclusions: This is the first study of the incidence of *E. coli* O157:H7 in cattle in Tunisia. Our finding proves the existence of STEC O157:H7 in healthy animals producing food for human consumption which could be a source of food-borne disease.

Key words: *Escherichia coli*; O157:H7; healthy cattle; antimicrobial susceptibility; virulence factors; Shiga toxins; Tunisia.

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Introduction

Escherichia coli is a common bacteria of the intestinal microbiota and an important pathogen in animals and human [1]. The pathogenic *E. coli* strains are classified into extraintestinal pathogenic strains (causing urinary tract infection, meningitis, diverse intra-abdominal infections, and pneumonia) and intestinal pathogenic (diarrheagenic) strains that cause gastroenteritis [2]. According to virulence determinants, diarrheagenic *E. coli* (DEC) are categorized as enterotoxigenic (ETEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteroaggregative (EAggEC), diffusely adherent (DAEC), and enteropathogenic *E. coli* (EPEC) [3].

Strains belonging to the subgroup of Shiga toxin-producing strains (STEC) are distinguished by certain EHEC serotypes, which are linked to outbreaks in humans and cause clinical sickness. STEC is a food-borne bacteria which have been associated with many epidemics across continents especially serotype O157:H7 [4]. Strains have been isolated from feces of healthy ruminants like cattle, goats, and sheep which can be natural reservoirs of these pathogens [5].

E. coli O157:H7 is the dominant serotype of the STEC group associated with human infections. The first identification of this serotype as a pathogen was in 1982 during an outbreak of hemorrhagic colitis in Oregon and Michigan, U.S.A. [6]. STEC O157:H7 can cause acute infections with a spectrum of human illnesses ranging from abdominal pain and bloody diarrhea to fatal diseases, like hemolytic-uremic syndrome (HUS) and hemorrhagic colitis (HC). The main STEC O157 infections are food-borne, particularly concerning cattle sources [7].

The STEC strains possess Shiga toxins (*stx1* and *stx2*) genes that are considered the major virulence factors of these strains. *Stx2* is associated more closely with the HUS sickness than *stx1* [8]. Other important

virulence determinants are: intimin protein, encoded by *eae* gene and important for attaching and effacing activity within the colonization of host intestinal mucosa and causing severe human infections, and enterohemolysin encoded by the plasmid- and phage-carried enterohemolysin (*ehxA*) gene [9].

STEC O157:H7 isolates have been detected in North Africa from humans, animals, and food products. An Algerian study identified a rate of 7% in the bovine carcasses [10]. In Morocco, the frequency of STEC O157:H7 was 9%, 9.1%, and 11.1% from raw meat products, dairy products, and marketed meat respectively [11,12]. A Tunisian study confirmed that 3.4% of *E. coli* isolates among human stool samples were STEC and the rate of *E. coli* O157:H7 was 0.3% [9]. In Egypt, a survey confirmed that the prevalence among beef samples, chicken samples, and lamb samples was 6%, 4%, and 4% respectively [13].

An increasing number of STEC O157 outbreaks are related to the human consumption of fruits and vegetables contaminated with domestic or wild animal feces. *E. coli* O157:H7 is transmitted to humans by the consumption of contaminated foods like raw meat, undercooked meat, and raw milk. Water and foods contaminated by fecal material and cross-contamination through food production and processing will lead to STEC infection [14]. Therefore, the objective of our study was to assess the incidence, virulence genes, and antimicrobial resistance profiles of *E. coli* O157:H7 in fecal samples of healthy cattle. To the best of our knowledge, this is the first report of *E. coli* O157:H7 in healthy cattle in Tunisia.

Methodology

Samples Collection

The samples analysed in this study were collected as part of a research project dedicated to the study of antibiotic resistance of bacteria isolated from the main five slaughterhouses in the region of the greater Tunis and from cattle farms located in the governorate of Bizerte, which provides 11% of the national production of red meat.

All the feces samples were collected by rectal swabbing, by rolling-rubbing the rectal mucosa. There are two types of samples; firstly, fecal samples from 160 cattle intended for slaughter were collected between December 2016 and April 2017. These samples were collected from five slaughterhouses in the greater Tunis, designated as A, B, C, D, and E. In the second samples category, a total of 100 fecal samples were gathered from healthy cattle between March and November 2018 from cattle farms located in the governorate of Bizerte. Samples were transported appropriately to the laboratory in ice-cooled containers for bacterial isolation and further investigations.

Selective isolation of E. coli O157:H7

Fecal samples were enriched in buffered peptone water overnight at 37 °C, then 10 µL were cultured by the streak plate technique on MacConkey agar and incubated for 18 to 24 hours at 37 °C. One putative colony was subcultured from each plate onto brain heart infusion agar for confirmation as *E. coli*. The identification of *E. coli* colonies was performed by API 20E galleries (bioMérieux). The bacterial colonies were cultivated onto sorbitol MacConkey agar (Oxoid) supplemented with cefixime-tellurite (CT-SMAC) and incubated for 18–24 hours at 37 °C. On this medium, most STEC O157:H7 are distinct from other STEC by their inability to ferment sorbitol. On each plate with sorbitol non-fermenting (straw color or colorless) colonies, one colony was subcultured as probably *E. coli* O157.

Agglutination test of O157

Each non-sorbitol fermenting colony isolated on SMAC plates was examined for the existence of the O157 antigens by agglutination latex reagent (DrySpot™ *E. coli* O157 Latex Agglutination Test, Oxoid).

Affirmation of E. coli O157 by PCR

All non-sorbitol fermenting *E. coli* isolated and O157 agglutination-positive were examined for the existence of *rfbEO157* gene and *fliCH7* by simplex PCR [15]. The PCR condition was as follows: initial denaturation at 94 °C for 5 minutes; 35 cycles of denaturation at 94 °C for 45 seconds, annealing at a specific temperature for 45 seconds (Table 1), extension at 72 °C for 45 seconds; and a final extension (72 °C, 7 minutes).

A multiplex PCR for *stx1*, *stx2*, *uidA*, *ehxA,* and *eae* was achieved for the O157:H7 strains, and primers are listed in table 1 [16]. The thermal cycling program of multiplex PCR was as follows: the denaturation: 95 °C for 5 minutes, followed by 25 cycles of 95 °C for 1 minute, annealing at 56 °C for 1 minute, the extension at 72 °C for 1 minute, and the final extension at 72 °C for 5 minutes. The gel electrophoresis was used to separate PCR products by using a 2 % agarose gel in a TBE buffer containing ethidium bromide.

The *stx1* and *stx2* amplifications were sequenced in order to prove that the amplicon matched the *stx1* and *stx2* sequences. The gained sequences were aligned

with the data sequences in NCBI (http://www.ncbi.nlm.nih.gov).

Virulence genes

PCR assay was used to study the presence of 13 virulence genes; *cdt3* (cytolethal distending toxin), *cnf1* (cytotoxic necrotizing factor), *hly* (hAemolysin), *aer* (aerobactin system), *papA* (P fimbriae), *bfpA* (bundle forming pilus), *papG allele III*, *fimH* (type 1 fimbriae), *traT* (serum survival gene), *ibeA* (invasion of brain endothelium), *sfa/foc* (S and F1C fimbriae), *iutA* (aerobactin system) and *fyuA* (yersiniabactin) [17] and resolved on agarose gels as described above**.**

Antimicrobial susceptibility testing

The antimicrobial susceptibility was determined by the disk-diffusion method on Mueller-Hinton agar plates (BioRad, Marne la Coquette, France) as recommended by the European Committee on Antimicrobial Susceptibility Testing guidelines (EUCAST) [18] using antibiotic disc panels comprising (µg/disk): twelve β-lactam [amoxicillin (25), amoxicillin/clavulanic acid (20/10), ticarcillin/clavulanic acid (75/10), cefotaxime (30), ceftazidime (30), cefepime (30), cefoxitin (30), aztreonam (30) ertapenem (10), piperacillin (30), cephalothin (30), cefuroxime (30)], and nine non-β-lactams [chloramphenicol (30), florfenicol (30), gentamicin (15), streptomycin (10), colistin (50), nalidixic acid (30), enrofloxacin (5), tetracycline (30) and sulfamethoxazole/trimethoprim (1.25/23.75)].

Detection of Phylogenetic groups

The phylogenetic groups (A, B1, B2, C, D, E, F) were detected among the isolates by the quadruplex PCR method developed by Clermont *et al.* [19]. The phylogroups were determined based on the presence of the *chuA*, *yjaA* genes, and *TspE4-C2* fragment detected by quadruplex PCR (A, B1, B2, D), and C, E were further identified using specific primer sets (Table 1).

Table 1. Primers for PCR amplification of *E. coli* O157:H7.

PCR reaction	Gene	Primer sequence (5'-3')	Size of PCR product (bp)	Annealing temperature (°C)	Reference
Phylogenetic genes					
Quadruplex	*chuA*	chuA.1b: ATGGTACCGGACGAACCAAC chuA.2: TGCCGCCAGTACCAAAGACA	288	60	Clermont *et al.,* 2013 [17]
	yjaA	yjaA.1b: CAAACGTGAAGTGTCAGGAG yjaA.2b: AATGCGTTCCTCAACCTGTG	211	60	Clermont *et al.,* 2013 [17]
	TspE4C2	TspE4C2.1b: CACTATTCGTAAGGTCATCC TspE4C2.2b: AGTTTATCGCTGCGGGTCGC	152	60	Clermont *et al.,* 2013 [17]
	arpA	AceK.f: AACGCTATTCGCCAGCTTGC AceK.r: TCTCCCCATACCGTACGCTA	400	60	Clermont *et al.,* 2013 [17]
Group E	*arpA*	ArpAgpE.f: GATTCCATCTTGTCAAAATATGCC ArpAgpE.r: GAAAAGAAAAAGAATTCCCAAGAG	301	57	Clermont *et al.,* 2013 [17]
Group C	*trpA*	trpAgpC.1: AGTTTTATGCCCAGTGCGAG trpAgpC.2: TCTGCGCCGGTCACGCCC	219	59	Clermont *et al.,* 2013 [17]
Internal control	*trpA*	trpBA.f: CGGCGATAAAGACATCTTCAC trpBA.r: GCAACGCGGCCTGGCGGAAG	489	57	Clermont *et al.,* 2013 [17]
Virulence factors					
Shiga toxin	*stx1*	F: CAGTTAATGTGGTGGCGAAGG R: CACCAGACAATGTAACCGCTG	348	56	Zhang *et al.,* 2006 [18]
Shiga toxin	*stx2*	F: ATCCTATTCCCGGGAGTTTACG R: GCGTCATCGTATACACAGGAGC	584	56	Zhang *et al.,* 2006 [18]
Enterohaemolysin	*ehxA*	F: GCATCATCAAGCGTACGTTCC R: AATGAGCCAAGCTGGTTAAGCT	534	56	Al-Ajmi *et al.,* 2020 [19]
Enteropathogenic attachment and effacement	*eae*	F : TGCGGCACAACAGGCGGCGA R : CGGTCGCCGCACCAGGATTC	629	56	Bannon *et al.,* 2016 [20]
Others					
Part of O-antigen 157	*O157*	F: CGGACATCCATGTGATATGG R: TTGCCTATGTACAGCTAATCC	259	52	Gannon *et al.,* 1997 [15]
Encoding H7 flagellar antigens	*fliCH7*	F: GCGCTGTCGAGTTCTATCGAGC R: CAACGGTGACTTTATCGCCATTCC	625	60	Gannon *et al.,* 1997 [15]
Beta-glucuronidase	*uidA*	F: ATCACCGTGGTGACGCATGTCGC R: CACCACGATGCCATGTTCATCTGC	486	56	Akanbi *et al.,* 2011 [21]

Results

In our study, 236 *E. coli* isolates were collected from the examination of 250 fecal samples of healthy cattle in Tunisia. Out of 236 *E. coli* isolates, 159 were from cattle in slaughterhouses and 77 from cattle farms. Of these *E. coli* strains, 100% were positive for methyl-red, lactose, and indol, and 100% were negative for urease, citrate, and H_2S production. The results revealed that 10 *E. coli* were sorbitol nonfermenting on CT-SMAC and these 10 (4.2%) strains were *E. coli* O157:H7. Out the 10 strains; 6 isolates were isolated from healthy cattle in slaughterhouses and the other from healthy cattle on farms.

All *E. coli* O157:H7 isolates were susceptible to amoxicillin/clavulanic acid, cefotaxime, cefepime, aztreonam, colistin, and sulfamethoxazole/trimethoprim. More than 80% of isolates were susceptible to ampicillin, cefoxitin, ticarcillin/clavulanic acid, ceftazidime, ertapenem, nalidixic acid, florfenicol, chloramphenicol, and enrofloxacin. However, resistance to cefuroxime, streptomycin, and tetracycline was 50%, 40%, and 30% respectively (Figure 1).

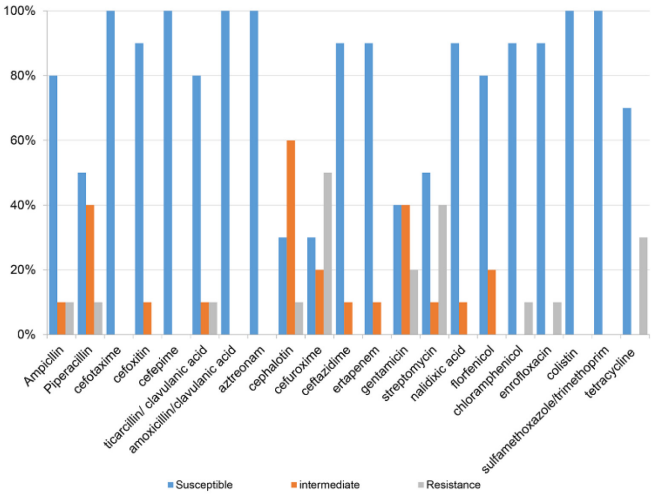
The confirmation of *E. coli* O157 by latex agglutination testing revealed that all isolates were O157 positive. All of these isolates were confirmed as *E. coli* O157:H7 via screening of *rfbO157* and *flicH7* genes by specific primers.

PCR analysis of the 10 *E. coli* O157 isolates reveals that *uidA*, *flicH7*, and *O157* genes were present in all strains. *Stx2* and *ehxA* genes were present in 7 isolates (70%), while *stx1* and *eae* were confirmed in six isolates (60%).

We found six isolates carrying three virulence genes as follows: three strains harbored *stx2*, *stx1* and *ehxA,* two strains harbored *stx2*, *eae,* and *ehxA*; one isolate harbored *stx1*, *eae* and *ehxA* (Table 2). All *E. coli* O157 isolates belong to the phylogroup E.

The O157 isolates were further tested for 13 virulence factors. All isolates carried at least one virulence gene tested. Out of 10 isolates, 60% carried more than three virulence genes tested. *FimH* was the most frequent virulence gene and was detected in 90% (9/10) of the isolates, followed by *sfa/focDE* in 60%. The frequency of *cdt3, traT,* and *iutA* among the isolates was 50%, 50%, and 40% respectively, whereas, *hly* was found in one isolate (Table 2). None of the isolates harbored *cnf1*, *aer*, *papA*, *bfpA*, *papG allele III*, *ibeA*, or *fyuA*.

Figure 1. Antimicrobial susceptibility of E. coli O157:H7 isolates.



Discussion

Human infections caused by STEC O157:H7 are associated with food of animal origin or plants contaminated with the feces of these animals. In particular, cattle, sheep, and goats have been demonstrated as the main natural reservoirs for STEC O157:H7 and play an important role in the public health concern [7]. This study was conducted to evaluate the incidence of *E. coli* O157:H7, antimicrobial profiles, and virulence genes in fecal samples of healthy cattle collected from slaughterhouses and cattle farms in

Table 2. Distribution of virulence genes and specific genes detected by PCR.

Bacterial code	Specific genes			STEC virulence markers				Other virulence factors
	uidA	*O157*	*fliCH7*	*stx1*	*stx2*	*eae*	*ehxA*	
T46	+	+	+	+	+	-	+	*cdt3, traT, fimH, sfa/focDE*
T48	+	+	+	+	+	-	+	*cdt3, sfa/focDE*
T51	+	+	+	-	+	+	-	*cdt3, fimH, sfa/focDE, iutA*
T109	+	+	+	-	+	+	+	*cdt3, fimH, sfa/focDE*
T125	+	+	+	-	+	+	+	*hly, cdt3, traT, fimH, sfa/focDE*
T132	+	+	+	+	+	-	+	*fimH*
BS10	+	+	+	+	-	+	+	*fimH*
BS37	+	+	+	-	+	-	+	*traT, sfa/focDE, fimH, iutA*
BS40	+	+	+	+	-	+	-	*traT, fimH, iutA*
BS43	+	+	+	+	-	+	-	*traT, fimH, iutA*

Tunisia. This is the first report concerning the presence of *E. coli* O157:H7 in cattle in Tunisia.

Our findings demonstrated that among 236 *E. coli* isolates, ten *E. coli* O157:H7 were detected at a rate of 4.2%. These isolates were cultured on CT-SMAC agar as non-sorbitol fermenters and were confirmed as STEC O157 by using latex agglutination and PCR. This is consistent with previous research that found *E. coli* O157: H7 in cattle feces samples and carcass swabs in slaughterhouses, with rates of 4.7% and 2.7%, respectively, in Ethiopia [7]. In a study in United Arab Emirates, the frequency of *E. coli* O157:H7 among slaughtered cattle was 1.4% [20]. An Algerian study reported an incidence of *E. coli* O157 in more than 7% of bovine carcasses [10]. In Morocco, the incidence of *E. coli* O157:H7 in dairy products and marketed meat products was 9.1% and 11.1% respectively [12]. In Tunisia, 327 *E. coli* strains were isolated from diarrheic and non-diarrheic people. By using PCR techniques, it has been demonstrated that 11 isolates (3.4%) express the *stx1* and *stx2* genes encoding for STEC and only one (0.3%) was confirmed as *E. coli* O157:H7 [9].

In this study, the rate of these bacteria among healthy cattle in slaughterhouses was higher than that of healthy cattle from farms, maybe the stress of transporting cattle from the farm to the slaughterhouse results in increased bacterial excretion. McCluskey *et al.* [21] confirmed that there were significantly higher rates of *E. coli* O157 in lambs that were transported and held for ≥ 18 hours. Furthermore, animals were not perfectly fed during the transition and holding prior to slaughter; withholding of food has the potential effect on colonization with *E. coli* O157.

In the present study, one putative colony per sample was selected for confirmation as *E. coli* O157:H7. Examination of up to ten colonies per plate [22] may have led to more O157:H7 isolates and this criterion was confirmed by many studies. Furthermore, some characteristics of these bacteria can change during lab manipulations. For example, *ehxA* gene is located on a plasmid that could be lost either naturally in the animal host, or during lab manipulations resulting in increased sensitivity of the method. On the other hand, immunomagnetic separation would certainly have improved the rate of isolation of STECs.

In Africa, the highest incidence of cattle was 31.2% as represented in two studies [23,24]. In Asian countries, the highest rate was 12.22% in Jordanian cattle [25] and the lowest (0.13%) was evaluated in cattle from Taiwan [26]. According to a meta-analysis of 40 studies, in several states of the USA the estimated incidence was 7.60% [27] while in California it was highly variable, from 0 to 90% [28].

Healthy cattle can be the main reservoir for prospecting human infection and play an important role in the epidemiology of STEC infections. Moreover, most human diseases caused by STEC bacteria originate from cattle [29]. The existence of STEC O157:H7 in our study among animal feces in slaughterhouses highlighted the possible contamination of meat products prepared for human consumption. On the other hand, identifying the STEC O157:H7 in humans is very important for public health objectives, like identifying outbreaks. Underlining the great scarcity of studies in Tunisia, the identification of STEC O157:H7 and non-O157 by Al-Gallas *et al.* [9] among humans has important benefits for public health and proves the need for epidemiological surveys on STEC infection in this country. The detection of *E. coli* O157:H7 in cattle and humans in Tunisia calls for further epidemiological assessment to detect whether a case is part of an outbreak, the outbreak source, and the spread prevention of it.

Antimicrobial resistance is considered a global health threat. Animal products have been demonstrated as reservoirs of antimicrobial resistant bacteria because the same genes encoded for antimicrobial resistance were demonstrated in the bacteria of animal food and in humans [30].

Our results show that all *E. coli* O157:H7 isolates were susceptible to amoxicillin/clavulanic acid, cefotaxime, cefepime, aztreonam, colistin, and sulfamethoxazole/trimethoprim. Previous studies in animals reported different antibiotic resistance profiles of *E. coli* O157:H7 isolates. One study reported that in Hawassa (Ethiopia), all *E. coli* O157:H7 isolates were susceptible to cefotaxime, ceftriaxone, gentamycin, kanamycin, and nalidixic acid [7]. A further report from United Arab Emirates showed that all isolates were susceptible to cefotaxime, chloramphenicol, ciprofloxacin, norfloxacin, and polymyxin B [20]. However, a Saudia study reported that the isolates were resistant to all tested antibiotics [31]. One study from Iran revealed that the resistance rate to gentamycin, ampicillin, erythromycin, amoxicillin, and tetracycline was 56.0%, 48.0%, 40.0%, 16.0%, and 12.0% respectively [32]. A UK study in humans showed that the resistance profile among 327 STEC O157 to ampicillin, streptomycin, trimethoprim/sulphonamide, and tetracycline was 5.8% followed by the resistance rate to ciprofloxacin (2.6%) and chloramphenicol (2.1%) [33].

A study conducted in Latin American countries has documented 78.5% sensitivity to all the antimicrobial agents in 14 O157 STEC strains from cattle. Resistance to streptomycin, trimethoprim, and sulfonamide was found in three strains [34].

Antimicrobial resistance variation might be due to the expression of resistance genes among bacteria in animals, the environment, or humans and this variation in resistance rates may also be an indicator of animal husbandry and agricultural use of antibiotics and antimicrobials [35].

On the other hand, more than 40% of the isolates were resistant to cefuroxime and streptomycin, perhaps via co-resistance or cross-resistance or inappropriate or wide use of these drugs for prophylactic purposes and treating infections. In fact, no multi-drug resistance was observed in all the strains tested, in agreement with a Turkish study, where a low resistance rate to cephalothin, streptomycin, and nalidixic acid, was detected [36]. Vali *et al.* [37] showed no multi-drug resistance among *E. coli* O157 strains isolated from beef cattle farms and identified a low prevalence of resistance to cephalothin, sulphamethoxazole, streptomycin, sulphonamide compounds, and nalidixic acid. However, some studies have demonstrated that there has been an increase in the antimicrobial resistance of STEC O157:H7 [38,39]. Previous studies conducted around the world revealed that the majority of antibiotic-resistant isolates were discovered in animal farms, which more commonly used antibiotics for prophylactic and treating purposes, but our findings of less resistance and no MDR were unexpected.

It is debatable whether it is safe to use antimicrobial drugs in humans to prohibit HUS due to lysis of the bacteria and release of the Shiga toxins in the gut. However, reports have revealed that using some antimicrobials in the early phase of the disease may prevent HUS advancement [36].

In our study, most strains exhibited an intermediate resistance pattern, suggesting the possibility of future resistance. The intermediate susceptibility profiles should be elevated and taken into consideration with resistance results because it means the organism may be on the way to becoming resistant. In fact, antibiotics are not recommended for O157:H7 infections as they can induce the bacteria to express more Shiga toxin and make the disease worse with the risk of triggering hemolytic-uremic syndrome [40]. However, knowing the antibiotic resistance of O157:H7 strains can help track them in an outbreak and be a useful tool for selective isolation.

Shiga toxins (*stx* genotypes) are important clinical outcome factors that correlate with HC and HUS, as well as higher pathogenicity in strains carrying the *stx2* genotype [41]. The *eae* gene encodes for an intimin protein, which is important for attaching and effacing activity in host intestinal cells and causes severe human illnesses, particularly HUS [42]. Furthermore, a hemolysin produced by STEC called enterohemolysin is encoded by the *hlyA* gene and causes erythrocyte lysis, which participates in iron intake in the intestine. This gene is commonly used as an epidemiological marker of STEC strains [43].

In the present study, the *stx2* gene was present in most isolates (7/10), and *eae* and *ehxA* were found in more than half of the isolates. Many studies have found that the virulence factors *stx2* and *eaeA* are clinically significant and are associated with the severity of human disease, particularly HUS [44,45]. In United Arab Emirates, Shiga toxin gene (*stx2*) was confirmed in all 24 *E. coli* O157 from camels, cattle, and goats. The *eaeA* and *hlyA* genes were present in 79.2% and 66.7%, respectively, whereas *stx1* was absent in all isolates [20].

The presence of *ehxA* + *eae* and *ehxA* + *eae* + stx_2 is significantly associated with HUS and O157:H7 isolates [46]. In our study, two isolates (T109 and T125) harbored *stx2*, *eae,* and *ehxA,* and one isolate (BS10) harbored *stx1*, *eae,* and *ehxA* suggesting that the existence of more than one virulence factor, particularly *eae,* and *ehxA* could be associated with more severe clinical outcomes in O157 infections. Hua *et al.* [46] mentioned that the presence of *ehxA* with *stx* and *eae,* can be used as a risk predictor for HUS in STEC infections.

An Ethiopian study revealed that the rate of *stx1*, *eae*, *hly,* and *stx2* among 14 *E. coli* O157:H7 detected among 157 isolates of *E. coli* was 11 (78.5%), 6 (42.8%), 3 (21.4%), and 11 (78.5%) respectively [7].

Generally, all *E coli* O157:H7 possess intimin (*eae*). Some strains may lose the Shiga toxin phage and be negative for *stx1* and *sxt2*, but the absence of *eae* in O157:H7 is very unusual. Furthermore, the lack of *ehxA* in O157:H7 is also unusual, although this gene is located on a plasmid that could be lost either naturally in the animal host or during lab manipulations. Negative results for the presence of *eae* and *ehxA* genes were verified by simplex PCRs with *eae* and *ehxA* primers instead of multiplex PCR.

The absence of *eae* and *ehxA* among our isolates recognizes these isolates as atypical O157:H7 which represents a less serious threat to public health. For typical STEC, the only reservoir is humans. However,

atypical STEC can have both animal and human reservoirs and may also be associated with human diarrhoeal disease [47].

In the same way, the atypical EPEC (enteropathogenic *E. coli*) strains may be less virulent than the typical isolates. One reason may be the lack of the adherence factor (EAF) plasmid among the atypical strains. However, atypical strains have not been confirmed to be less pathogenic, and these bacteria have other virulence factors that may compensate for the absence of the EAF plasmid [48].

This study showed that 9 STEC strains harbored *fimH* and half the isolates harbored *sfa/focDE*, *cdt3, traT,* and *iutA*. These factors were identified in a previous study on *E. coli* from dairy farms in the USA [49]. In an Iranian study of STEC, they found *papA*, *cnf1*, *traT*, and *cnf2* were the most common virulence genes [50]. The detected factors contribute to virulence, which affects host cell processes and contributes to bacterial pathogenesis. The findings of these virulence factors in our isolates in association with the high frequency of *stx1*, *stx2*, and *ehxA* suggest that STEC O157 in Tunisian calves may pose a serious public health concern.

The findings of our study revealed that all *E. coli* O157:H7 isolates belonged to phylogroup E which usually corresponds to commensal strains. This was identical to the report of Tenaillon *et al.* [51]. A study in Brazil demonstrated that *E. coli* belonging to phylogroups E and B1 were isolated from cattle, whereas phylogroups A and F were from poultry, and B2 and D were associated with isolates from water buffalo [52].

Conclusions

The frequency of *E. coli* O157:H7 in healthy cattle indicates a possible risk for a public health concern. The detection of STEC O157:H7 in this study among cattle and previously in humans in Tunisia have important benefits for public health and calls for the public health system in our country to track food-borne outbreaks. The existence of STEC O157:H7 in animals intended for slaughter highlighted the possible contamination of meat products prepared for human consumption. The high prevalence of *stx1*, *stx2,* and *ehxA* together with other virulence factors suggest that STEC O157 in Tunisian calves may pose a serious public health concern. Our study reveals the necessity of regular screening animals for *E. coli* O157:H7 in order to control this pathogen. Moreover, the frequency of O157:H7 in slaughterhouse animals indicates that the risk is significant for public health. Therefore, it is important to take the necessary precautions during the slaughter and skinning of animals to prevent cross contamination of meat by this pathogen. Clinical results must be obtained to evaluate the actual influence of food contamination on humans in Tunisia.

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Authors' Contributions

Ghassan Tayh designed the study, performed the experimental work (the microbiological and molecular tests), collected the data, analyzed and interpreted the data and drafted the manuscript. Salma Mariem Boubaker and Rym Ben Khedher collected samples and helped in performing the experimental part of the manuscript. Mounir Jbeli collected samples. Faten Ben Chehida, Aymen Mamlouk and Monia Dâaloul-Jedidi participated in the project design. Lilia Messadi designed and supervised the study, and contributed to final writing and editing the manuscript. All authors read and approved the final version of the manuscript.

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