Prevalence, Virulence Genes and Antimicrobial Profiles of Escherichia Coli O157:H7 Isolated from Healthy Cattle

Ghassan Tayh (✉ ghassan.tayh@gmail.com)
Universite de la Manouba

Salma Mariem Boubaker
Universite de la Manouba

Rym Ben Khedher
Universite de la Manouba

Mounir Jbeli
Universite de la Manouba

Faten Ben Chehida
Universite de la Manouba

Aymen Mamlouk
Universite de la Manouba

Monia Dâaloul-Jedidi
Universite de la Manouba

Lilia Messadi
Universite de la Manouba

Research Article

Keywords: Escherichia coli O157:H7, Healthy cattle, Antimicrobial susceptibility, Virulence factors, Shiga toxins, Tunisia.

DOI: https://doi.org/10.21203/rs.3.rs-580804/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License
Abstract

**Background:** Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is associated with intestinal infection in human and considered a main cause of food-borne diseases. It was isolated from animals, human and food. 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 farms (n=100).

**Methods:** *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 to detect non-sorbitol fermenting colonies that were examined for the presence of O157 antigen by latex agglutination, and positive bacteria were screened for the existence of stx1, stx2, eaeA and ehxA by PCR as well as rfbEO157 and fliC7 genes specific for serotype O157. All isolates were examined for the susceptibility against 21 antibiotics discs.

**Results:** Of the 236 *E. coli* isolates, 4.2% (10/236) were positive for STEC O157:H7. Shiga toxin gene (stx2) was present in 70% of isolates, stx1 and ehxA were confirmed in 60% of the isolates, whereas eae was identified in two isolates. Other virulence factors screened (fimH, sfa/focDE, cdt3, traT, iutA and hly) 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.

**Conclusion:** 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 human contamination.

Introduction

*Escherichia coli* is a common bacteria of the intestinal microbiota and an important pathogen in animals, human and public health (Tayh et al. 2016). The pathogenic *E. coli* strains are classified into extraintestinal pathogenic strains (causing urinary tract infection, meningitis, diverse intraabdominal infections and pneumonia) and intestinal pathogenic (diarrheagenic) strains that causing gastroenteritis (Johnson, Russo 2002). 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) (Hashish et al. 2016).

Strains belonging to the subgroup of shiga toxin-producing strains (STEC) are distinguished by certain EHEC serotypes, which are considerably linked to outbreaks in humans and causes clinical sickness. STEC is a food-borne bacteria which have been associated to many epidemics in all continents especially serotype O157:H7 (Karmali 1989). STEC strains were isolated from faeces of healthy ruminants like cattle, goats and sheep which can be natural reservoirs of these pathogens (Persad, Lejeune 2015).

*E. coli* O157:H7 is the dominant serotype of 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 (Riley et al. 1983). The STEC O157:H7 can cause acute infections, with a spectrum of human illnesses ranging from abdominal pain, bloody diarrhea to fatal disease, like hemolytic-uremic syndrome
(HUS) and hemorrhagic colitis (HC). The main STEC O157 infections are food borne more particularly concerning cattle sources (Atnafie et al. 2017).

The STEC strains possess shiga toxins (stx1 and stx2) genes which consider the major virulence factors of these strains. Stx2 is associated more closely with the sickness than stx1 (García-Aljaro et al. 2004). 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 cause severe human infections, and enterohemolysin is encoded by the plasmid- and phage-carried enterohemolysin (ehxA) gene (Al-Gallas et al. 2006).

STEC O157:H7 isolates have been detected in north Africa from humans, animals and food products. An Algerian study identified a rate of 7% from bovine carcasses (Chahed et al. 2006). In Morocco, a prevalence of STEC O157:H7 was 9%, 9.1% and 11.1% from raw meat products, dairy products and marketed meat respectively (Beneduce et al. 2008, Benkerroum et al. 2004). A Tunisian study confirmed that 3.4% of E. coli isolates among human stool samples were STEC and 0.3% was E. coli O157:H7 (Al-Gallas et al. 2006). In Egypt, a survey confirmed that the prevalence among beef samples, chicken samples and lamb samples was 6%, 4% and 4% respectively (Abdul-Raouf et al. 1996).

An increasing rate of STEC O157 outbreaks, is related to the human consumption of fruits and vegetables contaminated with domestic or wild animal faeces. E. coli O157:H7 is transmitted to human by consumption of contaminated foods like raw meat, undercooked meat and raw milk. Contaminated water and foods by faecal material and cross-contamination through food production and processing, will lead to STEC infection (Lupindu 2018). 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 detection report of E. coli O157 in healthy cattle in the Tunisia.

**Materials And Methods**

**Samples Collection**

The sample collection in this study was conducted on two types; firstly, faecal samples from 160 cattle intended for slaughter collected between December 2016 and April 2017. These samples were collected from five slaughterhouses in the greater Tunis, namely: El Ouardia slaughterhouse, Momag slaughterhouse, Fouchana slaughterhouse, Khelidia slaughterhouse and Ezzahra slaughterhouse. In the second sampling method, a total of 100 faecal samples were gathered from healthy cattle between March and November 2018 from cattle farms located in the governorate of Bizerte.

**Selective isolation of E. coli O157:H7**

Fecal samples were enriched in buffered peptone water overnight at 37°C, then cultured on MacConkey agar for 18 to 24 hours at 37°C. The identification of E. coli colonies was performed by classical biochemical methods. The bacterial colonies were cultivated onto sorbitol MacConkey agar (Oxoid) supplemented with cefixime - tellurite (CT-SMAC) and incubated for 18–24 h at 37°C. All sorbitol nonfermenters (straw color or colorless) colonies each were picked 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 (Oxoid).

Affirmation of E. coli O157 by PCR

All non-sorbitol fermenting E. coli isolates and O157 agglutination-positive were examined for the existence of rfbEO157 gene and fliCH7 by simplex PCR (Gannon et al. 1997). The PCR condition was as follows: initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 45 sec, annealing at specific temperature for 45 sec (Table 1), extension at 72°C for 45 sec; and a final extension (72°C, 7 min).

A multiplex PCR for stx1, stx2, uidA, ehxA and eae was achieved for the O157:H7 strains and primers are listed in Table 1 (Al-Ajmi et al. 2020). The thermal cycling program of multiplex PCR was as follows, the denaturation: 95°C for 5 min followed by 25 cycles of 95°C for 1 min, annealing at 56°C for 1 min and the extension at 72°C for 1 min and the final extension at 72°C for 5 min. The gel electrophoresis was used to separated PCR products by using 2 % agarose gel containing ethidium bromide.

The stx1 and stx2 amplifications were sequenced in order to prove that the amplicon matched to 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 (hemolysin), 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).

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility was determined by the disk-diffusion method on Mueller-Hinton agar plates as recommended by the Antibiogram Committee of the French Society (CA-SFM; www.sfm-microbiologie.org) 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), and piperacillin (30), cefalotin (30), cefuroxime (30)), and nine non-β-lactam (chloramphenicol (30), gentamicin (15), colistin (50), nalidixic acid (30), enrofloxacin (5), tetracycline (30) and sulfamethoxazole/trimethoprim (1.25/23.75), streptomycin and florfenicol).

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. (Clermont et al. 2013). The phylo-groups determination established on the existence
of the *chuA*, *yjaA* genes and *TspE4-C2* fragment by the quadruplex PCR to detect (A, B1, B2, D) and C, E were further identified by using specific primer sets (Table 1).
| PCR reaction | Gene | Primer sequence (5’-3’) | Size of PCR product (bp) | Annealing temperature (°C) | Reference |
|--------------|------|-------------------------|-------------------------|---------------------------|-----------|
| **Phylogenetic genes** | | | | | |
| Quadruplex | chuA | chuA.1b: ATGGTACCGGACGAACCAAC | 288 | 60 | (Clermont et al. 2013) |
| | | chuA.2: TGCCGCCAGTACAAAGACA | | | |
| | yjaA | yjaA.1b: CAAACGTGAAGTGTCAGGAG | 211 | 60 | (Clermont et al. 2013) |
| | | yjaA.2b: AATGCGTTTCCTCAACCTGTG | | | |
| | TspE4C2 | TspE4C2.1b: CACTATTCGTAAGGTGATCC | 152 | 60 | (Clermont et al. 2013) |
| | | TspE4C2.2b: AGTTTATCGCTGCGGTACG | | | |
| | arpA | AceK.f: AACGCTATTCGCCAGCTTGC | 400 | 60 | (Clermont et al. 2013) |
| | | AceK.r: TCTCCCATACCGTGCTAGTA | | | |
| Group E | arpA | ArpAgpE.f: GATTCCATCTTGCAAAAATATGCC | 301 | 57 | (Clermont et al. 2013) |
| | | ArpAgpE.r: GAAAGAAAAAGAAATTCCAAAGAG | | | |
| Group C | trpA | trpAgpC.1: AGTTTATGCCAGTTGCCGAG | 219 | 59 | (Clermont et al. 2013) |
| | | trpAgpC.2: TCTGCGCCGGTCACGCCC | | | |
| Internal control | trpA | trpBA.f: CGCGCATAAACGACATCTCAGC | 489 | 57 | (Clermont et al. 2013) |
| | | trpBA.r: GCAACGCGCCCTGGCAGGAAG | | | |
| **Virulence factors** | | | | | |
| Shiga toxin | stx1 | F: CAGTTAATGTGGTGCGGAAGG | 348 bp | 56 | (Sjöling et al. 2015) |
| | | R: CACCAGACAATGGTAACGCCGTG | | | |
| Shiga toxin | stx2 | F: ATCCTATTCGCGGAGGTTTACG | 584 bp | 56 | (Sjöling et al. 2015) |
| | | R: GCGTCATCGTATAACAGGAGC | | | |
| PCR reaction                                      | Gene                  | Primer sequence (5'-3')                                                                 | Size of PCR product (bp) | Annealing temperature (°C) | Reference                        |
|--------------------------------------------------|-----------------------|----------------------------------------------------------------------------------------|--------------------------|----------------------------|----------------------------------|
| Enterohaemolysin                                 | ehxA                  | F: GCATCATCAAGCGTACGTTCC<br>R: AATGAGCCAAGCTGGTTAAGCT                                      | 534 bp                   | 56                         | (Grispoldi et al. 2017)          |
| Enteropathogenic attachment and effacement       | eae                   | F: TGCGGCACAAACAGGCCGCGCA<br>R: CGTGCACCCGACCCAGGATTCC                                       | 629 pb                   | 56                         | (Ranjbar et al. 2017)            |
| **Others**                                       |                       |                                                                                        |                          |                            |                                  |
| Part of O-antigen 157                            | O157                  | F: CGGACATCCATGTGATATGG<br>R: TTGCTATGTACAGCTAAATCC                                         | 259 bp                   | 52                         | (Mohamed 2018)                   |
| Encoding H7 flagellar antigens                   | fliCH7                | F: GCGCTGTGAGTTCTATCGAGC<br>R: CAAACGTGACTTTATCGCCATTCC                                          | 625 bp                   | 60                         | (Mohamed 2018)                   |
| Beta-glucuronidase                               | uidA                  | F: ATCACCGTGGTGACGCATGC<br>R: CACCACGATGCCATGTTTCATGTC                                          | 486 bp                   | 56                         | (Heininger et al. 1999)          |

**Results**

In our study, 236 *E. coli* isolates were collected from the examination of 250 faecal samples of healthy cattle in Tunisia. Out of 236 *E. coli* isolates, 159 were from cattle in slaughterhouses and 77 from cattle from farms. Of these *E. coli* strains, 100% were positive for methyl-red, lactose and indol, and 100% were negative for urease, citrate and H2S. The results revealed that 10 *E. coli* were nonfermenting of sorbitol 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 4 from healthy cattle from 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, fleroxacin, chloramphenicol and enrofloxacin. However, resistance to cefuroxime, streptomycin and tetracycline was 50%, 40% and 30% respectively (Fig. 1).

The confirmation of *E. coli* O157 by latex agglutination testing reveal 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* gene was present in 7 isolates (70%), *stx1* and *ehxA* were confirmed in six isolates (60%) whereas *eae* was identified in two isolates.
We found four isolates carrying three virulence genes as follow; three strains harbored stx2, stx1 and ehxA and one strain harbored stx2, eae and ehxA (Table 2). All E. coli O157 isolates belong to the phylo-group 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 gene tested. The fimH was the most frequent virulence gene and was detected in 90% (9/10) of the isolates, followed by sfa/focDE 60%. The frequency of cdt3, traT, and iutA among the isolates was 50%, 50%, and 40% respectively, whereas, hly was the lowest virulence genes of E. coli isolates which was found in one isolates (Table 2). None of the isolates harbored cnf1, aer, papA, bfpA, papG allele III, ibeA and fyuA.

### Table 2

| Bacterial code | Specific genes | STEC virulence markers | Virulence factors |
|----------------|----------------|------------------------|-------------------|
|                | uidA | O157 | fliCH7 | stx1 | stx2 | eae | ehxA |
| T46            | +    | +    | +      | +    | +    | -   | +    |
|                |      |      |        |      |      |     |      |
| T48            | +    | +    | +      | +    | +    | -   | +    |
|                |      |      |        |      |      |     |      |
| T51            | +    | +    | +      | -    | +    | -   | -    |
|                |      |      |        |      |      |     |      |
| T109           | +    | +    | +      | -    | +    | -   | +    |
|                |      |      |        |      |      |     |      |
| T125           | +    | +    | +      | -    | +    | +   | +    |
|                |      |      |        |      |      |     |      |
| T132           | +    | +    | +      | +    | +    | -   | +    |
|                |      |      |        |      |      |     |      |
| BS10           | +    | +    | +      | +    | -    | -   | +    |
|                |      |      |        |      |      |     |      |
| BS37           | +    | +    | +      | -    | +    | -   | +    |
|                |      |      |        |      |      |     |      |
| BS40           | +    | +    | +      | +    | -    | -   | -    |
|                |      |      |        |      |      |     |      |
| BS43           | +    | +    | +      | +    | -    | -   | -    |
|                |      |      |        |      |      |     |      |

### Discussion

Human infections caused by STEC O157:H7 have particularly been distinguished to be originated from foods that come from animals. Particularly, cattle, sheep, and goats have been demonstrated as main natural reservoirs for STEC O157:H7 and play an important role in the public health concern (Atnafie et al. 2017).

The high morbidity of this serotype around the world has been focused as a major public health threat. It can cause acute human infections and outbreaks. The STEC O157 infection might involve abdominal pain, bloody diarrhea, hemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS) (Zhang et al. 2006). The majority of E. coli O157 infections in human are food borne and concerning with cattle sources.

A total of 236 E. coli isolates were collected from faecal samples of healthy cattle in Tunisia during a five-month time period in 2017 and nine months in 2018, and were evaluated for the incidence of E. coli O157 and antimicrobial profiles. This is the first report concerning the presence of E. coli O157:H7 in cattle in Tunisia.
Our finding exhibited that among 236 E. coli isolates, ten E. coli O157:H7 were detected with 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 in agreement with other studies investigating E. coli O157:H7 among cattle feces samples and carcass swabs in slaughterhouses where the prevalences were reported as 4.7% and 2.7% respectively in Ethiopia (Atnafu et al. 2017). In a study in United Arab Emirates, the prevalence of E. coli O157:H7 among slaughtered cattle was 1.4% (Al-Ajmi et al. 2020). An Algerian study reported an occurrence of E. coli O157 in more than 7% of bovine carcasses (Chahed et al. 2006). In Morocco, the incidence of E. coli O157:H7 in dairy products and marketed meat products was 9.1% and 11.1% respectively (Benkerroum et al. 2004). 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 stx gene encoding for STEC (EHEC) and only one (0.3%) was confirmed as E. coli O157:H7 (Al-Gallas et al. 2006).

In Africa, the highest incidence in cattle was 31.2% representative in four studies. In Asian countries, the highest rates was 12.22% in Jordanian cattle and the lowest (0.13%) was evaluated in Taiwan. In Europe, the highest estimated occurrence was demonstrated from Italy (10.45%) and the lowest from Norway (0.25%). Furthermore, the USA incidence estimate was 7.60% among forty studies (Islam et al. 2014).

Healthy cattle can be a main reservoir for prospect human infection, and it plays an important role in the epidemiology of STEC infections. Moreover, most human diseases by STEC bacteria originate from cattle (Mead, Griffin 1998). 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 human is very important for public health objective, like finding outbreaks.

Antimicrobial resistance is considered as a global health threat. Food animals 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 (Founou et al. 2016).

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 antibiotics resistance profiles of E. coli O157:H7 isolates. One study found that all E. coli O157:H7 isolates were susceptible to cefotaxime, ceftriaxone, gentamycin, kanamycin and nalidixic acid (Atnafu et al. 2017). Further report showed that all isolates were susceptible to cefotaxime, chloramphenicol, ciprofloxacin, norfloxacin, and polymyxin B (Al-Ajmi et al. 2020). However, a Saudian study reported that the isolates were resistant to all used antibiotics (Al-Wabel 2007). One study in Iran revealed that resistance rate to gentamycin, ampicillin, erythromycin, amoxicillin and tetracycline was 56.0%, 48.0%, 40.0%, 16.0% and 12.0% respectively (Rahimi, Nayebpour 2012). A UK study in human showed that resistance profile among 327 STEC O157 to ampicillin, streptomycin, trimethoprim/sulphonamide and tetracycline was 5.8% followed by the resistance rate in ciprofloxacin (2.6%) and chloramphenicol (2.1%) (Day et al. 2016).

A study conducted in Latin American countries has documented 78.5% sensitivity to all the antimicrobial agents in 14 O157 STEC strains from cattle. Three strains were resistant to streptomycin, trimethoprim and sulfonamide (Bastos et al. 2006).

Antimicrobial resistance variation might be due to expression of resistance genes among bacteria in animals, environment or humans (Reuben, Owuna 2013).
On the other hand, more than 40% of the isolates were resistant to cefuroxime and streptomycin, perhaps via inappropriate or wide use of drug for prophylactic purpose and treating infections.

In our study, most strains exhibited an intermediate resistance pattern, suggesting the possibility for future resistance. The intermediate susceptibility profiles should be elevated and take in consideration with resistance results because it means the organism may be at the way to resistant.

Shiga toxins (stx genotypes) are important factors of the clinical outcome which correlate with HC and HUS and the pathogenicity higher in the strains harbouring stx2 genotype (Kawano et al. 2008). The eae gene encoding for an intimin protein, which is important for attaching and effacing activity in host intestinal cells and cause severe human illnesses particularly HUS (Cornick et al. 2002). Furthermore, a hemolysin produced by STEC called enterohemolysin is encoded by hlyA gene and cause erythrocyte lysis which participate in iron intake in the intestine. This gene is commonly used as epidemiological marker of STEC strains (Schwidder et al. 2019).

In this study, stx2 gene was present in most isolates, eae and ehxA were found in more than half of isolates. Many studies mentioned that virulence factors stx2 and eaeA are clinically significant and linked with the acuteness of human disease, particularly HUS (Friedrich et al. 2002, Beutin et al. 2004). In UAE, shiga toxin gene (stx2) were confirmed in all twenty four 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 (Al-Ajmi et al. 2020).

An Ethiopian study revealed that prevalence of stx1, eae, hly and stx2 among 157 isolates E. coli were 11 (78.5%), 6 (42.8%), 3 (21.4%) and 11 (78.5%) respectively (Atnafie et al. 2017).

Our study showed that 9 STEC strains harbored fimH and half isolates harbored sfa/focDE, cdt3, traT, and iutA. These factors were identified in a previous study among E. coli from dairy farms in America (Pereira et al. 2011). In an Iraian study of STEC, they found papA, cnf1, traT and cnf2 the highest virulence genes (Momtaz et al. 2012). The detected factors contribute to virulence which affect of host cell processes and contribute to bacterial pathogenesis. The findings of these virulence factors in our isolates in associated with high prevalence 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 isolates belonged to phylogroup E. This was identical to the report of Tenaillon et al. (Tenaillon et al. 2010). 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 (Morcatti Coura et al. 2015).

**Conclusions**

The prevalence of E. coli O157:H7 in healthy cattle with some antibiotics resistance indicate a possibly risk to public health concern. The existence of STEC O157:H7 in animal feces intended to slaughter highlighted the possible contamination of meat products prepared for human consumption. The high prevalence of stx1, stx2 and ehxA with other virulence factors suggest that STEC O157 in Tunisian calves may pose a serious public health concern. Our study reveals the necessity for a regular screening of E. coli O157:H7 in animal in order to control this pathogen. It is important to take necessary measures in the slaughterhouse during the slaughter and skinning of animals to prevent cross contamination of meat by this pathogen.
Declarations

Funding: This work was supported by the research project PEER 7-349 funded by the USAID "Monitoring of antimicrobial resistance of bacteria for a better health of animals in Tunisia". Prof. Lilia Messadi is the recipient of the funding (number PEER 7-349).

Conflicts of interest/Competing interests: The authors declare that they have no conflicts of interest.

Code availability: The datasets generated during and analysed during the current study are available in this manuscript.

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-Jedidiparticipated 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.

Ethics approval: Not applicable.

Consent to participate: All the authors consented to participate in this study. All authors read and approved the final manuscript.

Consent for publication: All the authors consent to publication of this article.

References

1. Tayh G, Sallem RB, Yahia HB, Gharsa H, Klibi N, Boudabous A, Slama KB (2016) First report of extended-spectrum β-lactamases among clinical isolates of Escherichia coli in Gaza Strip, Palestine. Journal of global antimicrobial resistance 6:17-21. doi:https://doi.org/10.1016/j.jgar.2016.01.013
2. Johnson JR, Russo TA (2002) Extraintestinal pathogenic Escherichia coli: "the other bad E coli". J Lab Clin Med 139:155-162. doi:https://doi.org/10.1067/mlc.2002.121550
3. Hashish EA, El Damaty HM, Tartor YH, Abdelaal AM (2016) Epidemiological Study of Diarrheagenic Escherichia coli Virulence Genes in Newborn Calves. Pak Vet J 36.
4. Karmali MA (1989) Infection by verocytotoxin-producing Escherichia coli. Clin Microbiol Rev 2:15-38. doi:https://doi.org/10.1128/cmr.2.1.15
5. Persad AK, Lejeune JT (2015) Animal reservoirs of Shiga toxin-producing Escherichia coli. In: Enterohemorrhagic Escherichia coli and Other Shiga Toxin-Producing E. coli. American Society of Microbiology (ASM), pp 231-244. doi: https://doi.org/10.1128/9781555818791.ch11
6. Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, Hebert RJ, Olcott ES, Johnson LM, Hargrett NT (1983) Hemorrhagic colitis associated with a rare Escherichia coli serotype. N Engl J Med 308:681-685. doi:https://doi.org/10.1056/NEJM198303243081203
7. Atnafie B, Paulos D, Abera M, Tefera G, Hailu D, Kasaye S, Amenu K (2017) Occurrence of Escherichia coli O157: H7 in cattle feces and contamination of carcass and various contact surfaces in abattoir and butcher
shops of Hawassa, Ethiopia. BMC Microbiol 17:24. doi:https://doi.org/10.1186/s12866-017-0938-1

8. García-Aljaro C, Muniesa M, Jofre J, Blanch AR (2004) Prevalence of the stx2 gene in coliform populations from aquatic environments. Appl Environ Microbiol 70:3535-3540. doi:https://doi.org/10.1128/AEM.70.6.3535-3540.2004

9. Al-Gallas N, Bahri O, Aissa RB (2006) Prevalence of shiga toxin-producing *Escherichia coli* in a diarrheagenic Tunisian population, and the report of isolating STEC O157: H7 in Tunis. Curr Microbiol 53:483-490. doi:https://doi.org/10.1007/s00284-006-0184-5

10. Chahed A, China B, Mainil J, Daube G (2006) Prevalence of enterohaemorrhagic *Escherichia coli* from serotype O157 and other attaching and effacing *Escherichia coli* on bovine carcasses in Algeria. J Appl Microbiol 101:361-368. doi:https://doi.org/10.1111/j.1365-2672.2006.02954.x

11. Beneduce L, Spano G, Nabi AQ, Lamacchia F, Massa S, Aouni R, Hamama A (2008) Occurrence and characterization of *Escherichia coli* O157 and other serotypes in raw meat products in Morocco. J Food Prot 71:2082-2086. doi:https://doi.org/10.4315/0362-028x-71.10.2082

12. Benkerroum N, Bouhlal Y, ATTAR AE, Marhaben A (2004) Occurrence of shiga toxin–producing *Escherichia coli* O157 in selected dairy and meat products marketed in the city of Rabat, Morocco. J Food Prot 67:1234-1237. doi:https://doi.org/10.4315/0362-028x-67.6.1234.

13. Abdul-Raouf U, Ammar M, Beuchat L (1996) Isolation of *Escherichia coli* O157: H7 from some Egyptian foods. Int J Food Microbiol 29:423-426. doi:https://doi.org/10.1016/0168-1605(95)00076-3

14. Lupindu AM (2018) Epidemiology of Shiga toxin-producing *Escherichia coli* O157: H7 in Africa in review. S Afr J Infect Dis 33:24-30. doi:https://doi.org/10.1080/23120053.2017.1376558

15. Gannon V, D’souza S, Graham T, King R, Rahn K, Read S (1997) Use of the agellar H7 gene as a target in multiplex PCR assays and improved specificity in identification of enterohemorrhagic *Escherichia coli* strains. J Clin Microbiol 35:656-662. doi:https://doi.org/10.1128/JCM.35.3.656-662.1997

16. Al-Ajmi D, Rahman S, Banu S (2020) Occurrence, virulence genes, and antimicrobial profiles of *Escherichia coli* O157 isolated from ruminants slaughtered in Al Ain, United Arab Emirates. BMC Microbiol 20:1-10. doi:https://doi.org/10.1186/s12866-020-01899-0

17. Clermont O, Christenson JK, Denamur E, Gordon DM (2013) The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep 5:58-65. doi:https://doi.org/10.1111/1758-2229.12019

18. Sjöling Å, Sadeghipoorjahromi L, Novak D, Tobias J (2015) Detection of major diarrheagenic bacterial pathogens by multiplex PCR panels. Microbiol Res 172:34-40. doi:https://doi.org/10.1016/j.micres.2014.12.003

19. Grispoldi L, Bertero F, Franceschini S, Mastrosimone F, Sechi P, Iulietto MF, Ceccarelli M, Cenci-Goga BT (2017) Prevalence and characterisation of shigatoxigenic *Escherichia coli* isolated from beef cattle fed with prebiotics. Ital J Food Saf 6. doi:https://doi.org/10.4081/ijfs.2017.6958

20. Ranjbar R, Masoudimanesh M, Dehkordi FS, Jonaidi-Jafari N, Rahimi E (2017) Shiga (Vero)-toxin producing *Escherichia coli* isolated from the hospital foods; virulence factors, o-serogroups and antimicrobial resistance properties. Antimicrob Resist Infect Control 6:4. doi:https://doi.org/10.1186/s13756-016-0163-y

21. Mohamed HS (2018) Detection of Virulence Factors of *Escherichia coli* Strains Isolated from Children with Diarrhea. EC Microbiology (ECMI) 14:475-486.
22. Heininger A, Binder M, Schmidt S, Unertl K, Botzenhart K, Döring G (1999) PCR and blood culture for
detection of *Escherichia coli* bacteremia in rats. J Clin Microbiol 37:2479-2482.
doi:https://doi.org/10.1128/JCM.37.8.2479-2482.1999.

23. Zhang W, Qi W, Albert TJ, Motiwala AS, Alland D, Hyytia-Trees EK, Ribot EM, Fields PI, Whittam TS,
Swaminathan B (2006) Probing genomic diversity and evolution of *Escherichia coli* O157 by single
nucleotide polymorphisms. Genome Res 16:757-767. doi:https://doi.org/10.1101/gr.4759706

24. Islam MZ, Musekiwa A, Islam K, Ahmed S, Chowdhury S, Ahad A, Biswas PK (2014) Regional variation in the
prevalence of *E. coli* O157 in cattle: a meta-analysis and meta-regression. PLoS One 9:e93299.
doi:https://doi.org/10.1371/journal.pone.0093299

25. Mead PS, Griffin PM (1998) *Escherichia coli* O157: H7. The Lancet 352:1207-1212.
doi:https://doi.org/10.1016/S0140-6736(98)01267-7

26. Founou LL, Founou RC, Essack SY (2016) Antibiotic resistance in the food chain: a developing country-
perspective. Front Microbiol 7:1881. doi:https://doi.org/10.3389/fmicb.2016.01881

27. Al-Wabel NA (2007) ANTIBIOTIC SUSCEPTIBILITY OF *E. COLI* O157: H7 ISOLATED FROM BEEFBURGER. B
PHARM SCI 30:131-134. doi:https://doi.org/10.21608/BFSA.2007.64179

28. Rahimi E, Nayebpour F (2012) Antimicrobial resistance of *Escherichia coli* O 157: H7/NM isolated from
feaces of ruminant animals in Iran. J Cell Anim Biol 6:104-108. doi:https://doi.org/10.5897/JCAB11.082

29. Day M, Doumith M, Jenkins C, Dallman TJ, Hopkins KL, Elson R, Godbole G, Woodford N (2016)
Antimicrobial resistance in Shiga toxin-producing *Escherichia coli* serogroups O157 and O26 isolated from
human cases of diarrhoeal disease in England, 2015. J Antimicrob Chemother 72:145-152.
doi:https://doi.org/10.1093/jac/dkwa371

30. Bastos FC, Vaz TMI, Irino K, Guth BEC (2006) Phenotypic characteristics, virulence profile and genetic
relatedness of O157 Shiga toxin-producing *Escherichia coli* isolated in Brazil and other Latin American
countries. FEMS Microbiol Lett 265:89-97. doi:https://doi.org/10.1111/j.1574-6968.2006.00472.x

31. Reuben R, Owuna G (2013) Antimicrobial resistance patterns of *Escherichia coli* O157: H7 from Nigerian
fermented milk samples in Nasarawa State, Nigeria. Int J Pharm Sci Invent 2:38-44.

32. Kawano K, Okada M, Haga T, Maeda K, Goto Y (2008) Relationship between pathogenicity for humans and
stx genotype in Shiga toxin-producing *Escherichia coli* serotype O157. Eur J Clin Microbiol Infect Dis 27:227-
232. doi:https://doi.org/10.1007/s10096-007-0420-3

33. Cornick NA, Booher SL, Moon HW (2002) Intimin facilitates colonization by *Escherichia coli* O157: H7 in
adult ruminants. Infect Immun 70:2704-2707. doi:https://doi.org/10.1128/iai.70.5.2704-2707.2002

34. Schwidder M, Heinisch L, Schmidt H (2019) Genetics, toxicity, and distribution of enterohemorrhagic
*Escherichia coli* hemolysin. Toxins 11:502. doi:https://doi.org/10.3390/toxins11090502

35. Friedrich AW, Bielaszewska M, Zhang W-L, Pulz M, Kuczis T, Ammon A, Karch H (2002) *Escherichia coli*
harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. J Infect Dis
185:74-84. doi:https://doi.org/10.1086/338115

36. Beutin L, Krause G, Zimmermann S, Kaulfuss S, Gleier K (2004) Characterization of Shiga toxin-producing
*Escherichia coli* strains isolated from human patients in Germany over a 3-year period. J Clin Microbiol
42:1099-1108. doi:https://doi.org/10.1128/JCM.42.3.1099-1108.2004.
37. Pereira RVV, Santos T, Bicalho M, Caixeta L, Machado V, Bicalho R (2011) Antimicrobial resistance and prevalence of virulence factor genes in fecal Escherichia coli of Holstein calves fed milk with and without antimicrobials. Int J Dairy Sci 94:4556-4565. doi:https://doi.org/10.3168/jds.2011-4337

38. Momtaz H, Farzan R, Rahimi E, Safarpoor Dehkordi F, Souod N (2012) Molecular characterization of Shiga toxin-producing Escherichia coli isolated from ruminant and donkey raw milk samples and traditional dairy products in Iran. Sci World J 2012. doi:https://doi.org/10.1100/2012/231342

39. Tenaillon O, Skurnik D, Picard B, Denamur E (2010) The population genetics of commensal Escherichia coli. Nat Rev Microbiol 8:207-217. doi:https://doi.org/10.1100/2012/231342.

40. Morcatti Coura F, Diniz SdA, Silva MX, Mussi JMS, Barbosa SM, Lage AP, Heinemann MB (2015) Phylogenetic group determination of Escherichia coli isolated from animals samples. Sci World J 2015. doi:https://doi.org/10.1155/2015/258424

**Figures**

**Figure 1**

Antimicrobial susceptibility of E. coli O157:H7 isolates.