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

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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 *fliCH7* genes specific for serotype O157. All isolates were examined for the susceptibility against 21antibiotics 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 comfirmed that 3.4% of *E. coli* isolates among human stool samples were STECand 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, Mornag 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 ofE. 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 probablly *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 ofE. 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 arelisted 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 piperacilline (30), cefalotine (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), streptomycine 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 *chu*A, *yja*A 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).

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)
	yjaA	yjaA.1b: CAAACGTGAAGTGTCAGGAG yjaA.2b: AATGCGTTCCTCAACCTGTG	211	60	(Clermont et al. 2013)
	TspE4C2	TspE4C2.1b: CACTATTCGTAAGGTCATCC TspE4C2.2b: AGTTTATCGCTGCGGGTCGC	152	60	(Clermont et al. 2013)
	arpA	AceK.f: AACGCTATTCGCCAGCTTGC AceK.r: TCTCCCCATACCGTACGCTA	400	60	(Clermont et al. 2013)
Group E	*arpA*	ArpAgpE.f: GATTCCATCTTGTCAAAATATGCC ArpAgpE.r: GAAAAGAAAAAGAATTCCCAAGAG	301	57	(Clermont et al. 2013)
Group C	*trpA*	trpAgpC.1: AGTTTTATGCCCAGTGCGAG trpAgpC.2: TCTGCGCCGGTCACGCCC	219	59	(Clermont et al. 2013)
Internal control	*trpA*	trpBA.f: CGGCGATAAAGACATCTTCAC trpBA.r: GCAACGCGGCCTGGCGGAAG	489	57	(Clermont et al. 2013)
Virulence factors					
Shiga toxin	*stx1*	F: CAGTTAATGTGGTGGCGAAGG R: CACCAGACAATGTAACCGCTG	348 bp	56	(Sjöling et al. 2015)
Shiga toxin	*stx2*	F: ATCCTATTCCCGGGAGTTTACG R: GCGTCATCGTATACACAGGAGC	584 bp	56	(Sjöling et al. 2015)

PCR reaction	Gene	Primer sequence (5'-3')	Size of PCR product (bp)	Annealing temperature (°C)	Reference
Enterohaemolysin	*ehxA*	F: GCATCATCAAGCGTACGTTCC R: AATGAGCCAAGCTGGTTAAGCT	534 bp	56	(Grispoldi et al. 2017)
Enteropathogenic attachment and effacement	*eae*	F : TGCGGCACAACAGGCGGCGA R : CGGTCGCCGCACCAGGATTC	629 pb	56	(Ranjbar et al. 2017)
Others					
Part of O-antigen 157	*O157*	F: CGGACATCCATGTGATATGG R: TTGCCTATGTACAGCTAATCC	259 bp	52	(Mohamed 2018)
Encoding H7 flagellar antigens	*fliCH7*	F: GCGCTGTCGAGTTCTATCGAGC R : CAACGGTGACTTTATCGCCATTCC	625 bp	60	(Mohamed 2018)
Beta-glucuronidase	*uidA*	F: ATCACCGTGGTGACGCATGTCGC R : CACCACGATGCCATGTTCATCTGC	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 healthly 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 ampicllin, cefoxitin, ticarcillin/clavulanic acid, ceftazidime, ertapenem, nalidixic acid, florfenicol, chloramphenicol and enrofloxacin. Howevere, 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, wherase, *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
Distribution of virulence genes and specific genes detected by PCR

Bacterial code	Specific genes			STEC virulence markers				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*

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 demonstraded 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 (Atnafie 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 occurence of *E. coli* O157 inmore 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 demonstraed 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% representive in four studies. In Asian countries, the highest rates was 12.22% in Jordian 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 fourty 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 orgininate 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 (Atnafie 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 exihibed 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 *stx*2 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, wherease 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

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Conflicts of 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.

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Figures

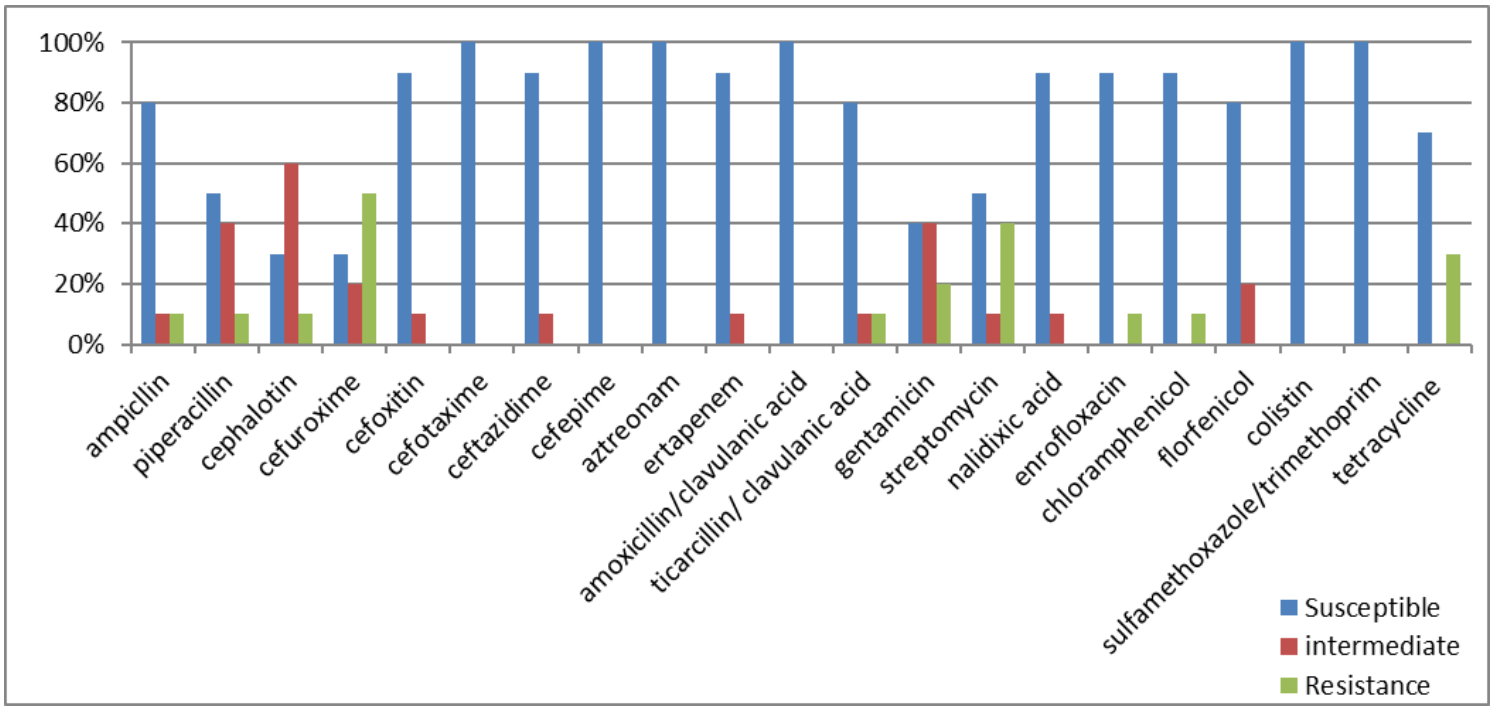

Figure 1

Antimicrobial susceptibility of E. coli O157:H7 isolates.