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Molecular analysis of multidrug resistance in Shiga toxin-producing *Escherichia coli* O157:H7 isolated from meat and dairy products

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ABSTRACT

Shiga toxin-producing *Escherichia coli* (STEC) O157:H7 is an important food-borne pathogen that has been implicated in numerous disease outbreaks worldwide. Little is known about the extent and molecular basis of antimicrobial resistance in STEC O157:H7 of food origin. Therefore, the current study aimed to characterize the genetic basis of multidrug resistance in 54 STEC O157:H7 strains isolated from 1600 food samples (800 meat products and 800 dairy products) collected from different street venders, butchers, retail markets, and slaughterhouses in Egypt. Thirty-one of 54 (57.4%) isolates showed multidrug resistance phenotypes to at least three classes of antimicrobials. The highest incidence of antimicrobial resistance was to kanamycin (96.8%), followed by spectinomycin (93.6%), ampicillin (90.3%), streptomycin (87.1%), and tetracycline (80.6%). PCR and DNA sequencing were used to screen and characterize integrons and antibiotic resistance genes, and 29.6% and 5.6% of isolates were positive for class 1 and class 2 integrons, respectively. β-Lactamase-encoding genes were identified in 63.0% of isolates as follows: bla_{TEM-1} and bla_{TEM-52} in 35.2% and 1.9% isolates respectively; bla_{CMY-2} in 13.0% isolates; bla_{CTX-M} in 5.6% isolates; bla_{SHV-12} in 5.6% isolates; and bla_{OXA-1} in 1.9% isolate. The plasmid-mediated quinolone resistance genes were identified in 13.0% of isolates as follows: *qnrB*, *qnrS*, and *aac(6′)-Ib-cr* in 5.6%, 3.7%, and 3.7% isolates, respectively. Finally, the florfenicol resistance gene *floR* was identified in 7.4% of isolates. This study demonstrated that meat and dairy products are potential sources of multidrug resistant STEC O157:H7. To our knowledge, this is the first report of the occurrence of class 2 integrons, *qnrB*, *qnrS*, and *aac(6′)-Ib-cr* in STEC O157:H7.

1. Introduction

Shiga toxin-producing *Escherichia coli* (STEC) is a group of *E. coli* that is defined by the ability to produce toxins called Shiga toxins (Stx) (Farrokh et al., 2013). Among the different STEC serogroups, O157:H7 is most frequently associated with food-borne outbreaks in North America, Japan, and parts of Europe (Farrokh et al., 2013). STEC O157:H7 causes food-borne illness, with symptoms ranging from mild diarrhea to life-threatening hemolytic-uremic syndrome (Karch et al., 2005). Foods associated with outbreaks of STEC include undercooked ground beef, fresh produce, unpasteurized juices, salami, cheese and raw (unpasteurized) milk (FDA, 2012).

Antimicrobial resistance is a global public health problem, and growing scientific evidence indicates that it is negatively impacted by both human and animal antimicrobial usages (Guardabassi et al., 2008). Therapeutic failures due to antimicrobial resistance increase morbidity and mortality rates, with serious impact at individual, social and economical levels. Furthermore, antimicrobial resistance limits the selection of therapeutic agents and increases the potential for treatment failures and adverse clinical complications (da Costa et al., 2013). Retail foods, especially meat and meat products, may be an important vehicle for community-wide dissemination of antimicrobial resistant *E. coli* and extraintestinal pathogenic *E. coli* (Johnson et al., 2005).

Although antimicrobial therapy is not the primary tool for treating infections caused by STEC O157:H7, multidrug-resistant (MDR) STEC O157:H7 is a public health issue as those strains participate to a reservoir of resistance genes that could be easily exchanged between Enterobacteriaceae in the host and in the environment. Many bacteria in the human gut that possess several antimicrobial resistance genes could be laterally transferred in the gut to potentially pathogenic bacteria (Rolain, 2013). Several studies have been conducted worldwide to characterize the molecular basis of antimicrobial resistance in clinical STEC O157:H7 isolates of human origin (Ahmed et al., 2005; Cergole-Novella et al., 2011; Morabito et al., 2002; Torpdahl et al., 2014; Van Meervenne et al., 2013), but little is currently known about the molecular basis of multidrug resistance in STEC O157:H7 isolates of food origin (Zhao et al., 2001). Therefore, the purpose of this study was to characterize MDR STEC O157:H7 strains isolated from retail meat and dairy products collected in a large-scale survey in Egypt by molecular screening for a wide range of antimicrobial resistance genes and integrons.

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2. Materials and methods

2.1. Bacterial isolates

Fifty-four STEC O157:H7 isolates (21 from beef, 4 from chicken, 20 from milk and 9 from cheese) were used in this study. All isolates were positive for *stx1* and/or *stx2* Shiga toxin virulence genes. They were isolated in Egypt from 800 meat products (480 beef and 320 chickens) and 800 dairy products (480 milk, 240 cheeses and 80 yogurts) as previously described (Ahmed and Shimamoto, 2014).

2.2. Antimicrobial susceptibility testing

The antimicrobial sensitivity phenotypes of bacterial isolates were determined using a Kirby-Bauer disk diffusion assay according to the standards and interpretive criteria described by Clinical and Laboratory Standards Institute (CLSI, 2011). The following antibiotics were used: ampicillin (AMP), 10 μg; amoxicillin-clavulanic acid (AMC), 20/10 μg; cefoxitin (FOX), 30 μg; cefotetan (CTT), 30 μg; cefotaxime (CTX), 30 μg; cefpodoxime (CPD), 10 μg; ceftriaxone (CRO), 30 μg; aztreonam (ATM), 30 μg; nalidixic acid (NAL), 30 μg; ciprofloxacin (CIP), 5 μg; chloramphenicol (CHL), 30 μg; gentamicin (GEN), 10 μg; kanamycin (KAN), 30 μg; oxacillin (OXA), 30 μg; streptomycin (STR), 10 μg; spectinomycin (SPX), 10 μg; sulfamethoxazole/trimethoprim (SXT), 23.75/1.25 μg, and tetracycline (TET), 30 μg. The disks were purchased from Oxoid (Basingstoke, UK) and the results were recorded based on CLSI guidelines (CLSI, 2011). The reference strain *E. coli* ATCC 25922 was included as a quality control.

2.3. Bacterial DNA preparation

DNA was prepared using boiled lysates, as previously described (Ahmed et al., 2013). All MDR STEC O157:H7 isolates (resistance to at least three classes of antimicrobials) were subcultured in Luria-Bertani broth medium. An overnight bacterial culture (200 μl) was mixed with 800 μl of distilled water and boiled for 10 min. The resulting solution was centrifuged, and the supernatant used as a DNA template. DNA was stored at −20 °C until used.

2.4. PCR screening for integrons and antimicrobial resistance genes

Conserved primers were used to detect and identify class 1 and class 2 integrons, as previously described (Ahmed et al., 2013). PCR screening for TEM, SHV, CTX-M, OXA, and CMY β-lactamase-encoding genes was performed using universal primers for the TEM, SHV, OXA, CTX-M, and CMY families (Ahmed et al., 2013). Other universal flanking gene primers were used for identification of the whole β-lactamase-encoding genes (except for TEM as the universal primers used for TEM family are already located in the flanking regions of the gene) as described previously (Ahmed et al., 2007). Furthermore, PCR amplification was used to screen for plasmid-mediated quinolone resistance genes, *qnrA*, *qnrB*, *qnrS*, and *aac(6′)-Ib-cr*, as described previously (Ahmed et al., 2013). Finally, the florfenicol resistance gene, *floR*, was detected using primers

Table 1
Primers used for PCR and DNA sequencing.

Primer	Sequence (5′ to 3′)	Target	Reference/GenBank accession no.
Integron/resistance genes			
Integrons			
5′-CS	GGCATCCAAGCAGCAAG	Class 1 integron	Ahmed et al. (2013)
3′-CS	AAGCAGACTTGACCTGA		
hep74	CGGGATCCCGGACGGCATGCACGATTTGTA	Class 2 integron	Ahmed et al. (2013)
hep51	GATGCCATCGCAAGTACGAG		
β-Lactamases			
TEM-F	ATAAAATTCTTGAAGACGAAA	bla_{TEM}	Ahmed et al. (2013)
TEM-R	GACAGTTACCAATGCTTAATC		
SHV-F	TT ATCTCCCTGTTAGCCACC	bla_{SHV}	Ahmed et al. (2013)
SHV-R	GATTTGCTGATTTCGCTCGG		
SHV-F-2	CGGCCTTCACTCAAGGATGTA	whole bla_{SHV}	Ahmed et al. (2007)
SHV-R-2	GTGCTGCGGGCCGGATAAC		
OXA-F	TCAACTTTCAAGATCGCA	bla_{OXA}	Ahmed et al. (2013)
OXA-R	GTGTGTTTAGAATGGTGA		
OXA-F-2	ATTAAGCCCTTTACCAAACCA	whole bla_{OXA}	J02967
OXA-R-2	AAGGGTTGGGCGATTTTGCCA		
CTX-M-F	CGCTTTGCGATGTGCAG	bla_{CTX-M}	Ahmed et al. (2013)
CTX-M-R	ACCGCGATATCGTTGGT		
CTX-M-F-2	CCAGAATAAGGAATCCCATG	whole bla_{CTX-M}	Ahmed et al. (2007)
CTX-M-R-2	GCCGTCTAAGGCGATAAAC		
CMY-F	GACAGCCTCTTTCTCCACA	bla_{CMY}	Ahmed et al. (2013)
CMY-R	TGGAACGAAGGCTACGTA		
CMY-F2	ACGGAACTGATTTCATGATG	whole bla_{CMY}	Ahmed et al. (2007)
CMY-R2	GAAAGGAGGCCCAATATCCT		
Florfenicol			
StCM-L	CACGTTGAGCCTCTATATGG	*floR*	Ahmed et al. (2013)
StCM-R	ATGCAGAAGTAGAACGCGAC		
Plasmid-mediated quiniolone			
qnrA-F	ATTTCTCACGCCAGGATTTG	*qnrA*	Ahmed et al. (2013)
qnrA-R	GATCGGCAAAGGTTAGGTCA		
qnrB-F	GATCGTGAAAGCCAGAAAGG ACGATGCCTGGTAGTTGTCC	*qnrB*	Ahmed et al. (2013)
qnrB-R			
qnrS-F	ACGACATTCGTCAACTGCAA TAAATTGGCACCCTGTAGGC	*qnrS*	Ahmed et al. (2013)
qnrS-R			
aac(6′)-Ib-F	TTGCGATGCTCTATGAGTGGCTA	*aac(6′)-Ib-cr*	Ahmed et al. (2013)
aac(6′)-Ib-R	CTCGAATGCCTGGCGTGTTT		

Table 2
Incidence of integrons and resistance genes in STEC *E. coli* O157:H7 isolated from meat and dairy products.

	Meat products (n = 25) (100%)					Dairy products (n = 29) (100%)					Total (n = 54) (100%)				
	Integrons		Antimicrobial resistance genes			Integrons		Antimicrobial resistance genes			Integrons		Antimicrobial resistance genes		
	Class 1	Class 2	β-Lactamases	Plasmid-mediated quinolone	*floR*	Class 1	Class 2	β-Lactamases	Plasmid-mediated quinolone	*floR*	Class 1	Class 2	β-Lactamases	Plasmid-mediated quinolone	*floR*
E. coli O157:H7	7 (28.0)	1 (4.0)	15 (60.0)	3 (12.0)	2 (8.0)	9 (31.0)	2 (6.9)	19 (65.5)	4 (13.8)	2 (6.9)	16 (29.6)	3 (5.6)	34 (63.0)	5 (9.3)	4 (7.40)

StCM-L and StCM-R, as described previously (Ahmed et al., 2013). The PCR products were subjected to electrophoresis in a 1.0% agarose gel, stained with ethidium bromide, and visualized under UV light. PCR fragments were then purified from the agarose gel using a QIAquick Gel Extraction Kit (Qiagen, Japan). Both DNA strands of the PCR product were sequenced using an ABI automatic DNA sequencer (Model 373; Perkin-Elmer). Of note, all above mentioned experiments were carried out on only MDR STEC O157:H7 isolates and primers are compiled in Table 1.

2.5. Computer analysis of the sequence data

A similarity search was carried out using the BLAST program, available at the NCBI BLAST homepage (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

3. Results

3.1. Incidence of MDR STEC O157:H7 in meat and dairy products

Thirty-one out of 54 (57.4%) STEC O157:H7 isolates showed multidrug resistance phenotypes (i.e. resistance to at least three classes of antimicrobials). The incidence of MDR STEC O157:H7 was 56.0% in meat products and 58.6% in dairy products. The most widespread resistance in MDR STEC O157:H7 was to kanamycin (96.8% of isolates), followed by spectinomycin (93.6%), ampicillin (90.3%), streptomycin (87.1%), and tetracycline (80.6%).

3.2. Incidence of class 1 and class 2 integrons in STEC O157:H7 from meat and dairy products

PCR identified class 1 integrons in 16 (29.6%) STEC O157:H7 isolates (Table 2). The incidence of class 1 integrons was 28.0% in meat products and 31.0% in dairy products (Table 2). DNA sequencing results of the inserted gene cassettes identified six types of class 1 integron, with eight different antimicrobial resistance gene cassettes (Table 3). The identified antimicrobial resistance genes were: dihydrofolate reductase types (*dfrA1* and *dfrA17*), which confer resistance to trimethoprim; aminoglycoside adenyltransferase types (*aadA1*, *aadA2*, *aadA5*, *aadA7*, and *aadA23*), which confer resistance to streptomycin and spectinomycin; and aminoglycoside acetyltransferase (*aac(3)-Id*), which confers resistance to gentamicin, sisomicin, and fortimicin (Table 3). In contrast, PCR identified only three (5.6%) STEC O157:H7 isolates containing class 2 integrons (Table 2). The incidence of class 2 integrons was 4.0% in meat products and 6.9% in dairy product (Table 2). DNA sequencing results for the inserted gene cassettes identified two types of class 2 integron (Table 3): the classical type, containing the three conserved resistance gene cassettes of class 2 integrons, *dfrA1*, *sat2*, and *aadA1*, which confer resistance to trimethoprim, streptothricin, and streptomycin/spectinomycin, respectively; and the short type of class 2 integron, containing only two gene cassettes, *dfrA1* and *sat2* (Table 3).

3.3. Incidence of β-lactamase-encoding genes in STEC O157:H7 from meat and dairy products

PCR identified β-lactamase-encoding genes in 34 (63.0%) STEC O157:H7 isolates (Table 2). The incidence of β-lactamase-encoding genes was 60.0% in meat products and 65.5% in dairy products (Table 2). DNA sequencing identified the following β-lactamase-encoding genes: narrow spectrum β-lactamase-encoding genes, $bla_{\text{TEM-1}}$ and $bla_{\text{OXA-1}}$ in 19 (35.2%) isolates and one (1.9%) isolate respectively; the extended-spectrum β-lactamase-encoding genes, $bla_{\text{CTX-M}}$ in three (5.6%) isolates (two $bla_{\text{CTX-M-3}}$ and one $bla_{\text{CTX-M-15}}$), $bla_{\text{SHV-12}}$ in three (5.6%) isolates, and $bla_{\text{TEM-52}}$ in one (1.9%) isolate; and AmpC β-lactamase-encoding gene, $bla_{\text{CMY-2}}$, in seven (13.0%) isolates (Table 2).

3.4. Incidence of plasmid-mediated quinolone resistance genes in STEC O157:H7 from meat and dairy products

Multiplex PCR screening identified plasmid-mediated quinolone resistance genes in seven (13.0%) STEC O157:H7 isolates (Table 2). The incidence of plasmid-mediated quinolone resistance genes was 12.0% in meat products and 13.8% in dairy products (Table 2). DNA sequencing identified the plasmid-mediated quinolone resistance genes *qnrB*, *qnrS*, and *aac(6′)-Ib-cr* in three (5.6%), two (3.7%), and two (3.7%) isolates, respectively (Table 3).

3.5. Incidence of the florfenicol resistance gene, floR, in STEC O157:H7 from meat and dairy products

PCR and DNA sequence analysis identified *floR* in four (7.4%) STEC O157:H7 isolates (two isolates from meat products (8.0%) and two isolates (6.9%) from dairy products) (Table 2).

4. Discussion

Integrons are gene-capture systems that play a fundamental role in dissemination of antimicrobial resistance genes, especially in Gram-negative bacteria (Rowe-Magnus and Mazel, 2002). In this study, class 1 and class 2 integrons with different antibiotic resistance gene cassettes were detected in STEC O157:H7 isolates from meat products. Our results showed a higher incidence of integrons than was previously reported in Japan, where 11.6% of the *E. coli* isolates recovered from retail chicken meat were positive for class 1 integrons, and 1.4% were positive for class 2 integrons (Ahmed et al., 2009). In the USA, Zhao et al. found that 18% of STEC (including O157:H7) isolated from cattle were positive for class 1 integrons (Zhao et al., 2001). In Norway, class 2 integrons were identified in 9.4% of *E. coli* isolated from meat and meat products (Sunde, 2005). Class 1 and class 2 integrons were also detected in STEC O157:H7 isolates, from dairy products in this study. In Egypt, class 1 and class 2 integrons have been reported in *E. coli* isolated from milk samples collected from bovine mastitis cases with 2.7% and 0.9%, respectively (Ahmed and Shimamoto, 2011). In USA, class 1 integrons were found in eight of 10 *E. coli* isolates (including one STEC O157:H7 from dairy farms and seven non-Stx-producing *E. coli* from dairy/bovine mastitis) (Murinda et al., 2005).

Production of β-lactamases is considered the main mechanism of resistance against penicillin-derivative antibiotics (β-lactams) in Gram-negative bacteria (Bradford, 2001). Extended-spectrum cephalosporins (ESCs) are an important class of drugs used in human and veterinary medicine. Resistance to ESCs in STEC O157:H7 is usually mediated by the production of AmpC β-lactamases, commonly encoded by bla_{CMY-2} genes (Folster et al., 2014). In this study, various types of narrow- and extended-spectrum β-lactamase-encoding genes were identified (including bla_{TEM-1}, bla_{OXA-1}, $bla_{CTX-M-3}$, $bla_{CTX-M-15}$, bla_{SHV-12}, and bla_{TEM-52}), in addition to AmpC β-lactamase-encoding gene, bla_{CMY-2}. Our results are similar to those of a previous report from Canada, in which bla_{TEM}, bla_{CMY}, and bla_{SHV} were found in 56%, 12%, and 4%, respectively, of ampicillin-resistant *E. coli* isolates recovered from a commercial beef processing plant (Aslam et al., 2009). However, the incidence of β-lactamase genes in isolates from our study is higher than in a recent report from Iran, in which 14.7% and 2.9% of STEC strains isolated from the external surfaces of chicken carcasses carried the bla_{TEM} and bla_{SHV} genes, respectively (Bagheri et al., 2014). In Japan, bla_{TEM-1} and bla_{CMY-2} were identified in 17.3% and 23.2% of *E. coli* isolates, respectively, from retail chicken meat (Ahmed et al., 2009). The presence of bla_{CMY-2} in meat products has great public health significance, as bla_{CMY-2} identified

Table 3
Resistance phenotype and incidence of integrons and resistance genes in STEC *E. coli* O157:H7 isolated from meat and dairy products.

No.	Isolate	Food product	Resistance phenotype	Integrons/resistance genes
1	O157-M1	Beef	AMC, AMP, ATM, CHL, CIP, CPD, CRO, CTT, CTX, FOX, GEN, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 1 (*dfrA1-aadA1*), bla_{TEM-1}, *qnrB*, bla_{CMY-2}, $bla_{CTX-M-3}$, *floR*
2	O157-M2	Chicken	AMC, AMP, ATM, CHL, CIP, CPD, CRO, CTT, CTX, FOX, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 1 (*dfrA1-aadA1*), bla_{TEM-1}, $bla_{CTX-M-15}$
3	O157-M3	Beef	AMP, ATM, CHL, CPD, CRO, CTT, CTX, FOX, GEN, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 1 (*dfrA1-aadA1*), bla_{CMY-2}
4	O157-M4	Beef	AMC, AMP, ATM, CHL, CIP, CPD, CRO, CTT, CTX, GEN, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 1 (*dfrA12-orf-aadA2*), bla_{TEM-1}, bla_{SHV-12}
5	O157-M5	Beef	AMP, CHL, CIP, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 1 (*dfrA12-orf-aadA2*), *qnrS*
6	O157-M6	Beef	AMP, ATM, CHL, CTT, FOX, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 1 (*dfrA17-aadA5*), bla_{TEM-1}
7	O157-M7	Beef	AMP, CHL, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 1 (*aadA1*), bla_{TEM-1}
8	O157-M8	Beef	AMP, CHL, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 2 (*dfrA1-sat2-aadA1*), bla_{TEM-1}
9	O157-M9	Beef	AMP, ATM, CHL, CTT, CTX, FOX, KAN, NAL, OXA, SPX, STR, SXT	bla_{CMY-2}
10	O157-M10	Beef	AMP, CHL, FOX, KAN, SPX, STR, SXT, TET	bla_{TEM-1}
11	O157-M11	Beef	AMP, KAN, NAL, SPX, STR, SXT	bla_{TEM-1}
12	O157-M12	Beef	AMP, KAN, SPX, STR	bla_{TEM-1}
13	O157-M13	Beef	GEN, KAN, SPX, TET	*floR*
14	O157-M14	Chicken	CIP, GEN, KAN, NAL, TET	*qnrB*
15	O157-D1	Milk	AMC, AMP, ATM, CHL, CTT, CIP, CPD, CRO, CTX, FOX, GEN, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 1 (*dfrA1-aadA1*), bla_{TEM-1}, *qnrB*, *floR* bla_{CMY-2}, $bla_{CTX-M-3}$
16	O157-D2	Cheese	AMC, AMP, ATM, CHL, CIP, CTT, CPD, CRO, CTX, FOX, GEN, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 1 (*dfrA1-aadA1*), bla_{TEM-1}, *qnrB*, $bla_{CTX-M-15}$, *aac(6′)-Ib-cr*
17	O157-D3	Milk	AMC, AMP, ATM, CHL, CIP, CTT, CPD, CRO, CTX, FOX, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 1 (*dfrA12-orf-aadA2*), bla_{TEM-1}, bla_{SHV-12}
18	O157-D4	Milk	AMP, CHL, CIP, GEN, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 1 (*dfrA12-orf-aadA2*), *aac(6′)-Ib-cr*, *floR*
19	O157-D5	Milk	AMC, AMP, ATM, CHL, CTT, CPD, CRO, CTX, FOX, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 1 (*dfrA17-aadA5*), bla_{TEM-1}, bla_{CMY-2}
20	O157-D6	Milk	AMP, CHL, CIP, CTX, GEN, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 1 (*aac(3)-Id-aadA7*), bla_{TEM-1}
21	O157-D7	Cheese	AMC, AMP, ATM, CHL, CPD, CRO, CTT, CTX, FOX, GEN, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 1 (*aadA23*), bla_{TEM-52}
22	O157-D8	Milk	AMC, AMP, ATM, CHL, CTT, FOX, GEN, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 1 (*aadA1*), bla_{CMY-2}
23	O157-D9	Milk	AMC, AMP, ATM, CHL, CTT, CPD, CRO, CTX, FOX, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 1 (*dfrA1-orf*), bla_{SHV-12}
24	O157-D10	Milk	AMP, CHL, CTT, FOX, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 2 (*dfrA1-sat2-aadA1*), bla_{TEM-1}
25	O157-D11	Milk	AMP, CHL, KAN, NAL, OXA, SPX, STR, SXT, TET	Class 2 (*dfrA1-sat2*), bla_{TEM-1}
26	O157-D12	Milk	AMP, CHL, KAN, NAL, SPX, STR, SXT, TET	bla_{TEM-1}
27	O157-D13	Cheese	AMP, CHL, KAN, SPX, STR, SXT	bla_{TEM-1}
28	O157-D14	Milk	AMP, KAN, SPX, TET	bla_{TEM-1}
29	O157-D15	Milk	AMP, ATM, CTT, FOX, KAN, OXA, SPX, STR	bla_{OXA-1}
30	O157-D16	Cheese	AMP, ATM, CTT, FOX, GEN, KAN, OXA, SPX	bla_{CMY-2}
31	O157-D17	Cheese	CIP, GEN, NAL, TET	*qnrS*

in *E. coli* from broiler meat in Europe has recently also been identified in clinical isolates from Swedish patients (Börjesson et al., 2013b). Regarding the types of bla_{CTX-M} gene, previously, British and Swiss studies reported $bla_{CTX-M-15}$ in *E. coli* isolates from animal meats (Geser et al., 2012). In Spain, bla_{SHV-12} is the most frequent extended-spectrum β-lactamase (ESBL) carried by *E. coli* isolates from raw poultry meat (Egea et al., 2012). More recently, it was found that 44% of Swedish chicken meat samples were contaminated with extended-spectrum or transferable AmpC β-lactamase-producing *E. coli* strains (Börjesson et al., 2013a). β-Lactamase-encoding genes were also detected in STEC O157:H7 isolates from dairy products in this study. These genes included bla_{TEM-1}, bla_{TEM-52}, bla_{CMY-2}, $bla_{CTX-M-15}$, bla_{SHV-12}, and bla_{OXA-1}. In Egypt, bla_{TEM-1} was identified in *E. coli* isolated from milk samples collected from bovine mastitis cases (Ahmed and Shimamoto, 2011). In Switzerland, only one *E. coli* isolate from mastitis milk samples contained $bla_{CTX-M-14}$ and bla_{TEM-1}, while all bulk tank milk samples were negative for β-lactamases (Geser et al., 2012). More recently, *E. coli* isolates containing $bla_{CTX-M-1}$ and bla_{CMY-2} were identified from dairy and beef cattle farms in Germany (Schmid et al., 2013). bla_{TEM-52} gene, encoding an extended-spectrum β-lactamase (ESBL), was first described in clinical isolate of *Klebsiella pneumoniae* isolated from the culture of a stool patient in 1998 in France (Poyart et al., 1998) and recently it also spread among *E. coli* isolated from cattle in France (Haenni et al., 2012).

Fluoroquinolones are broad-spectrum antimicrobials used in medicine and veterinary practice to treat infectious diseases caused by enteric bacteria. In this study, the plasmid-mediated quinolone resistance genes *qnrB* and *qnrS* were identified in 12.0% of tested STEC O157:H7 isolates from meat products. Recently, *qnrS* and *aac(6)-Ib-cr* were identified in *E. coli* isolates from retail chicken and ground pork in China (Xu et al., 2014). Furthermore, *qnrB*, *qnrS*, and *aac(6)-Ib-cr* were identified in 13.8% of STEC O157:H7 isolates from dairy products. In Egypt, *qnrA*, *qnrB*, *qnrS*, and *aac(6)-Ib-cr* were identified in 14.3% of Gram-negative bacteria isolated from bovine mastitis cases (Ahmed and Shimamoto, 2011). To the best of our knowledge, this is the first report of the detection and identification of plasmid-mediated quinolone resistance genes in STEC O157:H7.

The resistance to florfenicol, a closely related drug to chloramphenicol, is mediated by *floR* resistance gene (Bischoff et al., 2002). Although florfenicol is not approved for human use, it is related to chloramphenicol, and can select for cross-resistance in bacterial pathogens. In this study, *floR* was identified in 8.0% of STEC O157:H7 from meat products and from 6.9% of isolates from dairy products. In the USA, *floR* was present in 43% of chloramphenicol-resistant *E. coli* isolates from retail meats (Zhao et al., 2012). In Egypt, *floR* was detected in 6.3% of Gram-negative bacteria isolated from cases of bovine mastitis (Ahmed and Shimamoto, 2011), and recently from avian pathogenic *E. coli* isolated from septicemic broilers (Ahmed et al., 2013). To the best of our knowledge, this is the first report of the detection and identification of *floR* in STEC O157:H7 isolated from food.

5. Conclusions

This study characterized the molecular basis of multidrug resistance in STEC O157:H7 isolated from retail meat and dairy products in Egypt. This is the first report of the occurrence of class 2 integrons, *qnrB*, *qnrS*, and *aac(6′)-Ib-cr* in STEC O157:H7. These data provide useful information to better understand the molecular basis of antimicrobial resistance in STEC O157:H7 of food origin. The study also highlights meat and dairy products as potential sources of MDR STEC O157:H7 harboring different classes of antimicrobial resistance genes and integrons. Although antimicrobials are not recommended to treat STEC infections, MDR STEC O157:H7 can laterally transfer these antimicrobial resistance genes to potentially pathogenic bacteria either in the host or in the Environment.

Conflict of interest

The authors declare no conflicts of interest.

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