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

Ashraf M. Ahmed [a], Tadashi Shimamoto [b,*]

[a] Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Sheikh 33516, Egypt
[b] Laboratory of Food Microbiology and Hygiene, Graduate School of Biosphere Science, Hiroshima University, Higashi-Hiroshima 739-8528, Japan

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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.

* Corresponding author. Tel./fax: +81 82 424 7897.
*E-mail address:* tadashis@hiroshima-u.ac.jp (T. Shimamoto).

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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 | | |