

Antibiotic resistance and integrons in Shiga toxin-producing *Escherichia coli* (STEC)

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Submitted: June 23, 2013; Approved: June 6, 2014.

Abstract

Shiga toxin-producing *Escherichia coli* (STEC) cause hemorrhagic colitis (HC) and hemolytic-uremic syndrome in humans (HUS). Cattle are the main reservoir of STEC and transmission to humans occurs through contaminated food and water. Antibiotics are used in pig production systems to combat disease and improve productivity and play a key role in the dissemination of antibiotic resistance genes to the bacteria. Integrons have been identified in resistant bacteria allowing for the acquisition and dissemination of antibiotic resistance genes. STEC strains isolated from humans and animals have developed antibiotic resistance. In our laboratory, 21 non-157 STEC strains isolated from pigs were analyzed to detect class 1 and 2 integrons by PCR. Eight carried integrons, 7 of them harbored *int12*. In another study 545 STEC strains were also analyzed for the presence of *int11* and *int12*. Strains carrying *int11* belonged to isolates from environment (n = 1), chicken hamburger (n = 2), dairy calves (n = 4) and pigs (n = 8). Two strains isolated from pigs harbored *int12* and only one *int11/int12*, highlighting the presence of *int12* in pigs. The selection for multiresistant strains may contribute to the emergence of antibiotic resistant pathogens and facilitate the spreading of the mobile resistance elements to other bacteria.

Key words: STEC, integrons, multiresistance, antibiotics, public health.

Shiga Toxin-Producing *Escherichia coli* (STEC)

STEC have been an important cause of foodborne illnesses worldwide that can cause gastrointestinal disease in humans, particularly infections that result in life-threatening complications such as hemolytic-uremic syndrome (HUS) and hemorrhagic colitis (HC) (Paton and Paton, 1998). HUS is a syndrome characterized for renal failure, hemolytic anemia and thrombocytopenia that affects children less than 5 years old (Voyer, 1996). This damage is produced by the action of cytotoxins: Stx1 and Stx2, encoded by *stx1* and *stx2* genes, respectively. The strains often carry the *eae* gene, encoding intimin an adherence membrane protein which is required for attachment to the host intestinal mucosa (Kaper *et al.*, 1994). These

strains produce putative accessory virulence such as a plasmid-encoded enterohemolysin (*ehxA*), a catalase-peroxidase (*katP*), an autoagglutinating adhesion (Saa), a subtilase cytotoxin (*subAB*), among others (Adu-Bovie *et al.*, 1998; Etcheverría and Padola, 2013).

Transmission of STEC to Humans

Transmission to people occurs primarily via ingestion of inadequately processed contaminated food or water and less frequently through contact with infected manure, animals and people (Hussein and Sakuma, 2005). Cattle are the main reservoir of STEC (Thomas *et al.*, 2012) but sheep are also considered a significant source of infection in humans (La Ragione *et al.*, 2009). Furthermore, STEC has

been isolated from goats, horses, dogs, rabbits and seagulls (Naylor *et al.*, 2005; Wetzel and LeJeune, 2006; Jay *et al.*, 2007). In addition, pigs are also involved in the transmission of STEC in several countries and some HUS outbreaks have been associated with the consumption of food products derived from pigs such as dry sausages (Notario *et al.*, 2000; Parma *et al.*, 2000).

Antibiotic Resistance: Integrons

Antibiotic resistance is recognized as a global problem in human and veterinary medicine. For more than 50 years, antibiotic agents have been an essential component of the infectious disease treatment, and the use of these has resulted in the development and spread of antibiotic multiresistance. Magiorakos *et al.* (2011) multidrug-resistant strain when bacteria are resistant to three or more antibiotic classes. In this work the term multiresistance is used as a synonymous of multidrug-resistant. Any kind of antibiotic used, be it for human, animal or plant health purposes can select for the emergence of resistance and further promote the dissemination of resistant bacteria and resistance genes (Newell *et al.*, 2010; Bush *et al.*, 2011). Bacteria are able to acquire antibiotic resistance genes that provide protection against most antibiotics. The dissemination and acquisition of such genes by horizontal gene transfer has led to the rapid emergence of antibiotic resistance among bacteria (Carattoli, 2013). A system described at the end of the 1980s has been referred to as bacterial integrons identified in multiple resistant bacteria that play an important role in the acquisition and dissemination of antibiotic resistance genes. Integrons are elements that contain the genetic determinants of the components of a site-specific recombination system that recognizes and captures the mobile genes cassette. The essential component of an integron is based on a functional platform (also called 5 conserved segment, 5CS), composed of three key elements: the *intI* gene encoding a site-specific recombinase, an adjacent site, *att*, that is recognized by the integrase and is the receptor site for the cassettes encoded gene and a promoter suitably oriented for the expression of the cassette (Hall and Collis, 1995). Integrons are not able to self-transfer, but can associate themselves with insertion sequences present in transposons and (or) conjugative plasmids that serve as a vehicle for their intra or inter-species transmission (Fluit and Schmitz, 1999; Waldor, 2010). It is known that the presence of integrase is potentially indicative of strains capable of recruiting antibiotic resistance genes (Di Conza and Gutkind, 2010).

Based on the aminoacid sequence of the IntI protein, five classes of integrons are known to play a role in the dissemination of antibiotic resistance genes (Mazel, 2006; Cambray *et al.*, 2010). Classes 1 and 2 are the most prevalent in clinical isolates and are largely implicated in the dissemination of antibiotic resistance and have also been the focus of numerous widespread studies (Ploy *et al.*, 2000a).

Class 1 integrons represents the most common structure and is characterized by the presence of two conserved segments, separated by a segment of variable length (sequence which includes inserted antibiotic resistance gene cassettes). The 5'-CS contains the *intI* gene, the *attI* site and the promoter, while the 3'-conserved segment (3'-CS) commonly codes for the *sulI* gene, conferring resistance to sulphonamides and the *qacEΔ1* gene, conferring resistance to quaternary ammonium compounds used as disinfectants (Stokes and Hall, 1989).

Class 2 integrons are embedded within transposable elements Tn7 and its derivatives. The gene coding for the integrase class 2 (*intI2*) is located at the 5-CS (Hansson *et al.*, 2002). Often the gene *intI2* is interrupted prematurely by a codon stop, and this makes it a pseudogene, which would explain the few arrangements that are described in the databases, compared to the number of integrons class 1 (Recchia and Hall, 1995). Its 3 conserved segment usually contains five *tns* genes involved in the movements of the transposon (*tnsA*, *tnsB*, *tnsC*, *tnsD*, *tnsE*) (Ploy *et al.*, 2000b).

STEC, Antibiotic Resistance and Integrons

Some reports indicate that STEC strains isolated from humans and animals have acquired antibiotic resistance genes almost twenty years ago (Farina *et al.*, 1996; Zhao *et al.*, 2001). Class 1 and 2 integrons are strongly associated with multiresistance to antibiotic in STEC isolated from different origins in the world (Guerra *et al.*, 2006; Vali *et al.*, 2007). On May 2011 in Germany, a large outbreak of HUS associated with the rare hybrid strain of Shiga toxin-producing *Escherichia coli* (STEC) and *Escherichia coli* enteroaggregative (EAEC) serotype O104:H4 occurred (Askar *et al.*, 2011). There are important differences between this outbreak and previous outbreaks of STEC infection as the majority of the cases of the HUS (90%) occurred in adults rather than in children, with the majority occurring in women. A total of 3816 cases (including 54 deaths) were reported, 845 of which (22%) involved HUS (Frank *et al.*, 2011). All isolates classified as the outbreak strain were resistant to all penicillins and cephalosporins and susceptible to carbapenems. This resistance pattern is typical for CTX-M-15 β-lactamases (Bielaszewska *et al.*, 2011). CTX-M-type enzymes were also described in other STEC serotype O111:H8 (Valat *et al.*, 2012) and O26:H11 (Ishii *et al.*, 2005).

Vali *et al.* (2007) tested 297 STEC O26, 152 STEC O103 and 13 STEC O145 isolated in two cohorts of Scottish beef cattle from two farms. Isolates were screened for the presence of class 1 and 2 integrons. *E. coli* O26 isolates were susceptible to apramycin, cefalexin, chloramphenicol and nalidixic acid. *E. coli* O103 isolates were susceptible to apramycin, nalidixic acid and neomycin. *E. coli* O145 isolates were susceptible to apramycin, cephalixin, chloram-

phenicol and nalidixic acid. Among STEC O26, O103 and O145 there were 4, 4 and 1 isolates, respectively, that contained class 1 integrans. Class 2 integrans were detected in only one O145 isolate.

Over a period of time between 1983 and 2003, a total of 105 epidemiologically unrelated STEC O111 isolates from humans and cattle from Germany were tested for susceptibility to 17 antibiotics agents. Resistant isolates were screened by molecular methods for class 1 and 2 integrans. Resistance was found in 76% of the isolates, with a prevalence of 72% for multiresistance. The most prevalent resistance patterns were to streptomycin, sulfamethoxazole and tetracycline (72-68%), followed by spectinomycin, ampicillin and kanamycin/neomycin (39-25%). Class 1 integrans were detected in 41% of the isolates while class 2 integrans were found in only one (Guerra *et al.*, 2006).

In the USA 93 (34%) of 274 STEC from poultry, cattle, swine, and humans were resistant to streptomycin, 89 (32%) to sulfamethoxazole, 83 (30%) to tetracycline, 48 (18%) to ampicillin, 29 (11%) to cefalothin, 22 (8%) to trimethoprim/sulfamethoxazole, 18 (7%) to gentamicin, 13 (5%) to chloramphenicol, and 10 (4%) to ceftiofur. Forty-three (16%) of the STEC isolates harbored class 1 integrans and 41 (95%) of STEC positive to class 1 integrans were resistant to one or more antibiotics (Sing *et al.*, 2005).

Another study was conducted in USA during 2005 on 24 *E. coli* isolates from dairy farms. From that, 14 *E. coli* were isolated from dairy cows with mastitis (ECDM), 9 STEC O157:H7 from dairy cow and only one from bulk tank milk. These strains were evaluated for sensitivity to 19 antibiotics used in human and/or veterinary medicine. Class 1 integrans were found only in eight of 10 isolates (one STEC O157:H7 and seven ECDM). Eight of 10 STEC O157:H7 and six of 14 ECDM were susceptible to all tested antibiotics (Murinda *et al.*, 2005).

In another research, antibiotic resistance patterns and molecular characteristics were determined in 32 STEC strains previously identified in São Paulo State, Brazil associated with 21 human infections and 11 cattle feces. The highest resistance was identified for tetracycline (100%),

streptomycin (78%) and trimethoprim-sulfamethoxazole (56%). Eleven STEC strains showed resistance to ampicillin. The class 1 integrase gene (*intI1*) was detected in seven (22%) strains, and five from seven (5/7) belonged to the O111:H8 serotype (Cergole-Novella *et al.*, 2011).

In other studies, a total of 50 isolates of STEC, including 29 O157:H7 and 21 non-O157 STEC strains were analyzed for antibiotic susceptibilities and the presence of class 1 integrans. Seventy-eight percent of the isolates exhibited resistance to two or more antibiotic classes. Multiresistance to streptomycin, sulfamethoxazole, and tetracycline was most often observed. Class 1 integrans were identified among nine STEC isolates, including serotypes O157:H7, O111:H11, O111:H8, O111:NM, O103:H2, O45:H2, O26:H11, and O5:NM (Zhao *et al.*, 2001).

Two different studies were carried out in our laboratory with isolates collected in Argentina. On the other hand, a total of 21 non-O157 STEC strains isolated from pig feces recovered from ten farms (named A to J) were analyzed to detect the 5'-conserved segment of integrans (called *intI1* and *intI2*) by PCR. Eight (38%) belonging to 4 different farms carried integrans. As a matter of interest, 7 of these isolates harbored *intI2* unlike with the studies cited above. The results of PCR and resistance phenotypes are summarized in Table 1.

Furthermore, a collection of 545 STEC strains from different origins were analyzed for the presence of *intI1* and *intI2*. The STEC strains carrying *intI1* belonged to isolates from environment (n = 1), chicken hamburger (n = 2), dairy calves (n = 4) and pigs (n = 8). Moreover, two strains isolated from pigs harbored *intI2* and another strain both *intI1/intI2*. Both local studies highlighted the presence of class 2 integrans in STEC isolated from pig samples (data not published).

The antibiotics mentioned above are used commonly in pigs production system to treat and prevent diseases. Antibiotics are often used in animal production with little or no veterinary consultation making it difficult to determine which drugs are used in what quantities (McEwen and Fedorka-Cray, 2002).

Table 1 - Results of PCR and resistance phenotypes.

Farm	Toxin subtype	5'-CS	Antibiotic resistance
A	<i>stx2e</i>	<i>intI1</i>	S-AMP-TET-CMP-TMS-DOX-FLOR
B	<i>stx2e</i>	<i>intI2</i>	S-TET-CMP-TMS-DOX-FLOR
B	<i>stx2e</i>	<i>intI2</i>	S-TET-CMP-DOX-FLOR
B	<i>stx2e</i>	<i>intI2</i>	TET-DOX-FLOR-CIP-NAL
B	<i>stx2e</i>	<i>intI2</i>	S-TET-CMP-TMP/SMX-DOX-FLOR
C	<i>stx2</i>	<i>intI2</i>	S-TET-TMP/SMX-DOX-FLOR
C	<i>stx2</i>	<i>intI2</i>	S-TET-TMP/SMX-DOX-NAL
D	<i>stx1</i>	<i>intI2</i>	S-TET-DOX-NAL

CIP: Ciprofloxacin. AMP: Ampicillin. TMP: Trimethoprim/Sulfamethaxazole. DOX: Doxycycline. CMP: Chloramphenicol. FLOR: Florfenicol. TET: Tetracycline. NAL: Nalidixic acid. S: Streptomycin (Colello *et al.*, 2012).

Taking into account the use of antibiotics in different stages of the pig production and the increase in the consumption of pig meat during the last years, it is of high interest the knowledge about the presence of STEC strains harboring integrons in this system of animal production. Only in Argentina, according to data obtained from the National Agricultural Trade Control (ONCAA) the pig production in 2009 was estimated at 287,121 tons, 4.9% more compare to the same period in 2008, showing the consumption of meat from pig continues increasing.

Thereby, the presence of STEC harboring integrons is of high significance because of the possibility of entrance into the food chain. Entrance into the food production implies a risk to the public health because the possibility of acquisition and transmission of the new genes conferring antibiotic resistance intra or inter species. Vignoli *et al.* (2005) have emphasized the importance of studying antibiotic multiresistance in pathogenic *E. coli*, such as STEC, from an epidemiological standpoint.

Concluding Remark

The emergence and dissemination of antibiotic resistance among STEC isolates may have potentially negative implications and the characterization of resistance mechanisms provides additional information about the epidemiology of the resistance.

Even though the antibiotic resistance itself is not a virulence factor, it has been proven that acts as a key factor in the development of the infection, therefor may be taken as a virulence like factor in specific ecological niches that could be colonized by antibiotic resistant bacteria (Beceiro *et al.*, 2013).

Although the degree of knowledge about integrons has changed in recent years and the information available today is extensive, there are still important points to understand and investigate. Many microorganisms carrying integrons can change and adapt quickly to different environmental niches and remain stable in several microbial lineages highlights not only its adaptive value, but also the potential for selection and dissemination of multiresistant bacteria and it is known that antibiotic resistant bacteria will increase a lot.

Even if hospitals were traditionally considered to be the major selective environment of antibiotic resistant bacteria and resistance genes, it is becoming clear that other reservoirs of resistance genes could exist (Avgustin, 2012).

Antibiotics are used in pig production systems to combat disease and improve animal productivity. Feed-based antibiotics consistently benefit productivity, increasing the ability of farms to maintain profitable margins (Cromwell *et al.*, 1996).

Although antibiotic therapy is generally not recommended for treatment of STEC infections in humans, the indirect selection for multiresistant strains contribute to the increase of emerging antibiotic resistant pathogens and fa-

cilitate the spread of these mobile resistance elements to other bacteria. Corogeanu *et al.* (2012) proposed that there is a chance for antibiotic treatment of patients in outbreaks of STEC with antibiotics that do not improve the release of Stx. However, an increased surveillance and the development of adequate prevention strategies are needed for public health reasons.

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Associate Editor: Elaine Cristina Pereira De Martinis

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