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**Original Article**

# Pathogenicity Islands in Shiga Toxin–Producing *Escherichia coli* O26, O103, and O111 Isolates from Humans and Animals

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**Abstract**

Non-O157 Shiga toxin–producing *Escherichia coli* (STEC) are increasingly recognized as foodborne pathogens worldwide. Serogroups O26, O111, and O103 cause most known outbreaks related to non-O157 STEC. Pathogenicity islands (PAIs) play a major role in the evolution of STEC pathogenicity. To determine the distribution of PAIs often associated with highly virulent STECs (OI-122, OI-43/48, OI-57, and high pathogenicity islands) among STEC O26, O103, and O111, a collection of STEC O26 ($n=45$), O103 ($n=29$), and O111 ($n=52$) from humans and animals were included in this study. Pulsed-field gel electrophoresis (PFGE) with *Xba*I digestion was used to characterize the clonal relationship of the strains. In addition, a polymerase chain reaction–restriction fragment length polymorphism assay was used to determine *eae* subtypes. Additional virulence genes on PAIs were identified using specific PCR assays, including OI-122: *pagC, sen, efa-1, efa-2,* and *nleB*; OI-43/48: t*erC, ureC, iha,* and *aidA-1*; OI-57: *nleG2-3, nleG5-2,* and *nleG6-2*; and HPI: *fyuA* and *irp2*. A PFGE dendrogram demonstrated that instead of clustering together with strains from the same O type (O111:H8), the O111:H11 ($n=14$) strains clustered together with strains of the same H type (O26:H11, $n=45$). In addition, O26:H11 and O111:H11 strains carried *eae* subtype $\beta$, whereas O111:H8 strains had *eae* $\gamma 2/\theta$. The O26:H11 and O111:H11 stains contained an incomplete OI-122 lacking *pagC* and a complete HPI. However, a complete OI-122 but no HPI was found in the O111:H8 strains. Additionally, *aidA-1* of OI-43/48 and *nleG6-2* of OI-57 were significantly associated with O26:H11 and O111:H11 strains but were almost missing in O111:H8 strains ($p<0.001$). This study demonstrated that H11 (O111:H11 and O26:H11) strains were closely related and may have come from the same ancestor.

## Introduction

Shiga toxin–producing *Escherichia coli* (STEC) are important foodborne pathogens due to their association with outbreaks and hemolytic uremic syndrome (Karmali *et al.*, 2003). *E. coli* O157:H7 is the most important STEC for its strong association with severe disease and outbreak. However, public health concerns of non-O157 STEC continue to increase, and more than 470 non-O157 serotypes have been associated with human diseases (Blanco *et al*., 2004). Among them, serogroups O26, O103, and O111 accounted for 67% cases of non-O157 STEC infection in the United States from 2000 to 2010 (Gould *et al*., 2013).

Pathogenicity islands (PAIs) carry various virulence genes that are usually absent in nonpathogenic strains (Karmali *et al.,* 2003). In STEC, locus of enterocyte effacement (LEE) is the most characterized PAI (Coombes *et al.,* 2008). Besides LEE, other PAIs such as OI-122 (encoding non-LEE-effectors and adhesins), OI-43/48 (encoding urease, tellurite resistance proteins, and adhesins), OI-57 (encoding non-LEE-effectors), and high pathogenicity island (HPI) (encoding an iron uptake system) have been found in STEC (Karch *et al.,* 1999; Nakano *et al*., 2001; Taylor *et al*., 2002; Karmali *et al*., 2003; Coombes *et al.,* 2008). In this study, we reported the PAIs distribution in STEC serogroups O26, O103, and O111 from animals and humans. In addition, their

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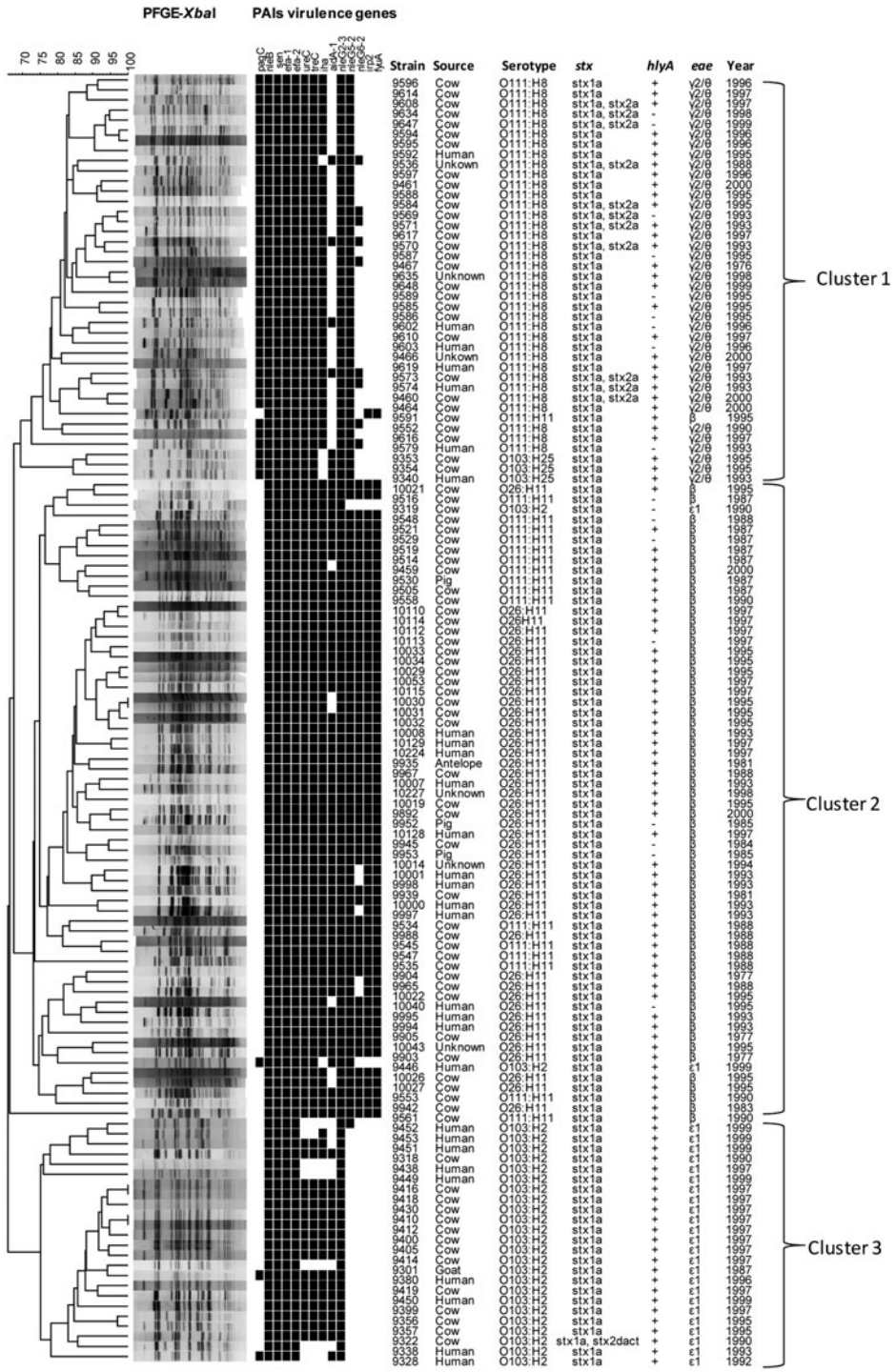


**FIG. 1.** Pulsed-field gel electrophoresis (PFGE) dendrogram of Shiga toxin–producing *Escherichia coli* (STEC) O26 ($n = 45$), O111 ($n = 52$), and O103 ($n = 29$) with *Xba*I digestion. The genetic relatedness of the STEC strains was analyzed by BioNumerics software (Applied Maths, Austin, TX) using unweighted pair group means with arithmetic averages to construct a dendrogram with a 1.5% lane optimization and 1.5% band position tolerance. *stx1* (Shiga toxin 1), *stx2* (Shiga toxin 2), *eae* (intimin encoding gene). OI-122: *pagC* (the *phoP*-activated gene C), *sen* (*Shigella flexneri* enterotoxin 2 similar gene), *nleB* (encoding a host immune response inhibitor), *efa-1* (encoding an adhesin), and *efa-2* (encoding an adhesin); OI-43/48: *ureC* (urease gene), *terC* (tellurite-resistant gene), *iha* (encoding an adhesin), *aidA-1* (encoding an adhesin); OI-57: *nleG2-3* (encoding a potential host immune response inhibitor), *nleG5-2* (encoding a potential host immune response inhibitor), *nleG 6-2* (encoding a potential host immune response inhibitor); high pathogenicity island (HPI): *fyuA* (HPI iron uptake–associated gene) and *irp2* (HPI iron uptake–associated gene). For each pathogenicity island (PAI) virulence gene, black square means positive and white square means negative.

genetic relatedness was analyzed using pulsed-field gel electrophoresis (PFGE).

## Materials and Methods

### *stx and* eae *subtyping*

*stx* gene subtype was determined as described (Ju *et al*., 2012b) using EDL933 (*stx1a* and *stx2a*), E32511 (*stx2c*), EH250 (*stx2d*), S1191 (*stx2e*), B2F1 (*stx2dact*), and N15018 (*stx1c*) as positive controls. A polymerase chain reaction (PCR)–restriction fragment length polymorphism was employed to identify *eae* subtypes as described (Tramuta *et al*., 2008) using TW07920 ($\varepsilon$), RDEC-1 ($\beta$), and TW01387 ($\gamma 2/\theta$) as positive controls. *E. coli* K12 was used as a negative control.

### *Presence of OI-122, OI-43/48, OI-57, and HPI*

PCR assays were used to determine the presence of 14 virulence genes in STEC OI-122 (*pagC, sen, nleB, efa-1,* and *efa-2*), OI-43/48 (*terC, ureC, iha* and *aidA-1*), OI-57 (*nle2-3*, *nleG6-2*, and *nleG5-2*) and HPI (*irp2* and *fyuA*) as described (Karch *et al.*, 1999; Nakano *et al.*, 2001; Taylor *et al.*, 2002; Karmali *et al.*, 2003; Coombes *et al.*, 2008). *E. coli* O157:H7 EDL933 (OI-122, OI-43/48, and OI-57) and O26:H11 SJ-13 (HPI) were used as positive controls, and *E. coli* K12 as a negative control.

### *PFGE*

PFGE was performed according to the PulseNet protocol (http://www.pulsenetinternational.org/SiteCollectionDocuments/pfge/5%201_5%202_5%204_PNetStand_Ecoli_with_Sflexneri.pdf). The genetic relationship of the STEC O26, O103, and O111 were analyzed with BioNumerics software (Applied Maths, Austin, TX) as described (Ju *et al.*, 2012b).

### *Statistical analysis*

Chi-square or Fisher's exact test were used to analysis the data by using SAS9.2 (SAS Institute, Cary, NC). A $p$-value of $< 0.05$ was considered statistically significant.

## Results and Discussion

STEC strains were separated into three major clusters by PFGE, and those in the same clusters tended to share similar PAI virulence gene profiles (Fig. 1 and Table 1). Cluster 1 mainly consisted of O111:H8 strains ($n = 35$) and contained *eae*-subtype $\gamma 2/\theta$. All but one strain in cluster 1 were positive for all OI-122 marker genes but appeared to lack *aidA-1* (OI-43/48), *nleG6-2* (OI-57), *fyuA* (HPI) and *irp2* (HPI). Most O111:H11 (14/16) and all O26:H11 (45/45) formed cluster 2 and carried *eae*-subtype $\beta$. The O111:H11 and O26:H11 strains appeared to have identical PAIs virulence gene profiles. O103:H2 contained *eae*-subtype $\varepsilon 1$, and most strains (24/26) belonged to cluster 3. The O103:H2 strains carried all OI-122 marker genes except for *pagC* but none of them contained *fyuA* or *irp2*; all but two O103:H2 were only positive for *nleG2-3* (OI-57).

Previously, we found that one or more PAIs virulence genes could be absent in STEC O157 (Ju *et al.*, 2013). In this study, similar findings have been observed in other STEC, especially in O103 strains, which indicated that PAIs may be unstable in STEC. In addition, none of O157 strains carried HPI, but all H11 strains (O26:H11 and O111:H11) contained this PAI.

Whittam *et al.* first reported that strains that carry the same H antigen (O55:H7 and O157:H7) are closely related (Whittam *et al.*, 1993). Previously, we reported the close relatedness of H11 strains (O26:H11 and O111:H11) based on whole genome-wide sequence study, and proposed that serotypes O26:H11 and O111:H11 may come from the same ancestor (Ju *et al.*, 2012a). In this study, we found that

Table 1. Prevalence of Virulence Genes of Pathogenicity Islands (PAI) OI-122, OI-43/48, OI-57, and High Pathogenicity Island in Shiga Toxin–Producing *Escherichia coli* O26, O103, and O111

| | | *% Isolates Positive for Virulence Gene* | | | | |
|---|---|---|---|---|---|---|
| *PAI* | *Virulence gene* | *O26:H11* (n = *45*) | *O111:H11* (n = *16*) | *O111:H8* (n = *36*) | *O103: H2* (n = *26*) | *O103:H25* (n = *3*) |
| OI-122 | | | | | | |
| | *pagC* | 0 | 0 | 100 | 12 | 100 |
| | *sen* | 100 | 100 | 100 | 100 | 100 |
| | *nleB* | 100 | 100 | 100 | 100 | 100 |
| | *efa-1* | 100 | 100 | 100 | 100 | 100 |
| | *efa-2* | 100 | 100 | 100 | 100 | 100 |
| OI-43/48 | | | | | | |
| | *terC* | 100 | 100 | 100 | 73 | 100 |
| | *ureC* | 100 | 100 | 100 | 73 | 100 |
| | *iha* | 100 | 100 | 100 | 73 | 0 |
| | *aid-1* | 91 | 94 | 8 | 73 | 100 |
| OI-57 | | | | | | |
| | *nleG2-3* | 100 | 100 | 100 | 100 | 67 |
| | *nleG6-2* | 89 | 94 | 28 | 0 | 0 |
| | *nleG5-2* | 100 | 100 | 100 | 8 | 67 |
| HPI | | | | | | |
| | *irp2* | 100 | 100 | 0 | 0 | 0 |
| | *fyuA* | 100 | 100 | 0 | 0 | 0 |

O26:H11 and O111:H11 contained an HPI and incomplete OI-122, and appeared to carry all marker genes for OI-43/48 and OI-57 (Fig. 1), which supported the hypothesis that the two serotypes might share a common ancestor. In addition, a recent clustered regularly interspaced short palindromic repeat (CRISPR) study by Yin *et al.* also support O26:H11 and O111:H11 might share a common ancestor (Yin *et al.*, 2013).

Strains from the same O group, however, did not belong to the same cluster based on PFGE, and had different PAIs virulence gene profiles. For example, the O111:H11 strains carried *eae*-subtype $\beta$ while O111:H8 contained $\gamma 2/\theta$; O111:H11 did not carry *pagC* but O111:H8 contained the gene; O111:H11 carried *fyuA* and *irp2* but O111:H8 did not; 14 (88%) O111:H11 but only 3 (8%) O111:H8 contained *aidA-1* ($p < 0.001$); and 14 (88%) O111:H11 but only 10 (28%) O111:H8 were positive of *nleG5-2* ($p < 0.001$). In addition, O103:H25 and O103:H2 were also separated in PFGE dendrogram and carried different virulence gene patterns (Fig. 1). Similar findings have been reported by other investigators. For example, Tarr *et al.* found that STEC O111 (O111:H9, O111:H21, and O111:H8) were located at three different linkages within the multilocus sequence typing (MLST) phylogenetic tree (Tarr *et al.*, 2008). O103 strains (O103:H2, O103:H11, and O103:H25) were shown to be at different branches based on the MLST phylogenetic tree, and had different intimin subtypes (Iguchi *et al.*, 2012).

In conclusion, our findings demonstrated that STEC O26:H11 and O111:H11 contain highly similar PAI virulence genes profiles, and appeared to be closely related. However, strains sharing the same O antigens appeared to be not closely related and had different PAIs virulence gene profiles, indicating that they may have had derived from different origins and have different pathogenic potentials.

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### Disclosure Statement

No competing financial interests exist.

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