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Distribution of the *saa* Gene in Strains of Shiga Toxin-Producing *Escherichia coli* of Human and Bovine Origins

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Certain strains of Shiga toxin-producing *Escherichia coli* (STEC) which do not have the locus of enterocyte effacement pathogenicity island carry the STEC autoagglutinating adhesin (*saa*) gene. The distribution of the *saa* gene in STEC isolates from patients with hemolytic-uremic syndrome (HUS), patients with less severe diarrheal disease, asymptomatic individuals, and healthy cattle was examined. *saa*-positive strains were detected more frequently ($P < 0.001$) in STEC strains from bovines (32 of 56 strains) than in those from humans (8 of 91 strains). No significant association ($P = 0.135$) was found between the *saa* gene and STEC isolated from patients with HUS (6 of 46 strains) or diarrhea (2 of 29 strains) and from healthy controls (0 of 16 strains).

Shiga toxin-producing *Escherichia coli* (STEC), also referred to as verocytotoxin-producing *E. coli*, cause a broad spectrum of disease in humans ranging from mild diarrhea to severe disease, such as hemorrhagic colitis and hemolytic-uremic syndrome (HUS) (2, 8, 20). A wide range of STEC serogroups can cause human disease, although the most commonly isolated serogroups from patients infected with STEC, notably O26, O103, O111, O145, and O157 (16, 21), are those harboring the locus of enterocyte effacement (LEE) (4). One of the genes located on the LEE is *eae* (*E. coli* attaching and effacing), which encodes intimin, an outer membrane protein involved in intimate attachment of the bacteria to intestinal host cells (4). However, the presence of the LEE is not essential for pathogenesis, as a number of cases of severe STEC disease, including HUS, have been caused by LEE-negative strains (6, 9, 12). Paton et al. recently described a novel autoagglutinating adhesin, designated Saa (STEC autoagglutinating adhesin), in a LEE-negative O113:H21 STEC strain responsible for an outbreak of HUS (13). Those authors presented evidence that the *saa* gene was plasmid encoded and associated with the STEC enterohemolysin genes (13, 14). The *saa* gene was also detected in certain other STEC isolates from humans, including other LEE-negative strains isolated from sporadic HUS cases (14). This raised the possibility that Saa might be a virulence factor for virulent LEE-negative STEC in humans, but there were insufficient numbers of strains available for testing to draw any firm conclusions. In the present study, we examined the distribution of the *saa* gene in a large collection of STEC isolates from patients with HUS and diarrheal disease and from a healthy control group to test whether there is any association between the presence of *saa* and severe human STEC disease. We also investigated the distribution of the *saa* gene in isolates from healthy cattle in the United Kingdom to determine whether this gene may be associated with bovine STEC.

The 91 strains of STEC from the culture collection of the Laboratory of Enteric Pathogens (LEP), London, United Kingdom, comprised 46 strains from patients with HUS (Table 1), 29 isolated from patients with diarrhea who did not develop HUS (Table 2), and 16 from a healthy control group (Table 3) (3, 8, 16, 18, 19). Sixty-one of these strains were differentiated by serotyping, *stx* typing, and the presence or absence of the *eae* and STEC enterohemolysin (*ehx*A) genes (5, 11, 15, 17). The strains were isolated between 1983 and 2000 either at the LEP from fecal samples submitted for detection of STEC or at hospital laboratories and then sent to the LEP for confirmation and further tests. Fecal pat samples collected from healthy cattle from 41 different farms in Scotland between 1996 and 1999 were examined for the presence of STEC strains other than those belonging to serogroup O157 (7). Fifty-six STEC strains were isolated from 423 fecal samples; 30 of these strains were differentiated by serotyping, *stx* typing, and the presence or absence of the *eae* and *ehx*A genes (5, 11, 15, 17) (Table 4). These strains were tested for the presence of *saa* by PCR with primers 5′-CGTGATGAACAGGCTATTGC and 5′-ATGGACATGCCTGTGGCAAC, which amplify a 119-bp portion of the *saa* gene (14). To test for differences in the detection of particular genes between the four groups of strains, generalized linear models with binomial errors were performed. In all cases, a P value of <0.05 was taken to indicate significance. Fisher's exact tests were performed to test the significance of the association between the detection of particular genes.

Forty (27%) of the 147 STEC strains in this study had the *saa* gene (Tables 1-3). None of these 40 *saa*-positive strains

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TABLE 1. Characterization of non-O157 STEC strains isolated from HUS patients[a]

Serotype (no. of strains)[b]	*stx* gene type	Presence or absence of:		
		eae	*ehxA*	*saa*
O2:H6 (1)	2	−	−	−
O5:H- (1)	1	+	+	−
O9ab:H- (1)	2	−	−	−
O26:H- (1)	2	+	+	−
O26:H- (1)	1	+	+	−
O26:H11 (1)	2	+	+	−
O26:H11 (3)	1	+	+	−
O55:H7 (1)	2	+	−	−
O55:H10 (1)	2	−	−	−
O91:H21 (1)	2	−	+	+
O104:H2 (1)	2	−	−	−
O105ac:H18 (1)	1+2	−	+	+
O111ac:H- (2)	1	+	+	−
O111ac:H- (5)	1+2	+	+	−
O113:H4 (1)	2	−	−	−
O115:H10 (1)	1	−	−	−
O121:H19 (1)	2	+	+	−
O128ab:H2 (1)	1+2	−	+	−
O128ab:H7 (1)	2	+	−	−
O128ab:H25 (1)	2	+	+	−
O134:H25 (1)	2	−	+	+
O145:H- (1)	1	+	+	−
O145:H25 (5)	2	+	+	−
O153:H25 (1)	2	−	−	−
O163:H19 (2)	2	−	+	+
O165:H25 (2)	2	+	+	−
O168:H- (1)	2	−	−	−
O172:H- (1)	2	+	+	−
O173:H21 (1)	2	−	+	+
O?:H4 (1)[c]	2	−	−	−
O?:H21 (2)	2	−	−	−
O?:H40 (1)	2	+	−	−

[a] See references 9, 18, 19, 20, and 21.
[b] Where the serotype is the same, isolates have been differentiated according to *stx* type and the presence or absence of the *eae* and *ehxA* genes.
[c] O?, could not be serogrouped using the current serotyping scheme.

TABLE 2. Characterization of non-O157 STEC strains isolated from patients with diarrhea but who did not develop HUS[a]

Serotype (no. of strains)	*stx* gene type	Presence or absence of:		
		eae	*ehxA*	*saa*
O4:H10 (1)	1+2	−	+	−
O5:H- (2)	1	+	+	−
O26:H11 (5)	1	+	+	−
O76:H7 (1)	2	+	+	−
O91:H- (1)	1+2	−	−	−
O105ac:H18 (1)	1+2	−	+	+
O118:H12 (3)	2	−	−	−
O121:H19 (1)	2	+	+	−
O128ab:H- (2)	1+2	−	+	−
O128ab:H2 (6)	1+2	−	+	−
O128ab:H8 (1)	1+2	−	−	+
O?:H- (1)[b]	1	+	+	−
O?:H10 (1)	2	−	−	−
O?:H19 (1)	1	−	−	−
Orough:H- (1)	2	−	−	−
Orough:H45 (1)	2	−	−	−

[a] See references 9, 18, 19, 20, and 26.
[b] O?, could not be serogrouped using the current serotyping scheme.

carried the *eae* gene, and 39 were *ehx*A positive; the exceptional strain (*saa* positive and *ehx*A negative) belonged to serotype O128ab:H8 (Table 2). The *saa* gene was detected in 6 (13%) of 46 strains from humans with HUS, and 28 (61%) of these 46 strains carried the *eae* gene, while 12 (26%) had neither (Table 1). Two (7%) of 29 strains from patients with diarrhea or bloody diarrhea who did not develop HUS were *saa* positive. The *eae* gene was detected in 10 (34%) strains from this group, and 17 (59%) had neither the *eae* nor the *saa* gene (Table 2). None of the isolates from the healthy control group had the *saa* gene (Table 3). Characterization of the 56 bovine strains showed that 32 (57%) carried the *saa* gene, 2 (4%) were *eae* positive, and 22 (39%) were negative for both *eae* and *saa* (Table 4). None of the strains harboring the *saa* gene had the *eae* gene.

Statistical analysis revealed a significant difference in the frequencies of *saa*-positive strains between the four groups ($\chi^2 = 45.45$; df = 3; $P < 0.001$). However, if the cattle strains are excluded from the analysis, then this significance was lost ($\chi^2 = 4.00$; df = 2; $P = 0.135$). Therefore, the number of cattle strains containing the *saa* gene is significantly higher than the number of *saa*-positive human strains, although there is no difference in the presence of the *saa* gene between the strains from patients with HUS, patients with diarrhea, and the asymptomatic individuals. Statistical tests also showed a significant difference in the frequencies of isolates containing the *eae* gene between the four groups ($\chi^2 = 55.89$; df = 3; $P < 0.001$), with the highest frequency of intimin-positive strains being found in the HUS group.

Variables over and above the group categories (HUS, diarrhea, healthy control, and cattle strains), such as toxin type and the presence of the *eae* and *ehx*A genes, and the fact that certain serotypes are represented by more strains than are others may influence the results. However, the panel of strains reflects the fact that some serotypes associated with HUS (e.g., O26:H11) or less severe diarrhea (e.g., O128ab:H2) are more commonly isolated than others (2, 9, 16, 20, 21). To date, the *saa* gene has not been found in STEC strains harboring the *eae*

TABLE 3. Strains of non-O157 STEC isolated from a healthy control group during a study of infectious intestinal disease[a]

Serotype (no. of strains)	*stx* gene type	Presence or absence of:		
		eae	*ehxA*	*saa*
O82:H2 (1)	1+2	−	+	−
O91:H- (1)	1	−	+	−
O91:H10 (1)	2	−	−	−
O115:H10 (1)	1	−	−	−
O118:H1 (1)	2	−	−	−
O128ab:H2 (3)	1+2	−	+	−
O146:H21 (2)	1+2	−	+	−
O162:H6 (1)	1+2	−	−	−
O162:H8 (1)	1+2	−	−	−
O?:H- (1)[b]	2	−	+	−
O?:H- (1)	1+2	−	−	−
O?:H18 (1)	1+2	−	+	−
O?:H21 (1)	1	−	+	−

[a] See reference 3.
[b] O?, could not be serogrouped using the current serotyping scheme.

TABLE 4. Characterization of non-O157 STEC strains isolated from Scottish cattle[a]

Serotype (no. of strains)[b]	*stx* gene type	Presence or absence of:		
		eae	*ehxA*	*saa*
O8:H16 (1)	1+2	−	+	+
O15:H- (2)	2	−	−	−
O15:H16 (5)	2	−	−	−
O22:H8 (8)	1+2	−	+	+
O87:H16 (2)	2	−	−	−
O91:H49 (1)	2	−	+	+
O103:H2 (1)	1	+	+	−
O105ac:H18 (1)	2	−	+	+
O105ac:H18 (6)	1+2	−	+	+
O113:H4 (1)	2	−	−	−
O113:H21 (3)	2	−	+	+
O136:H- (1)	2	−	−	−
O159:H7 (1)	2	−	+	−
O172:H21 (1)	1+2	−	−	−
E11362/78:H- (1)[c]	2	−	−	−
E43478/86:H8 (1)[c]	2	−	+	+
E43478/86:H12 (1)[c]	2	−	+	+
E8686/77:H16 (2)[c]	2	−	−	−
E54071/88:H19 (1)[c]	1+2	−	+	+
E7477/77:H25 (1)[c]	1	+	+	−
O?:H- (2)[d]	2	−	−	−
O?:H2 (1)	2	−	−	−
O?:H7 (1)	2	−	+	+
O?:H11 (1)	2	−	+	+
O?:H16 (3)	2	−	−	−
O?:H19 (3)	2	−	+	+
O?:H19 (1)	1+2	−	+	+
O?:H25 (1)	2	−	+	+
O?:H40 (1)	2	−	+	+
O?:H49 (1)	2	−	+	+

[a] See reference 7.
[b] Where the serotype is the same, isolates have been differentiated according to *stx* type and the presence or absence of the *eae* and *ehxA* genes.
[c] Provisional new serotypes (formal "O" group pending).
[d] O?, could not be serogrouped using the current serotyping scheme.

gene, and the association of *saa* with bovine strains may be due to the fact that there are so few intimin-positive strains in this group. However, statistical analysis of only the intimin-negative strains showed that there was still a significantly higher frequency of *saa*-positive strains in STEC strains from cattle than in those from humans ($P < 0.001$, Fisher's exact test). Thirty-nine of 40 (98%) *saa*-positive strains also carried the *ehx*A genes, and 39 of 51 (76%) strains that were negative for the *saa* and *eae* genes were also negative for the *ehx*A gene. Tests showed that for the *eae*-negative strains, there was a significant association between the presence of the *saa* and *ehx*A genes ($P < 0.001$, Fisher's exact test). These results are consistent with the observation of Paton et al. that the *saa* gene is plasmid encoded and associated with the plasmid-encoded *ehx*A gene (13). Only one strain of serotype O128ab:H8 was *saa* positive but *ehx*A negative, and further work is being carried out to determine whether the *saa* gene is plasmid encoded in this strain.

The only *saa*-positive serotype common to both human and bovine STEC strains in this study was O105ac:H18. Carriage of the *saa* gene appeared to be specific to certain serotypes within a serogroup, e.g., O113:H21 was *saa* positive, whereas O113:H4 was *saa* negative. Of the 22 bovine serotypes that have yet to be designated as a formal "O" type (i.e., provisional types and "O?"), 13 carried the *saa* gene. This highlights the need to extend the serotyping scheme to include new and emerging STEC serogroups.

Our results showed that there was no significant association between strains of STEC isolated from patients with HUS and the *saa* gene, although they do support the suggestion that the *eae* gene is detected more frequently in STEC from this group of patients (1, 10). However, the *saa* gene was significantly associated with bovine STEC strains, suggesting that Saa may have a role in attachment to the bovine gut. Further work is being carried out, both in vitro and in vivo, to examine the role of Saa in attachment to the bovine gut mucosa.

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