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Evolution of the *iss* Gene in *Escherichia coli*▽†

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The increased serum survival gene *iss* has long been recognized for its role in extraintestinal pathogenic *Escherichia coli* (ExPEC) virulence. *iss* has been identified as a distinguishing trait of avian ExPEC but not of human ExPEC. This gene has been localized to large virulence plasmids and shares strong similarities with the *bor* gene from bacteriophage λ. Here, we demonstrate that three alleles of *iss* occur among *E. coli* isolates that appear to have evolved from a common λ *bor* precursor. In addition to the occurrence of *iss* on the ColV/BM virulence plasmids, at least two *iss* alleles occur within the *E. coli* chromosome. One of these alleles (designated type 3) was found to occur in the genomes of all currently sequenced ExPEC strains on a similar prophage element that also harbors the Sit iron and manganese transport system. When the prevalence of the three *iss* types was examined among 487 *E. coli* isolates, the *iss* type 3 gene was found to occur at a high frequency among ExPEC isolates, irrespective of the host source. The plasmid-borne *iss* allele (designated type 1) was highly prevalent among avian pathogenic *E. coli* and neonatal meningitis-associated *E. coli* isolates but not among uropathogenic *E. coli* isolates. This study demonstrates the evolution of *iss* in *E. coli* and provides an additional tool for discriminating among *E. coli* pathotypes through the differentiation of the three *iss* allele types and *bor*.

Extraintestinal pathogenic *Escherichia coli* (ExPEC) is one of the most diverse *Escherichia coli* pathotypes, with members that cause a variety of diseases in both humans and animals (50). Because of its diversity, ExPEC is further divided into several subpathotypes, including uropathogenic *E. coli* (UPEC), neonatal meningitis-associated *E. coli* (NMEC), and avian pathogenic *E. coli* (APEC). ExPEC strains contain a variety of virulence factors, and it has been suggested that a mix and match of these traits results in the ability of an ExPEC strain to cause disease (16). In fact, efforts to identify universal ExPEC virulence factors, present among all ExPEC strains but absent in other *E. coli* pathotypes or commensal strains, have proved mostly futile (2, 9, 30). One promising virulence gene in this regard is the increased serum survival gene, *iss*, identified as significantly more associated ($P < 0.0001$) with the APEC strains than with fecal isolates from healthy birds (44, 46) and found to occur in around 60% of UPEC and NMEC strains (21, 46) but present in few of the human fecal commensal *E. coli* isolates examined (T. J. Johnson, unpublished data). *iss* was previously localized to pathogenicity islands (PAIs) occurring on colicin-encoding (ColV/BM) plasmids, which commonly occur among APEC strains (33, 35). Since such plasmids are less common among human ExPEC strains (21, 46), the location of *iss* within the human ExPEC genome is unclear.

The *iss* gene was first identified in a human septicemic *E. coli* isolate (6, 7) and was associated with a 20-fold increase in complement resistance and a 100-fold increase in virulence toward 1-day-old chicks (6, 7, 12). The Iss protein from an APEC isolate has been purified and expressed (23), has had monoclonal antibodies made against it (22), and has been shown to protect against homologous and heterologous APEC challenges in birds (38). *iss* shares nucleotide and predicted protein similarities with the phage λ gene *bor*, whose product is also an outer-membrane lipoprotein involved in serum resistance (3). Iss and Bor were both found to be surface-exposed proteins (40). Similarly, both of the *iss* and *bor* mutant strains were attenuated in their abilities to resist the killing effects of host complement (39). However, unlike *iss*, *bor* is present on a cryptic prophage within the chromosome of many types of *E. coli*, pathogenic and nonpathogenic alike (8, 10, 11, 34, 43, 56). The *iss* sequence was first described by Chuba et al. and was identified on large transmissible plasmids (6, 7, 12). Recent plasmid genome sequencing efforts have localized *iss* to the highly conserved region of a ColV/BM-encoded PAI (33, 35), thus making it a useful marker of these loci.

Speculation as to the evolutionary relationship between *iss* and *bor* has been presented, but no further studies have explored this relationship (3, 27). Several recent studies have identified *iss* as occurring commonly among APEC isolates, associated with the presence of a ColV/BM virulence plasmid (19, 21, 28, 44, 47, 57, 58). However, some recent studies involving human *E. coli* have reported conflicting results regarding the prevalence of *iss* among different *E. coli* populations (1, 5, 20, 46, 47, 53). Using *iss* as a marker for ExPEC, Bekal et al. reported that the false-positive reactions they obtained for *iss* could be attributed to its homology with *bor* (5). Anjum et al. (1) recently identified UPEC CFT073 as possessing *iss*, even though its annotation listed no such presence (56) and the cited GenBank reference actually described *iss* from an APEC isolate (27). In an effort to resolve these discrepancies and to avoid future errors in the detection of *iss*, we have performed a more comprehensive comparison of *iss* and *bor*.

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TABLE 1. Primers used in PCR studies

Gene	Forward (F) and reverse (R) primers	Amplicon size (bp)	Multiplex allele detection panel
iss type 1	F 5′-CAGCAACCCGAACCAC TTGATG-3′ R 5′-TTCTGCCGCTCTGGCA ATGCT-3′	323	1
iss type 2	F 5′-GGTAACCCTGCGTCTG TCAGCACA-3′ R 5′-CCAGCGGAGTATAAA TGCCTAAAG-3′[a]	581	2
iss type 3	F 5′-GTCCCCAACTTCCTCC AATAGTCT-3′ R 5′-CCAGCGGAGTATAAA TGCCTAAAG-3′[a]	390	2
bor	F 5′-CCCGTCAGGGCTGTGG ACATAGTT-3′ R 5′-GGGCCAGCGCAGTAG CGAGTAG-3′	201	1

[a] The same reverse primer was used for *iss* types 2 and 3.

MATERIALS AND METHODS

Bacterial strains used in this study. A total of 487 bacterial strains were used in this study. These isolates included 91 NMEC strains (31), 91 UPEC strains (46), 91 APEC strains (44, 46, 47), 30 strains isolated from cases of bovine septicemia (necrotoxigenic *E. coli* [NTEC]), 92 strains isolated from the feces of healthy chickens and turkeys (44), and 92 strains isolated from the feces of healthy humans. All bacterial isolates were stored in glycerol at −80°C until used.

Analysis of *iss*- and *bor*-containing regions. The genomes of 13 available *E. coli* sequences were searched for the presence of *bor* or *iss*, to determine the number of copies and location(s) in the chromosome. These sequences were extracted in silico from the 13 chromosomes and from available plasmid and phage sequences in the NCBI database. Nucleotide sequences were aligned using a ClustalW algorithm with DNASTAR software (Lasergene, Madison, WI). A phylogenetic tree was constructed based upon the *iss* or the *bor* nucleotide sequence. Additionally, a 1,700-bp sequence surrounding *iss* or *bor* was extracted from each of the available genomes and plasmids and aligned using a ClustalW algorithm (Lasergene software package). The nucleotide homology of each sequence, compared to the 1,700-bp region from pAPEC-O1-ColBM (33), was determined for every 100 bp within the 1,700-bp sequence. Phage regions were aligned using Mauve software (15).

PCR prevalence of *iss* and *bor*. Sequence analysis revealed that the *E. coli* genome contains at least three *iss* alleles. The prevalence of these alleles and the *bor* gene was determined among 487 isolates, using two multiplex reactions, screening for *iss* type 1 and *bor* (panel 1) and *iss* types 2 and 3 (panel 2) (Table 1). Templates were prepared as previously described (32). Reactions were performed in 25-μl reaction mixtures using 4 mM $MgCl_2$, 0.125 mM of each deoxynucleoside triphosphate, 0.3 μM each primer, and 1.25 U Amplitaq Gold (Applied Biosystems, Inc.). Cycling parameters were 94°C for 5 min, and then 25 cycles of 94°C for 30 s, 94°C for 30 s, and 94°C for 3 min, with a final extension at 72°C for 10 min.

Phylogenetic typing. All isolates were examined for the presence of three amplicons corresponding to phylogenetic types A, B1, B2, and D, according to the methods described by Clermont et al. (13).

Biostatistics. Fisher's exact test was used to compare differences in prevalence among the populations studied. A P value of less than 0.05 was considered statistically significant.

RESULTS

At least three *iss* alleles exist in the *E. coli* strains. Our analyses of the regions annotated as Bor in ExPEC strains revealed discrepancies in the annotations (Table 2). In CFT073 and UTI89, the gene is annotated as a 342-base-pair gene with a product description of a Bor protein homolog. The UPEC 536 annotation describes a 294-bp gene as a Bor protein precursor. The APEC O1 annotation describes a 309-bp gene as Bor. However, a reannotation performed here revealed that the best gene prediction and annotation of this coding region, in all cases, is a 309-bp gene that should be described as *iss*.

A ClustalW alignment of all of the available *iss* and *bor* sequences revealed three genetically distinct alleles of *iss*, which we have designated *iss* types 1 to 3 (Fig. 1 and 2). Compared to the plasmid-borne *iss* type 1 that was described previously in the literature, types 2 and 3 are 94.2% and 95.5% similar, respectively (Table 3). *iss* type 3, previously annotated as *bor*, occurs on a prophage in the chromosomes of all sequenced ExPEC strains. *iss* type 2 was found on the chromosomes of draft genome sequences of enteroaggregative *E. coli* (EAEC) strain 101-1 and enterotoxigenic *E. coli* (ETEC) strain B7a (Fig. 3). This the first report of the presence of *iss* on the bacterial chromosome. The *bor* gene was identified only within the complete chromosomes of the *E. coli* K-12 and EHEC O157:H7 strains.

***iss* and *bor* occur in multiple locations in the *E. coli* genome.** Within the sequenced *E. coli* genomes, the *iss* alleles were identified in three different loci (Fig. 3). Some *E. coli* genomes instead harbored *bor* in these same loci. The genomes of the *E. coli* strains K-12 MG1655 (8) and K-12 W3110 (24), EAEC strain 101-1 (GenBank accession no. NZ_AAMK00000000), EPEC strain B171 (GenBank accession no. NZ_AAJX00000 000), and EPEC strain E22 (GenBank accession no. NZ_AAJV 00000000) contained *bor* or *iss* type 2 on a prophage element near the *adk* gene. Strains O157:H7 EDL933 (43) and O157:H7 Sakai (25) and ETEC strain B7a (GenBank accession no. NZ_AAJT00

TABLE 2. *iss* and *bor* in sequenced genomes

Organism genome or plasmid	Start position (bp)	Annotated protein description	Annotated gene length (bp)	Reannotated gene size (bp)	Reannotated gene
E. coli K-12 MG1655	578116	Bacteriophage λ Bor protein	294	294	*bor*
E. coli O157:H7	1359194				
EDL933	1712116	Putative Bor protein precursor	294	294	*bor*
UPEC CFT073	1423221	Bor protein homolog	342	309	*iss* type 3
UPEC 536	1205810	Bor protein precursor	294	309	*iss* type 3
UPEC UTI89	1259756	Bor-like protein	342	309	*iss* type 3
APEC O1	1197110	λ prophage Bor protein	309	309	*iss* type 3
pAPEC-O1-ColBM	126545	Increased-serum-survival protein	309	309	*iss* type 1
pAPEC-O2-ColV	137931	Increased-serum-survival protein	309	309	*iss* type 1

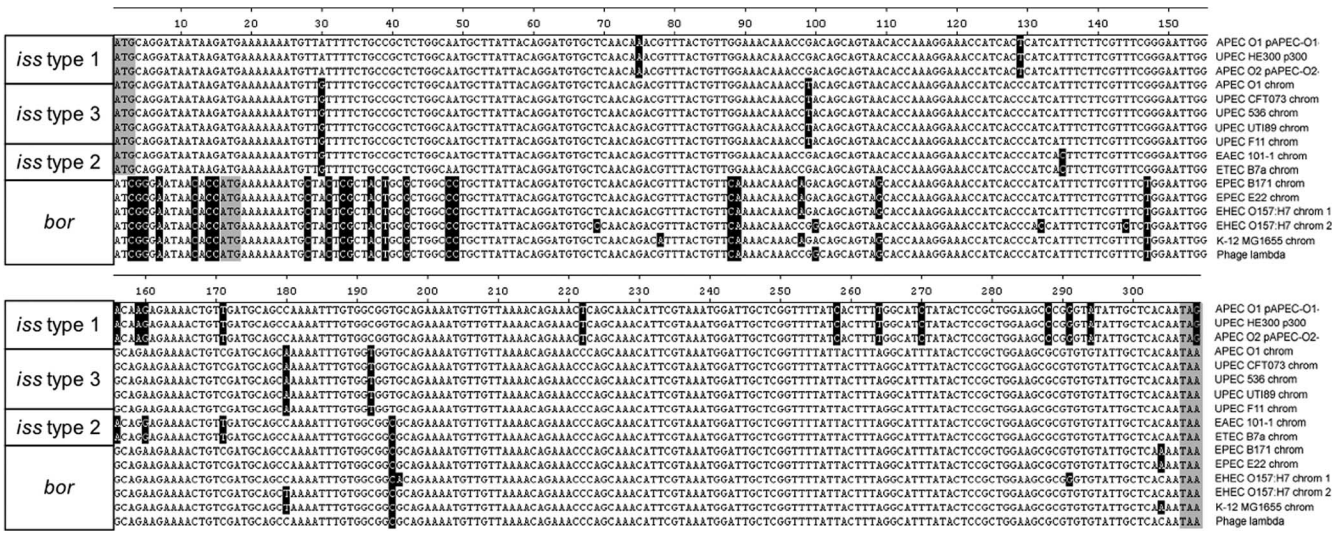

FIG. 1. Alignment of the *iss* and *bor* nucleotide sequences. The three different *iss* types and *bor* are identified based upon nucleotide and amino acid differences. Black shading indicates those nucleotides that are different from the consensus sequence, and gray shading indicates the start and stop codons for either *bor* or *iss*.

000000) contained *bor* or *iss* type 2 on a prophage between the *aspC* and *icd* genes. The sequenced ExPEC strains examined included APEC O1 (34), UPEC 536 (10), UPEC UTI89 (11), UPEC CFT073 (56), and UPEC F11 (GenBank accession no. NZ_AAJU00000000). These strains and the O157:H7 genomes (EDL933 and Sakai) contained a *bor* or an *iss* type 3 on a prophage element between *icd* and *fumC*. The location of *iss* was also analyzed within the extrachromosomal elements. The virulence plasmids pAPEC-O2-ColV (35), p300 (54), pAPEC-O1-ColBM (33), pAPEC-1 (17, 18, 51), and pVM29188 (*Salmonella enterica* serovar Kentucky strain CVM29188, GenBank accession no. NZ_ABAK00000000) contained *iss* within a highly conserved region of the ColV/BM PAI, between the salmochelin siderophore system (the *iroBCDEN* genes) and the *repFIB* replicon region.

***iss* occurs on the *E. coli* chromosome.** Nucleotide and protein alignments were performed between all of the available *iss* and *bor* sequences (Fig. 1). Of the 12 analyzed chromosomal sequences thought to be *bor*, only 6 sequences appeared to be the *bor* gene upon reannotation. Chromosomes containing *bor* included those for *E. coli* K-12 (both MG1655 and W3110), EHEC O157:H7 (both EDL933 and Sakai), and EPEC strains B171 and E22 (draft sequences) (Fig. 3). In the K-12 and EPEC strains, *bor* was present near the *adk* gene. *E. coli* O157:H7 strains EDL933 and Sakai each contained two copies of *bor*. One copy was found on the BP-933W prophage located between the *aspC* and *icd* housekeeping genes, and the second copy was on the DLP12-like prophage located between *icd* and *fumC* (25, 43). In the K-12 strains, *bor* was found within the DLP12 prophage (8, 24).

The remaining six sequences originally thought to be *bor* were reannotated as *iss* (Fig. 2), when analyzed for DNA sequence homology and protein sequence homology and the predicted start and stop sequences. Those sequences reannotated as *iss* types 2 and 3 had predicted protein lengths of 103 amino acids and had 94 to 95% nucleotide homology and 97 to 98% protein homology with the ColV/BM plasmid-borne *iss* type 1 that was originally described as *iss* (Table 3) (27). Sequences reannotated as *bor* had a shorter predicted protein

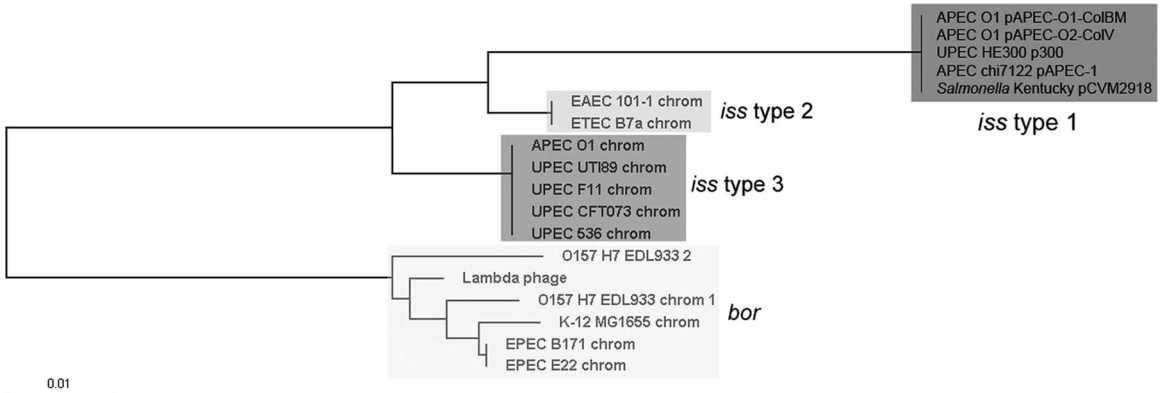

FIG. 2. Phylogenetic tree based upon *iss* and *bor* alignment. Tree displays the three *iss* types (1, dark gray; 2, light gray; 3, medium gray) and *bor* based upon nucleotide differences.

TABLE 3. Similarities between *iss* and *bor*

Gene	Location	Nucleotide differences	% Nucleotide difference	Amino acid differences	% Amino acid difference
iss type 1	ColV/BM plasmids	0	100	0	100
iss type 2	Chromosome	18	94.2	3	97.1
iss type 3	ExPEC chromosome	14	95.5	2	98.1
bor	Chromosome	36	88.4	11	88.8

length of 98 amino acids due to nucleotide variations within the first 15 base pairs of the sequences, resulting in an alternative start site. These sequences also had an alternative stop codon (TAA in *bor*; TAG in *iss*) and shared approximately 88% nucleotide homology and 89% protein homology with *iss* type 1 (12, 27).

***iss* occurs more frequently among *E. coli* strains than previously reported.** A multiplex panel was designed to detect and differentiate between the different *iss* alleles and *bor* gene among *E. coli* strains. The panel was verified by using several fully sequenced *E. coli* strains (Table 4) and by sequencing the products obtained for each primer pair. The occurrence of plasmid-borne *iss* type 1 was highest among populations of NMEC and APEC strains (66% and 78%, respectively), suggesting a high prevalence of the ColV/BM plasmids with a PAI among these populations (Fig. 4 and Table S1 in the supplemental material). The prevalence of type 1 *iss* was significantly lower ($P < 0.05$) among the UPEC, NTEC, and fecal strains examined, ranging from 8 to 15%. The occurrence of *iss* type 2 was lowest among the ExPEC populations and fecal strains examined (15 to 27%) but was significantly higher among NTEC strains (47%). The occurrence of *iss* type 3 was highest among human ExPEC strains (62 to 80%), lower among APEC (48%) and fecal (31 to 42%) strains, and even lower among NTEC (20%) strains than all other populations. The occurrence of *bor* was low among the human ExPEC and human fecal strains examined (4 to 9%) but was significantly higher among APEC (25%), NTEC (50%), and avian fecal (22%) strains. The occurrence of at least one *iss* type ranged from 75 to 91% among the ExPEC strains examined, from 49 to 57% among the fecal strains examined, and in 52% of the NTEC strains examined. Overall, screening for the multiple *iss* alleles resulted in an enhanced detection of *iss* sequences compared to that reported previously (44, 46, 47).

Distribution of the *iss* types and *bor* among the *E. coli* phylotypes was also examined (Fig. 5 and Table 4). The occurrences of *iss* type 1 were similar (34 to 45%) among all phylotypes examined. The prevalence rate of *iss* type 2 ranged from 10 to 33% among all populations examined, with group D containing a significantly higher proportion of *iss* type 2 than the other groups. The occurrences of *iss* type 3 were different among all populations examined. Its prevalence rate was highest among the B2 phylotype (77%), lower among the A phylotype (52%), and even lower among the B1 (14%) and D (33%) phylotypes. The occurrences of *bor* were highest among the A (37%) and B1 (25%) phylotypes and significantly lower among the B2 (3%) and D (6%) phylotypes. Overall, the occurrence of at least one type of *iss* was highest among the B2 (85%), A (73%), and D (72%) phylotypes and slightly lower among the B1 (50%) phylotype.

Homology between plasmid-borne and chromosomal *iss* regions accounts for diagnostic difficulties. A nucleotide comparison of the 1,700-bp regions surrounding *iss* and *bor* in the sequenced *E. coli* strain revealed that the homology between the plasmid-borne *iss* and the chromosomal *iss* types extends beyond the gene itself (Fig. 6). Two predicted genes of unknown function flanked *iss* type 1 and shared strong homology to the respective regions within the ExPEC chromosome. This extended homology is likely the source of the problems encountered when attempts were made to identify *iss* and distinguish it from *bor* (Fig. 6). This is particularly true for hybridization-based techniques, with which it would be virtually impossible to avoid false positives with strains containing *bor* when using primers within the *iss* gene. In fact, the PCR primers used previously for identifying *iss* actually produce an amplicon spanning *iss* and its adjacent upstream gene (35, 44, 46, 47). Prior to the sequencing of multiple ExPEC genomes, it would have appeared as though these primers would not detect chromosomal *iss*/*bor* sequences. However, the ExPEC-borne *iss* type 3 has more homology with the ColV/BM *iss* region than do the *bor*-containing regions of K-12 or O157:H7; therefore, false positives with previous primer sets would have been likely to occur. Table 1 lists primers that are suggested to be specific for the plasmid and chromosomal variants of *iss* and for *bor*. These primer sets contain one primer within the sought-for *iss* or *bor* sequence and a primer outside of the sequence that is specific for a particular location within the genome. Though the complete *E. coli* genomic sequences that are available at present do not contain both *bor* and *iss*, our PCR identified several isolates that do, as well as every other possible combination of the four traits of interest. Thus, while these primer sets have been verified and used effectively to analyze *E. coli* populations for the presence of *bor* and the different *iss* alleles, it will be necessary to review these regions with care as more *E.*

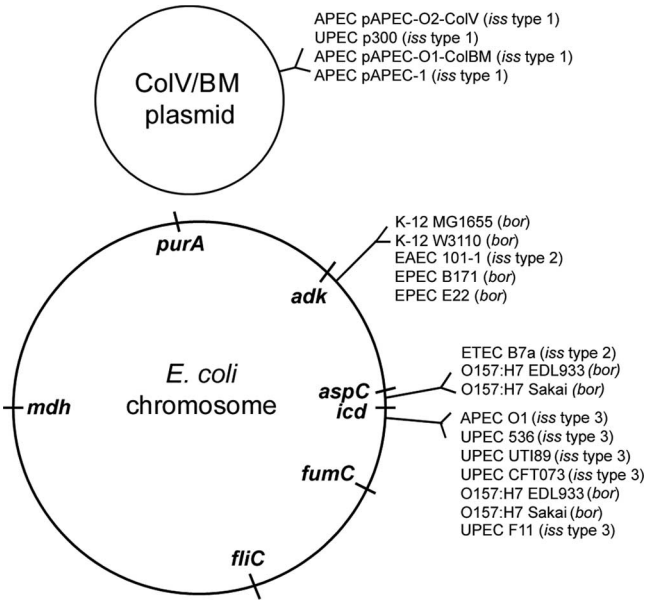

FIG. 3. Locations of *iss* and *bor* in the *E. coli* chromosome. Housekeeping genes were used as markers of the locations of *bor*- and *iss*-containing prophage in the *E. coli* chromosome.

TABLE 4. Comparison of *iss* prevalence data, using Fisher's exact test

Gene type	Strain or phylotype	Significance of *iss* prevalence (*P* value) among:									
		E. coli strains				*E. coli* fecal isolates		Phylotypes			
		NMEC	UPEC	APEC	NTEC	Human	Avian	A	B1	B2	D
iss 1	NMEC		<0.0001	0.4926	<0.0001	<0.0001	<0.0001				
	UPEC			<0.0001	0.6856	1	0.2657				
	APEC				<0.0001	<0.0001	<0.0001				
	NTEC					0.6792	0.1898				
	Human fecal isolates						0.1715				
	Avian fecal isolates										
iss 2	NMEC		0.3801	0.0144	0.0011	0.3818	0.3818				
	UPEC			0.1161	0.0117	0.5908	0.5908				
	APEC				0.2163	0.1145	0.1145				
	NTEC					0.0112	0.0112				
	Human fecal isolates						1				
	Avian fecal isolates										
iss 3	NMEC		0.3022	0.0429	0.0023	0.0112	0.0004				
	UPEC			0.322	0.0178	0.1318	0.0126				
	APEC				0.0667	0.6916	0.1306				
	NTEC					0.1403	0.4942				
	Human fecal isolates						0.3226				
	Avian fecal isolates										
bor	NMEC		1	0.0006	<0.0001	0.3744	0.0021				
	UPEC			0.0006	<0.0001	0.3744	0.0021				
	APEC				0.099	0.012	0.7355				
	NTEC					0.0003	0.0548				
	Human fecal isolates						0.0421				
	Avian fecal isolates										
Any *iss* type	UPEC			0.38	0.7407	0.2922	0.0932				
	APEC				0.3304	0.0533	0.011				
	NTEC					0.8645	0.486				
	Human fecal isolates						0.5337				
	Avian fecal isolates										
iss 1	A								0.5977	0.9035	0.7584
	B1									0.4328	0.8521
	B2										0.5085
	D										
iss 2	A								0.6336	0.3149	0.0626
	B1									0.1396	0.3031
	B2										0.0015
	D										
iss 3	A								0.0027	0.1047	0.1352
	B1									<0.0001	0.0818
	B2										0.0013
	D										
bor	A								0.4445	<0.0001	0.0003
	B1									0.0002	0.0239
	B2										0.4772
	D										
Any *iss* type	A								0.2859	0.4784	1
	B1									0.076	0.2728
	B2										0.4455
	D										

coli genomes are sequenced so that these primer sets may be refined accordingly.

Proposed evolution of *iss* and *bor*. Based upon our analyses of the *iss* and *bor* genes and a comparison of the prophage elements on which they reside (Fig. 7), we propose a scheme for the evolution of *iss* (Fig. 8). It appears that the different *iss*- and *bor*-containing prophage elements may have been derived from a common λ phage precursor containing *bor*. A major

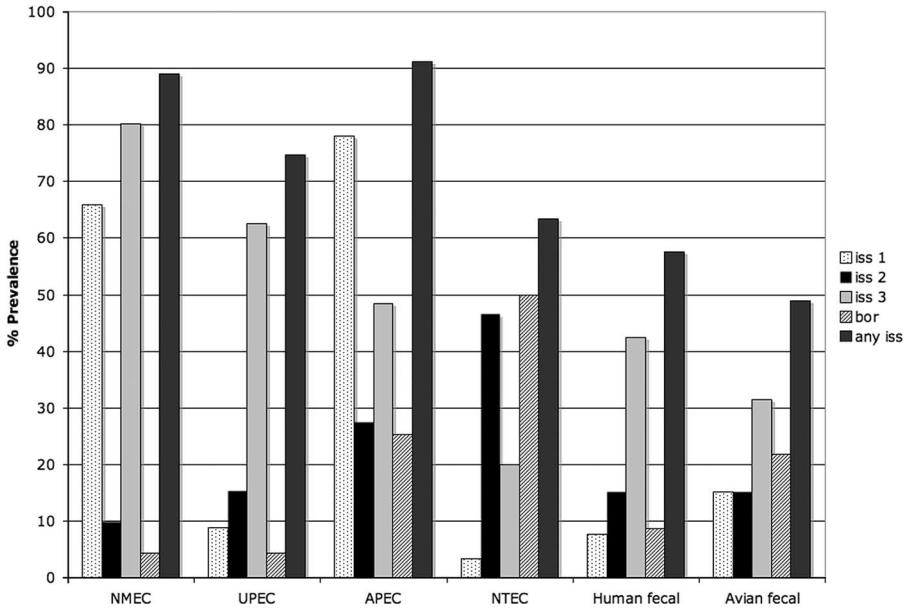

FIG. 4. Prevalence of the three *iss* types and the *bor* gene among *E. coli* populations.

evolutionary divergence likely occurred at an early time point when two phage types apparently split from the λ phage ancestor. Subsequently, the *bor*-containing phage precursor may have inserted into the genomes of K-12 and EHEC/EPEC, possibly at different time points. It is also likely that a further divergence of the *bor*-containing phage occurred prior to this integration to form the DLP12 phage of K-12 and the Shiga toxin (Stx)-like phage of the EHEC strains. These phage then apparently introduced *bor* into the *E. coli* chromosome in separate events. Another important event was the evolution of *bor* into *iss*. This likely occurred on a separate phage element after its early divergence from the λ phage ancestor. This *iss*-containing phage then integrated into multiple *E. coli* loci, in the ExPEC chromosome at one locus and in EAEC/ETEC chromosomes at other loci. In the ExPEC chromosome, *iss* type 3 was introduced by a phage that also appears to have carried the Sit iron and manganese transport system (48, 49, 51). The association of both *iss* types 1 and 3 with Sit provides further evidence that a common phage element harboring *iss* (and Sit) was responsible for the evolution of the three *iss* alleles. A final key event in the history of *iss* was its integration into the ColV/BM plasmid. The significance of this recombinational event, possibly involving IS*2* elements, was that *iss* then became readily transmissible to

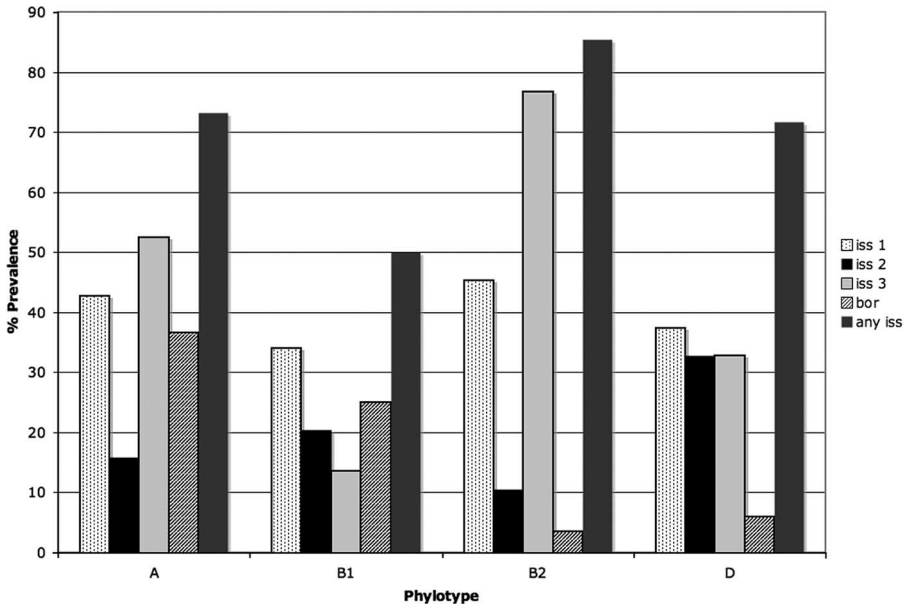

FIG. 5. Prevalence of the three *iss* types and *bor* among *E. coli* phylotypes.

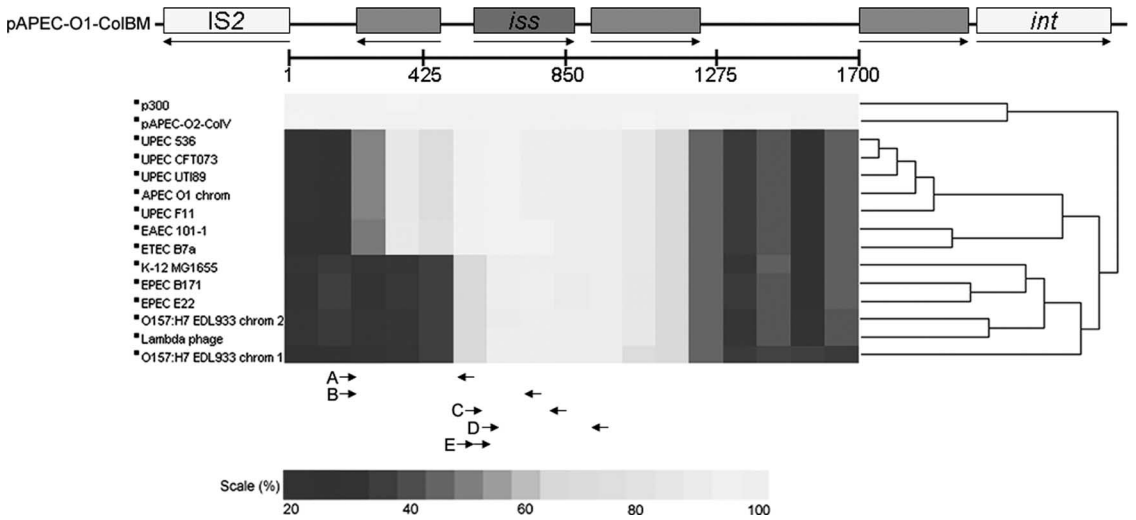

FIG. 6. Nucleotide homology comparison of 15 *bor*- and *iss*-containing regions to that of pAPEC-O1-ColBM (33). Genes are indicated by rectangles, with their orientation depicted by arrows. The scale below the genes depicts the 1,700-bp region in which BLASTn was performed. Below the homology map, primers are given from several recent studies: A to C for *iss* in PCR studies (20, 32, 44, 46, 47); D for *bor* in this PCR study; and E for *iss* in an oligonucleotide-based microarray study (1).

enteric bacterial recipients in the gut. Overall, these key evolutionary events have shaped the emergence of the three allelic *iss* variants.

As mentioned above, in addition to the introduction of *iss* type 3 on prophage, some ExPEC genomes have also been affected by the acquisition of the ColV/BM plasmids harboring *iss* type 1. This *iss* type likely evolved independently of the chromosomally acquired *iss* types, and the introduction of *iss* could have involved recombination between an ancestral plasmid type and one of the different phage types harboring *iss* or *bor*. A recent study by Jeziorowski and Gordon supported the idea that *iss* type 1 is significantly associated with the presence of genes of the ColV/BM PAI and that the conserved region of this PAI has arisen in multiple plasmid types (ColV, ColBM, and ColIa) on separate occasions (29). Overall, the incorporation of *iss*-containing phage and plasmids into the ExPEC

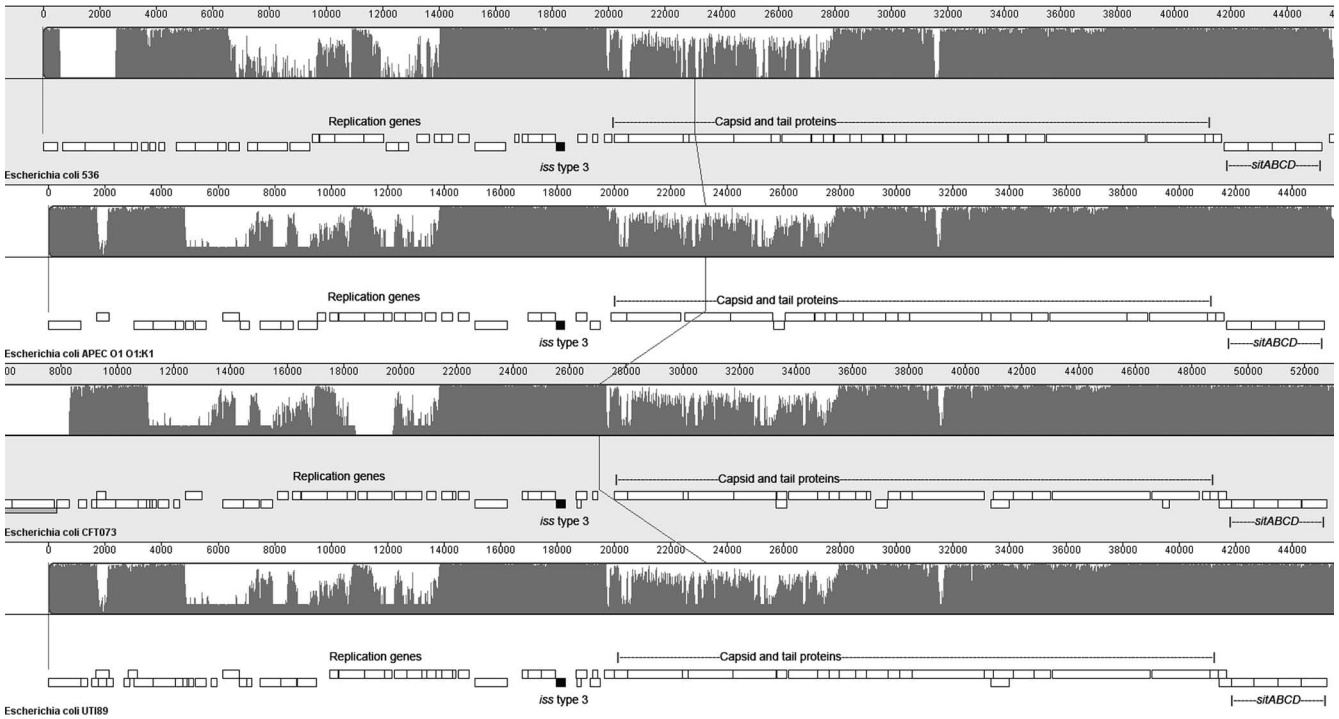

FIG. 7. Nucleotide alignment of ExPEC strain *iss*-containing prophage using MAUVE software (15). The *iss* type 3 gene is shaded black. The overall homology is indicated by spiked lines across sequences. The vertical line indicates the center of each sequence.

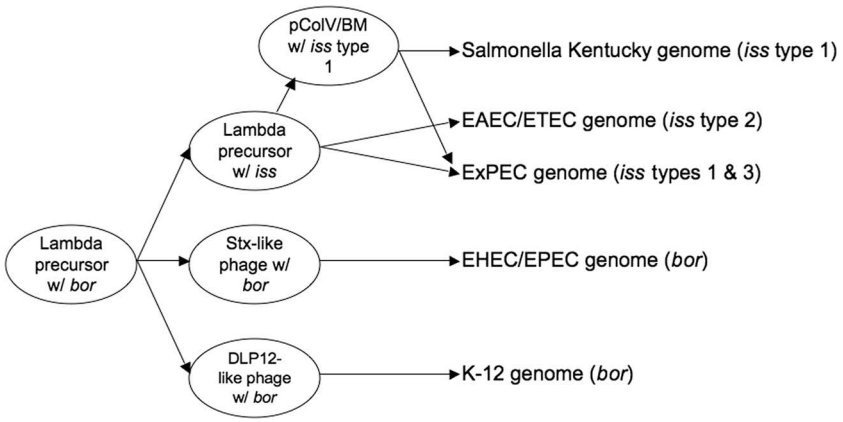

FIG. 8. Proposed evolution of the *iss* types and *bor*. Phage types carrying the *iss* types or *bor* are thought to have evolved from a common λ phage precursor. The *iss* variants were likely introduced by a similar phage element at different points in time, with the differences in the three *iss* alleles due to their independent evolution postintegration.

genome may have contributed to its evolution toward an extraintestinal lifestyle.

The K-12, EHEC, and EPEC genomes harboring *bor* on prophage likely arose independently of any *iss*-containing phage or prophage. The K-12 genome contains *bor* within the DLP12 prophage, which may be the closest relative of all the sequenced prophage to a λ phage precursor. Similarly, the EHEC and EPEC strains appear to have acquired *bor* from Stx-like phage arising from a common λ phage precursor. Interestingly, sequenced EHEC strains possess multiple copies of *bor* on different prophages. One loci of prophage insertion is the same that is occupied by ExPEC prophage harboring *iss* type 3. The other locus of insertion for EHEC *bor*-containing prophage is the same as that occupied by an ETEC B7a prophage containing *iss* type 2. Speculations aside, it is evident that the *bor*-containing prophage, the *iss* type 2-containing prophage, and the *iss* type 3-containing prophage are distinct from one another and appear to have arisen independently of one another via acquisitional events. Also apparent is that there appears to be certain hot spots within the *E. coli* genome for phage integration, as evidenced here by the apparent integration of multiple phage types within three loci at different points in time.

Finally, there is evidence that the dissemination of *iss* via horizontal gene transfer continues. The recent emergence of *Salmonella* serovar Kentucky strains among poultry and the strains' presence among cattle and swine and their presence within retail meats have been reported without plausible explanation (4, 26, 36, 37, 41, 42, 45, 52, 55, 59). However, recent genome sequencing efforts have identified the ColV plasmid as a component of the genome of a multidrug-resistant *Salmonella* serovar Kentucky isolate (GenBank accession no. NZ_ABAK00000000). This plasmid, harboring a PAI containing *iss* type 1, was very similar to the plasmids possessed by APEC strains. The spread of this plasmid, its PAI, and the *iss* gene to non-*E. coli* strains is of particular concern because these elements encode virulence-related traits, fitness, and multidrug resistance. Perhaps recent transfer events have resulted in the acquisition of ColV plasmids by some *Salmonella* species, and such an acquisition is responsible for the increased prevalence of *Salmonella* serovar Kentucky among production animals and within retail meats. The apparent emergence of *Salmonella* serovar Kentucky strains might have implications for human health. It is therefore tempting to speculate that the introduction of the ColV plasmids might have resulted in an increased pathogenic and zoonotic potential for these strains (14, 37, 55). Such findings emphasize the need for continued monitoring of changes occurring in the food production environments.

While the identification of multiple *iss* alleles among *E. coli* genomes is interesting from an evolutionary standpoint, what is its significance? It has been reported that both Iss and Bor confer complement resistance. However, Lynne et al. recently reported that an *iss* mutant resulted in a significantly greater attenuation of complement resistance capabilities than did a *bor* mutation in the same strain (39). Therefore, it appears that Iss and Bor confer different biological properties on their hosts. Whether these differences in conferred properties are due to structural differences between Iss and Bor remains to be addressed. An alternative explanation for the differences between Iss and Bor could be their different genomic locations. Perhaps the ColV/BM plasmid-borne *iss* type 1 undergoes different regulation than the chromosomal *iss* types, resulting in a differentially expressed protein. Future work may answer these and other related questions.

In sum, this study provides an explanation for the recent discrepancies observed while screening for the presence of *iss* among *E. coli* strains. Surprisingly, many ExPEC strains have *iss* within their chromosomes, and screening for the different *iss* alleles results in an enhanced detection of *iss* among *E. coli* strains. Analysis of these allelic variants helps to shed light on the evolution of *iss* and perhaps on the evolution of *E. coli* virulence in general. The different *iss* types appear to have evolved from a *bor*-containing phage precursor, with several key events leading to the current *iss* alleles present on different prophage elements and conjugative plasmids. From these analyses, it also appears that more ExPEC strains possess *iss* than previously thought. Because *iss* is highly prevalent among ExPEC and other pathotypes, is transmitted on mobile ele-

ments, and has been implicated in *E. coli* virulence, further analysis of the *iss* types should be undertaken to explore the possibility that this gene and the protein it encodes are useful diagnostic tools or vaccine targets for the prevention of various ExPEC-caused diseases.

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