Escherichia albertii Pathogenesis

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ABSTRACT *Escherichia albertii* is an emerging enteropathogen of humans and many avian species. This bacterium is a close relative of *Escherichia coli* and has been frequently misidentified as enteropathogenic or enterohemorrhagic *E. coli* due to their similarity in phenotypic and genetic features, such as various biochemical properties and the possession of a type III secretion system encoded by the locus of enterocyte effacement. This pathogen causes outbreaks of gastroenteritis, and some strains produce Shiga toxin. Although many genetic and phenotypic studies have been published and the genome sequences of more than 200 *E. albertii* strains are now available, the clinical significance of this species is not yet fully understood. The apparent zoonotic nature of the disease requires a deeper understanding of the transmission routes and mechanisms of *E. albertii* to develop effective measures to control its transmission and infection. Here, we review the current knowledge of the phylogenic relationship of *E. albertii* with other *Escherichia* species and the biochemical and genetic properties of *E. albertii*, with particular emphasis on the repertoire of virulence factors and the mechanisms of pathogenicity, and we hope this provides a basis for future studies of this important emerging enteropathogen.

HISTORY, NOMENCLATURE, AND CLASSIFICATION

Escherichia albertii is a Gram-negative, facultative anaerobic bacillus that belongs to the family *Enterobacteriaceae*. The first representative of the species was isolated from a 9-month-old diarrheic child in Bangladesh (strain 19982; referred to as the type strain in this manuscript), where it was classified by biochemical assays as *Hafnia alvei* (1). Afterward, six additional *H. alvei* strains were isolated from diarrheic children in Bangladesh (2). These strains resembled enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) in that they promoted attaching-effacing (AE) lesions in rabbit ileum and carried the *eae* gene encoding intimin (locus of enterocyte effacement [LEE]-encoded adhesin), which is responsible for the intimate bacterial attachment seen in these lesions. Subsequently, the *eae*-positive (*eae+*) *H. alvei* isolates were reclassified as a novel species in the genus *Escherichia*

based on biochemical and genetic features ([3](#)–[6](#)) and named *E. albertii* in recognition of M. John Albert, who described the first isolate of the species ([7](#)).

The difficulty in discriminating *E. albertii* from other species of *Enterobacteriaceae* by biochemical methods, as well as the presence of the *eae* gene, explains the frequent misidentification of *E. albertii* as EPEC or EHEC ([8](#)–[10](#)). Unfortunately, although *E. albertii* has now been recognized as a human enteropathogen as well as an avian pathogen ([7](#), [11](#)), misidentification of *E. albertii* strains is still a concern due to their underappreciation as an emerging agent of diarrhea ([12](#)).

PHYLOGENETIC POSITION IN THE GENUS *ESCHERICHIA* AND INTRASPECIES PHYLOGENOMICS

In the early genomic era, multilocus sequence analysis of seven housekeeping genes was employed to infer that *E. albertii* is phylogenetically distinct from other *Escherichia* species and cryptic clades ([8](#), [11](#), [13](#)). Recently, phylogenetic analysis of 34 genome-sequenced *E. albertii* strains with 64 genomes of other *Escherichia* species and cryptic clades was performed based on the sequences of 111 single-copy genes that are fully conserved in these *Escherichia* species/clade strains ([14](#)). This analysis provided clear evidence showing the phylogenetic position of *E. albertii* in the genus *Escherichia* ([Fig. 1](#)). Average nucleotide identities (ANIs) between *E. albertii* and other *Escherichia* species range from 86 to 90%, while those among *E. albertii* strains are >98% ([14](#)–[16](#)), supporting the classification of *E. albertii* as a distinct species in the genus *Escherichia*.

In the above-mentioned phylogenomic study ([14](#)), which analyzed a relatively small number of *E. albertii* strains, the strains were divided into five phylogroups. However, a more recent study analyzing a much larger number of genome-sequenced *E. albertii* strains ($n = 225$) based on the sequences of 2,128 core genes provided a more comprehensive view of the phylogenetic diversity and population structure of *E. albertii* ([17](#)). The 225 *E. albertii* strains sequenced so far are divided into two clades (clades 1 and 2), but the presence of multiple deep branching sublineages is also evident in each clade, particularly in clade 2 ([Fig. 2](#)).

PHENOTYPIC FEATURES

Although the original Bangladeshi *E. albertii* strains shared various biochemical properties with *E. coli*, they
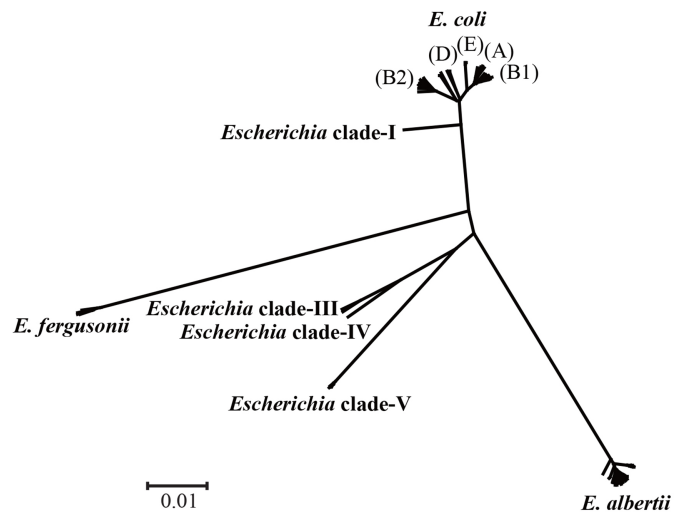

Figure 1 The phylogenetic position of *E. albertii* in the genus *Escherichia*. The neighbor-joining tree was constructed using the sequences of 111 single-copy genes that are fully conserved in the analyzed genomes of *E. albertii* ($n = 34$), *E. coli* ($n = 44$), *E. fergusonii* ($n = 5$), and other *Escherichia* species ($n = 15$). These genes show a low probability of recombination. The labels "A, B1, B2, D, and E" in *E. coli* indicate the major phylogroups of *E. coli*. These phylogroups include *E. coli* strains of various pathotypes. The figure was taken from our published article ([14](#)) with modifications.

differed from typical *E. coli* strains in that they were all nonmotile and unable to produce indole from tryptophan or to hydrolyze lactose ([5](#), [7](#), [18](#)). As novel isolates from different sources and geographic regions were identified, biochemical properties shared by and variable between *E. albertii* isolates have become evident ([5](#), [7](#), [8](#), [10](#), [14](#), [18](#), [19](#)). For example, although all isolates are nonmotile at ~35°C and the large majority do not ferment lactose, some *E. albertii* strains have been shown to produce tryptophanase, an enzyme that catalyzes L-tryptophan degradation, resulting in indole production ([10](#), [11](#)). Other features shared by most *E. albertii* strains are the inability to use citrate as the only carbon source, the production of β-D-glucuronidase, and the use of xylose, cellobiose, adonitol, myoinositol, and 2-ketogluconate ([8](#), [18](#), [19](#)). Moreover, *E. albertii* strains are negative for oxidase, Voges-Proskauer test, and hydrogen sulfide production, and they are unable to promote oxidative deamination of L-tryptophan or to hydrolyze urea.

In addition, a biogroup classification has been proposed, and three biogroups have been described so far: biogroup 1 (indole-negative/lysine-positive), biogroup 2 (indole-positive/lysine-negative), and biogroup 3 (indole-positive/lysine-positive) ([20](#), [21](#)). Consequently, it has been recommended that nonmotile isolates, which are negative
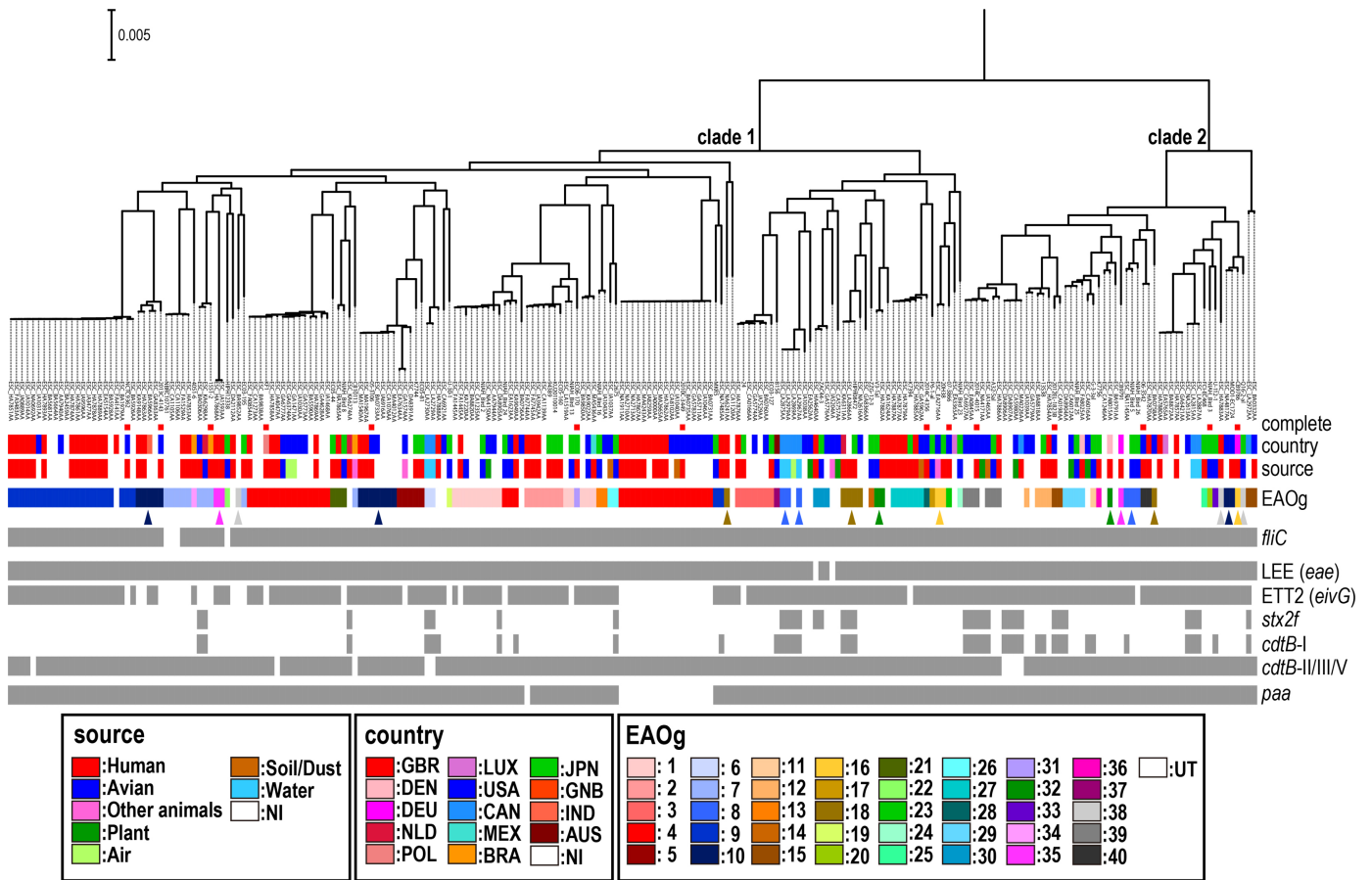
Figure 2 The phylogenetic relationship of the 225 *E. albertii* strains genome-sequenced so far. The maximum-likelihood tree was reconstructed based on the core gene sequences ([17](#)). Strain names are indicated at each tip, and 14 completely sequenced strains are indicated. The information on the geographic distribution and isolation sources of the strains and the distribution of 40 EAOgs and virulence-related genes are also shown. The 7 EAOgs identified in both clades are indicated by triangles. The presence of LEE and ETT2 regions were determined by a tblastn search of the *eae* and *eivG* genes, respectively, as marker genes for each region.

for hydrogen sulfide production, xylose fermentation, and β-glucuronidase activity, with profiles of indole and lysine decarboxylase production consistent with one of the three biogroups, should be further analyzed using *E. albertii*-specific PCR assays ([20](#)). This biotyping may be useful for identifying *E. albertii* in diagnostic laboratories. It may be important to investigate this biogroup classification scheme in the context of phylogeny in the future.

DIVERSITY AND GENOTYPING OF O-ANTIGEN GENE CLUSTERS

The chemical composition and structure of O-antigen are highly variable even in the same species ([22](#), [23](#)), and this variation has long been used for serotyping of strains in many Gram-negative bacteria. Although it is known that some *E. albertii* strains exhibit a genetic or serological similarity to known *E. coli*/*Shigella* serotypes ([13](#), [24](#),

[25](#)), no serotyping systems specific to *E. albertii* are available. Recently, on the basis of the genetic structures of O-antigen gene clusters (O-AGCs), 40 *E. albertii* O-genotypes (named EAOg1 to EAOg40) were defined ([17](#), [25](#)–[30](#)), 8 of which (EAOg1 to EAOg8) were characterized for their chemical structures ([25](#)–[30](#)). A multiplex PCR-based O-genotyping system for *E. albertii* has also been developed on the basis of these findings ([17](#)).

Analysis of the 40 EAOgs revealed that as many as 20 EAOgs showed significant genetic and serological similarities to the O-AGCs of known *E. coli*/*Shigella* serotypes ([17](#)). Intriguingly, eight of the 20 EAOgs exhibited particularly high nucleotide sequence identity (mostly >98%) to their *E. coli*/*Shigella* counterparts ([Fig. 3](#)). This finding indicates the recent interspecies transfer of these O-AGCs between *E. albertii* and *E. coli* because the ANI values between *E. albertii* and *E. coli* are much lower than 98%
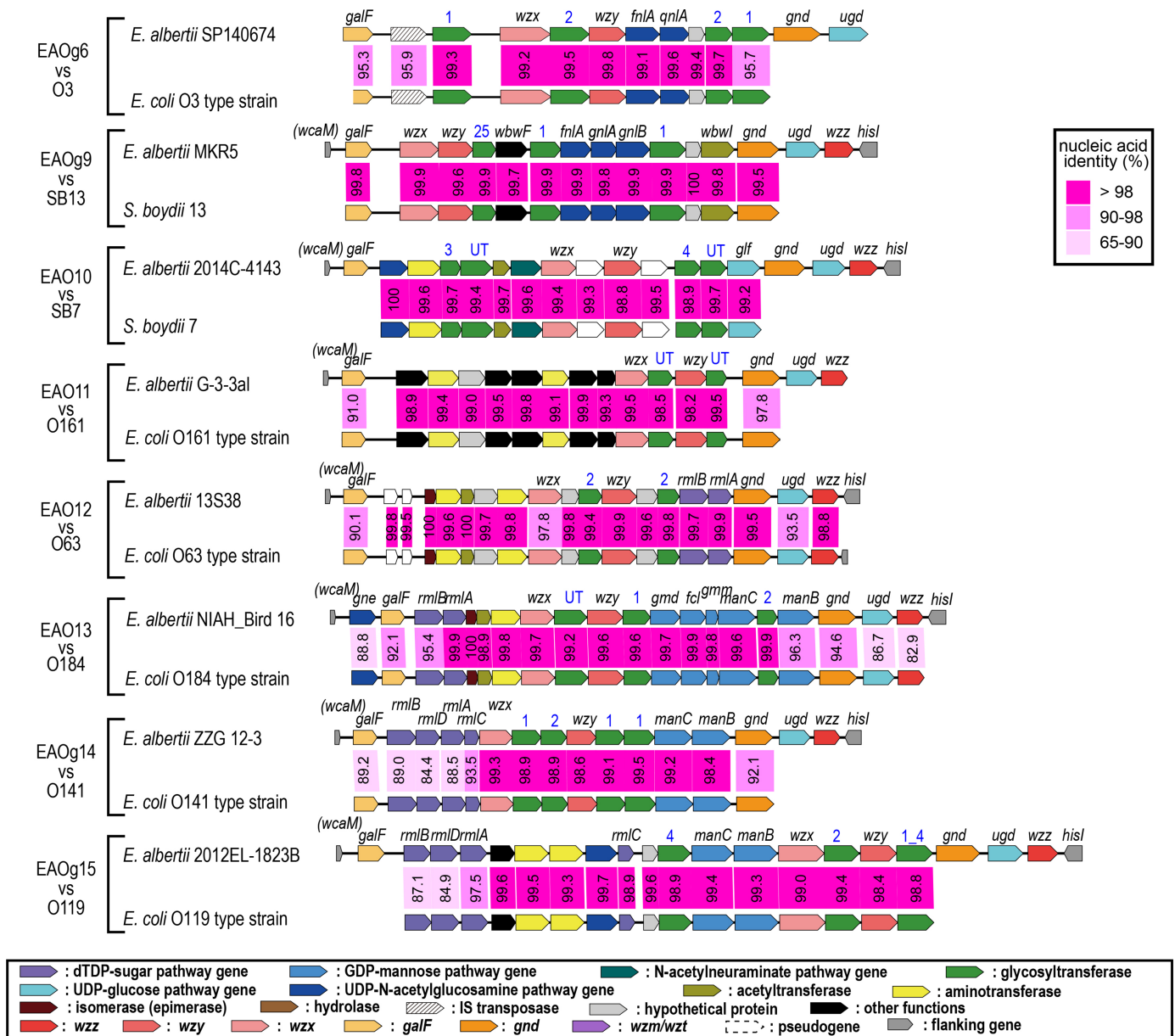
Figure 3 Eight EAOgs highly homologous to the O-AGCs of known *E. coli/Shigella* O-serotypes. Shading and numbers between O-AGCs indicate the nucleotide sequence identities (%) of each gene. The figure was taken from our published article (17) with modifications.

(around 90%). The remaining 20 EAOgs showed no or low similarity in genetic structure and/or nucleotide sequence identity with those of *Escherichia/Shigella* lineages, including *Escherichia fergusonii* and the cryptic clades of *Escherichia* species. Thus, they can be regarded as being unique to *E. albertii* in the current database. In addition, analysis of the distribution of the 40 EAOgs in the entire *E. albertii* lineage revealed that seven of them were distributed in two phylogenetically distinct clades (Fig. 2), suggesting a frequent intraspecies transfer of O-AGCs in *E. albertii* (17).

The multiplex PCR-based O-genotyping system for *E. albertii* (EAO-genotyping PCR) was developed based on the finding that the *wzx* genes are highly divergent in sequence between the 40 EAOgs. This system is similar to that for *E. coli* (31) but includes the primers to detect an *E. albertii*-specific gene (17) to distinguish *E. albertii* from *E. coli* and other species, because the *wzx* sequences of several EAOgs show >95% identity to those of *E. coli/Shigella* serotypes. An *in silico* EAO-genotyping analysis using this system successfully genotyped 81.2% of 186 *E. albertii* genome-sequenced strains (151/186), which

were isolated in various countries and registered in the EnteroBase website ([32](#)). A PCR-genotyping analysis of 92 Japanese *E. albertii* strains also genotyped 82.6% of the tested strains (76/92), demonstrating the utility of this O-genotyping system. However, the fact that approximately 18% of the strains could not be assigned to any of the 40 EAOgs in both evaluation tests indicates that the system needs to be further improved. Analyses of untyped strains are required not only to improve the current O-genotyping system but also to better understand the diversity of O-AGCs in *E. albertii*.

DISEASES, CLINICAL SIGNIFICANCE, AND EPIDEMIOLOGY

Since *E. albertii* strains are currently unidentified or misidentified as *E. coli*, *H. alvei*, *Shigella boydii*, or *Yersinia ruckeri* by biochemical tests ([1](#), [8](#), [18](#)), limited information is available regarding the clinical relevance, prevalence, reservoirs, transmission routes, and epidemiology of the species.

A strong correlation between *E. albertii* and disease outbreaks has been demonstrated in humans ([8](#), [14](#), [33](#)–[35](#)). In some of these outbreaks, the agent was mistakenly identified as EPEC or EHEC in initial investigations ([8](#), [34](#), [36](#)). Furthermore, a report suggested that *E. albertii* can cause bacteremia, since a strain was isolated from the blood of a febrile 76-year-old woman who had multiple comorbidities, including gastric dysplasia, but this patient recovered without complications ([37](#)). This finding suggests that *E. albertii* may have alternative portals of entry because the bacteremic patient had no recent history of diarrhea or gastrointestinal discomfort and had no recent contact with potential *E. albertii* reservoirs, such as infected humans and birds ([37](#)).

In addition to humans, *E. albertii* has been implicated as an etiologic agent of disease in diverse domestic and migratory species of birds, occasionally causing epidemics worldwide ([11](#), [38](#)). As *E. albertii* can colonize various healthy wild bird species ([38](#)), it has been suggested that *E. albertii* may be maintained by subclinical bird carriers and cause disease outbreaks in birds under circumstances of elevated bacterial burdens or enhanced stress ([11](#)).

The first patient confirmed as carrying *E. albertii* exhibited symptoms associated with gastroenteritis, including watery diarrhea, mild dehydration, abdominal distension, fever, and vomiting ([1](#)). Later studies of patients infected with *E. albertii* reported similar symptoms, with nausea being a nonnegligible symptom ([34](#), [39](#)). The incubation period for human infections by *E. albertii* has been estimated to be in the range of 12 to 24 h ([39](#)).

Studies conducted so far have shown that *E. albertii* strains are potentially both food- and waterborne enteric pathogens, since they have been isolated from chicken, processed meat food products (pork, duck, mutton) ([34](#), [40](#), [41](#)), and contaminated water ([36](#)). *E. albertii* strains have also been detected in water distribution systems ([42](#)). Some outbreaks have been suspected to be transmitted by boxed lunches ([34](#)) and restaurant foods ([39](#), [43](#)), with chickens being one of the key vehicles in the transmission of *E. albertii* to humans ([38](#)).

GENOMIC FEATURES

As of November 2019, 14 complete and 229 draft genome sequences of *E. albertii* have been deposited in public databases ([14](#), [17](#), [32](#), [44](#)–[47](#)). Since the 14 completely sequenced strains are widely distributed in the entire phylogeny of *E. albertii*, as shown in [Fig. 2](#), these strains represent roughly the entire *E. albertii* lineage.

General Genomic Features

[Table 1](#) summarizes the general genomic features of the 14 complete genomes. Their chromosome size ranges from 4,551 to 4,940 kb (median, 4,805 kb), and thus, the genomes are relatively smaller than those of *E. coli* (those of 44 completely sequenced strains range from 4,639 to 5,697 kb with a median of 5,132 kb), as suggested by an early genomic comparison ([14](#)). The estimated genome size of the 229 draft genomes ranges from 4,401 to 5,321 kb (median, 4,810 kb), but some genomes may include plasmid sequences. The GC content is approximately 50.0%, very similar to that of *E. coli*.

Plasmids

Among the 14 completely sequenced strains, eight strains possess one to six plasmids (4 to 137 kb in length). Although the genetic features of these plasmids, such as replicon type and similarity to plasmids of other species, have not yet been well characterized, three plasmids encode multiple antimicrobial resistance genes ([Table 1](#)). In other strains, it has been reported that three of the six Bangladeshi strains carried one large plasmid each (80 or 130 MDa), and two of the three strains additionally harbored three small plasmids of different sizes ([2](#)), although

TABLE 1 Summary of the genome information of 14 completely genome-sequenced *E. albertii* strains

Strain name	Source	Country	Clade	*stx2f*[a]	Intactness of the ETT2 region	Total genome size (kb)	Chromosome			Plasmid(s)			Reference
							Size (kb)	% GC content	Accession no.	Size (kb)	Antimicrobial resistance-related genes	Accession no.	
KF1	Human	Bangladesh	1	–	No	4,874	4,702	49.7	CP007025	85, 77, 6, 4		Not available	45
CB9786	Human	Germany	2	–	Yes	4,599	4,599	49.8	AP014856				14
NIAH_Bird 3	Bird	Japan	2	–	Yes	4,561	4,561	49.8	AP014855				14
EC06-170	Human	Japan	1	–	Yes	4,657	4,657	49.8	AP014857				14
1551-2	Human	Brazil	1	–	No	4,731	4,731	49.9	CP025317				44
2014C-4356	Human	USA	1	–	Yes	5,337	4,852	49.8	CP024282	128, 124, 114, 60, 40, 19	128 [*sul1, aadA*, AAC (3)-Via, *tet(A)*] 124 [APH (6)-Id, APH(3'')-Ib]	CP024283-CP024288	46
2010C-3449	Human	USA	1	–	No	4,924	4,924	50.0	CP034212				47
NCTC 9362	Human	Egypt	1	–	No	4,591	4,551	50.2	CP034213	40		CP034214	47
2013C-4143	Human	USA	1	–	No	4,660	4,660	49.8	CP030787				47
05-3106	Human	USA	1	–	Yes	4,858	4,720	49.8	CP030778	81, 57	81 [*sul1*, TEM-1, *dfrA15, tet(A)*]	CP030779, CP030780	47
2012EL-1823B	Human	USA	2	+	Yes	5,097	4,810	49.7	CP030783	106, 100, 81		CP030784-CP030786	47
07-3866	Human	USA	1	–	Yes	5,044	4,940	49.8	CP030781	104		CP030782	47
2014C-4015	Human	USA	2	+	Yes	4,921	4,624	49.8	CP034166	137, 96, 64		CP034164, CP034165, CP034167	47
06-3542	Human	USA	2	–	Yes	4,805	4,709	49.8	CP034162	96		CP034163	47

[a]–, negative; +, positive.

the first *E. albertii* strain identified in Bangladesh was devoid of plasmids ([1](#)). Analysis of six Brazilian *E. albertii* strains showed that only one strain (0621-6/89) harbored two plasmids, a 200-kb (pMLP1; IncHI2) and a 120 kb (pMLP2) plasmid ([10](#)). pMLP1 encodes a tetracycline resistance and a functional conjugation system, and pMPL2 possesses two virulence-related genes, *sitA* and *traT*, which encode an iron uptake-associated protein necessary for the virulence of *Salmonella enterica* serovar Typhimurium in infected BALB/c mice ([48](#)) and an outer membrane protein that mediates serum resistance ([49](#)), respectively. Altogether, these data demonstrate that *E. albertii* strains can carry virulence- and antibiotic resistance-encoding plasmids, some of which may be self-transferable by conjugation, thus contributing to the acquisition and spread of novel phenotypes to other *E. albertii* and even *E. coli* strains.

Other Mobile Genetic Elements

Other mobile genetic elements, such as prophages, other integrative elements, and insertion sequences, have been analyzed in only three complete genomes ([14](#)). This analysis identified relatively small numbers of prophages (4 to 7 per strain) and insertion sequences (7 to 11 copies per strain), but detailed analyses of other complete genomes are required to understand the overall features of the mobilome of *E. albertii*.

VIRULENCE-RELATED GENE REPERTOIRES

Type III Secretion Systems

In addition to the LEE-encoded type III section system (T3SS) responsible for the ability of *E. albertii* to form AE lesions, genome analysis has revealed that *E. albertii* strains contain a gene cluster encoding another T3SS called *E. coli* type III secretion system 2 (ETT2) ([14](#)).

LEE is highly conserved among *E. albertii* and is absent in only a very limited number of strains ([Fig. 2](#)). All *E. albertii* strains examined so far contain LEE at the *pheU* locus ([14](#)), showing a clear contrast to EPEC and EHEC strains, in which LEEs are present at either the *pheU*, *pheV*, or *selC* locus, dependent on the lineages of the strains. Although the integration site and the genetic structure of the LEE core region are highly conserved in *E. albertii*, there is a significant sequence variation in the *eae* gene, which is widely used as a genetic marker of LEE ([8](#)). In EPEC and EHEC strains, the sequence variation in the *eae* gene is used for subtyping the LEEs (intimin

subtypes) ([50](#)). Importantly, many of the intimin subtypes identified in *E. albertii* strains have been rare subtypes in EPEC/EHEC or novel subtypes (not identified in EPEC/EHEC) ([8](#)). However, since a limited number of *E. albertii* strains have been examined for their intimin subtypes so far, more systematic analysis is required to fully understand the diversity of intimin subtypes in *E. albertii*. As for the repertoires of LEE-encoded T3SS-dependent effectors, *E. albertii* strains contain a large set of effector genes, like EPEC and EHEC strains; for example, the genome analysis of 29 *E. albertii* strains identified 19 to 51 genes in each strain (38 on average) ([14](#)), which is comparable to those in EPEC and EHEC ([51](#)–[53](#)). In EPEC and EHEC, many of the effectors are encoded on prophages and integrative elements, and these effectors are called non-LEE effectors ([51](#)–[53](#)). However, more than half of the non-LEE effector-encoding loci identified in three completely sequenced *E. albertii* strains have been located in chromosomal regions showing no signatures of prophages and integrative elements ([14](#)). This feature indicates the possibility that the LEE-encoded T3SS of *E. albertii* has an evolutionary history different from (and/or longer than) that of EPEC and EHEC.

ETT2, which is related, but distantly, to the *Salmonella* T3SS encoded on *Salmonella* pathogenicity island 1, was originally identified as a cryptic second T3SS in the *E. coli*/*Shigella* lineage ([54](#)). However, the ETT2 locus has been highly degraded in most *E. coli* strains ([55](#)). An entero-aggregative *E. coli* strain (EAEC strain 042) appears to possess a nearly complete ETT2 region, but one gene (*eivJ*) is apparently disrupted. In contrast, many *E. albertii* strains contain an apparently intact ETT2 locus, which is shown in [Fig. 4A](#) in comparison with the ETT2 loci of representative *E. coli* strains ([14](#)). Among the 243 genome-sequenced *E. albertii* strains ([Fig. 2](#)), ETT2 is widely distributed in *E. albertii* as inferred by the presence of the *eivG* gene, which was shown to be highly conserved in the *E. albertii* ETT2 loci ([14](#)). However, compared to LEE, ETT2 shows a poorer conservation. In addition, an *E. albertii* strain lacking ETT2 has been isolated from a patient with diarrhea ([10](#)). Therefore, the roles of ETT2 in the pathogenicity and physiology of *E. albertii* remain unknown despite the expression of ETT2 genes being experimentally demonstrated ([14](#)). Effectors secreted by ETT2 are also yet to be identified.

Shiga Toxins

Some *E. albertii* strains possess the *stx* genes encoding Shiga toxins (Stxs). Although Stxs are classified into
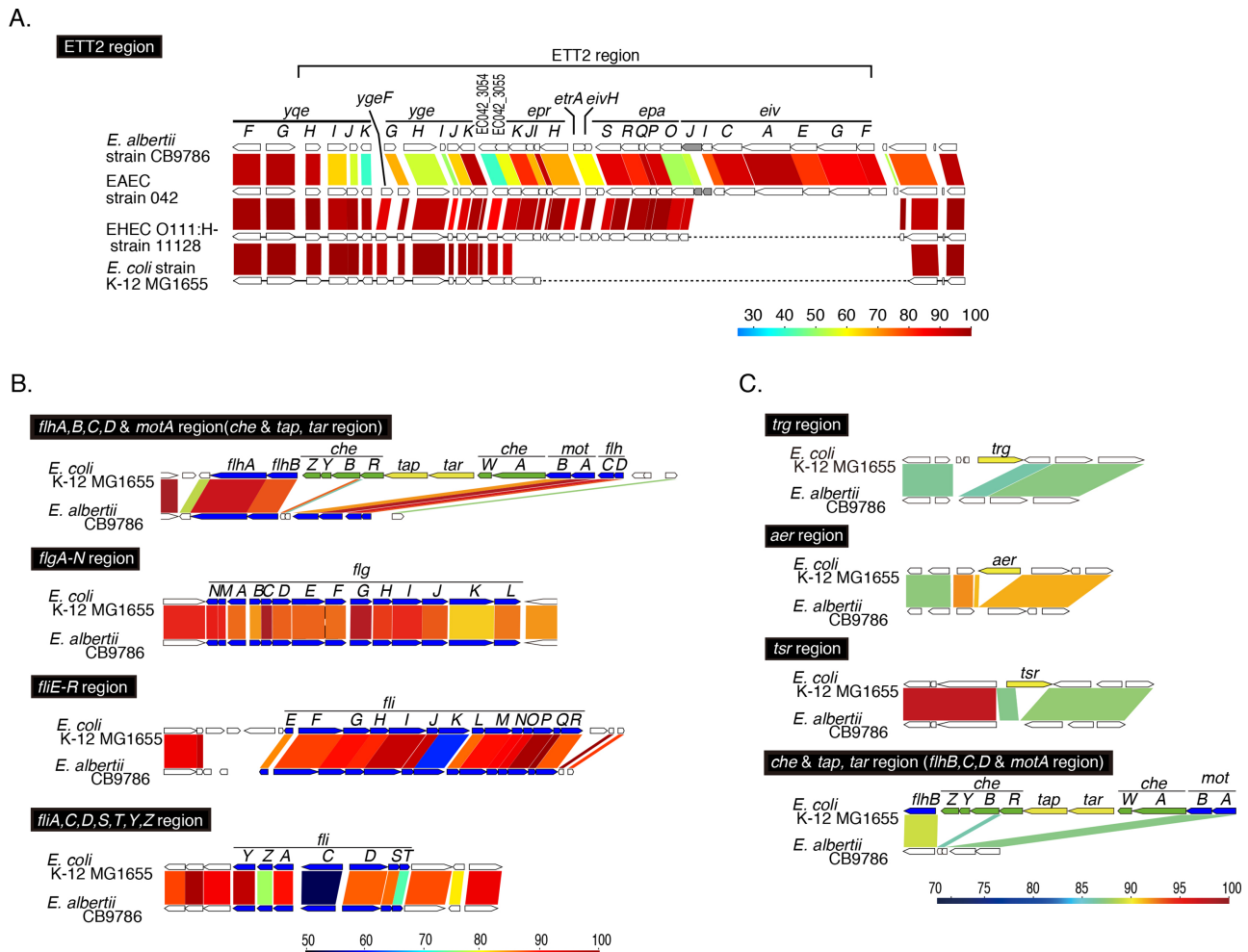
Figure 4 Comparison of the gene organizations of the ETT2 region (A) and the genomic loci encoding flagellar biosynthesis- and chemotaxis-related genes (B and C, respectively) between *E. albertii* and *E. coli*. The amino acid sequence identities (A and C) and the nucleotide sequence identities (B) are presented. The dashed lines in (A) indicate the regions missing in each strain. The figure was taken from our published article (14) with modifications.

two types, Stx1 (subtyped as 1a, 1c, and 1d) and Stx2 (subtyped as 2a, 2b, 2c, 2d, 2e, 2f, and 2g) (56), all *stx*-positive *E. albertii* strains reported so far contain the *stx2f* gene (8, 57–60), with the single exception of an *stx2a*-positive isolate in Norway (61). Consistent with these observations, the 243 genome-sequenced *E. albertii* strains include 32 *stx2f*-positive strains but no *stx2a*-positive strains (Fig. 2). Although the *stx* genes are exclusively encoded on prophage genomes in *E. coli*, the genomic locations of the *stx2f* genes in *E. albertii* have not been investigated. However, sporadic distribution of the *stx2f* gene across the entire *E. albertii* lineage (Fig. 2) suggests that it is also carried by mobile genetic elements. In fact, the *stx2f* genes in the two completely sequenced strains in Table 1 are located in prophage regions (our unpublished observation).

Other Potential Virulence Factors

Cytolethal distending toxin (CDT), which is encoded by the *cdtABC* operon, was originally identified in *E. coli* (62, 63) and has been classified into five subtypes on the basis of sequence variation in the *cdtB* gene (*cdtB*-I to *cdtB*-V) (64). Although the *cdtABC* operon is present in only a subset of *E. coli* strains, most *E. albertii* strains possess the *cdtB* gene belonging to the II/III/V subtype group, and some strains possess a second *cdtB* gene, which belongs to subtype I (8, 10, 11, 13, 65). In fact, among the 243 genome-sequenced strains, 232 (95%) possess the *cdtB* gene belonging to the *cdtB*-II/III/V subtype group, and only 37 (15%) contain the *cdtB*-I gene (Fig. 2). Intriguingly, Fig. 2 clearly indicates that the presence of the *cdtB*-I and *stx2f* genes is highly correlated, suggesting the colocalization of these genes on the same mobile genetic element.

Consistent with this presumption, the two above-mentioned completely sequenced strains carrying the *stx2f* gene also contain the *cdtB-I* gene, and these two genes are located in the same prophage region in both strains (our unpublished observation).

The *paa* gene, which encodes the porcine attaching-effacing associated protein, was also originally identified in porcine EPEC (66). Although this gene is present in a subset of EPEC and ETEC strains, it is highly conserved in *E. albertii* strains (10). Among the 243 genome-sequenced *E. albertii* strains, only 22 strains lack the *paa* gene. Although ETT2 is missing in many of the 22 strains, the *paa* gene is not linked to the ETT2 locus, and most *paa*/ETT2 double-negative strains are very closely related strains isolated in the United Kingdom and the United States (Fig. 2).

Genes for Flagellar Biosynthesis and Chemotaxis

As described in the section on phenotypic features, *E. albertii* is known to be nonmotile (8, 11, 19, 57). However, gene clusters related to flagellar biosynthesis and its regulation are conserved in most *E. albertii* strains (Fig. 4B) (14). Among these genes, the *fliC* gene, which encodes flagellin, the major subunit of flagella, has remarkable sequence diversity (65 to 85%) between strains, suggesting that flagellin is under immunological selection in the host. Expression of these genes was also experimentally confirmed (14). Curiously, however, the genes encoding chemotaxis-related proteins (CheA-Z), four methyl-accepting chemotaxis proteins, and the aerotaxis receptor protein (Aer) identified in *E. coli* are not present in *E. albertii* strains, indicating that these genes are selectively missing in *E. albertii* (Fig. 4C). These data suggested that, while *E. albertii* is nonmotile under standard culture conditions, it may express flagella or flagella-related structures in some environments or hosts (14). This hypothesis has been proven by two very recent studies (67, 68) showing that flagella are produced at lower temperatures as well as in media containing low concentrations of nutrients. It is still unknown whether *E. albertii* displays chemotaxis or chemotaxis-like behavior.

Genes for Metabolism

As also described in the section on phenotypic features, the inabilities to ferment xylose, lactose, or rhamnose and to produce β-D-glucuronidase are common biochemical properties of this species (8, 19, 57). These properties are useful for discriminating *E. albertii* from *E. coli*, although some strains are positive for lactose fermentation. Consistent with these phenotypes, most *E. albertii* strains lack the genes responsible for these metabolic functions, namely the *xylBAFGHR*, *rhaMDABSRT*, and *uidCBAR* operons (14). Interestingly, while the *lacA/Y/I* genes are missing, the *lacZ* gene selectively remains; however, its physiological function is unknown.

PATHOGENESIS

To prove that the *E. albertii* type strain, which was obtained from the stool sample of a diarrheic child, could cause diarrhea, Albert and coworkers used the RITARD (removable intestinal tie-adult rabbit diarrhea) assay, which employs an animal model previously used to investigate the diarrheagenic properties of a variety of microorganisms, such as *Vibrio cholerae*, ETEC, and *Aeromonas* (69, 70). In this assay, the type strain produced diarrhea in 8 of 12 rabbits. Moreover, by oral inoculation, it caused diarrhea in 1 of the 2 rabbits tested. In both assays, the strain colonized the gut with displacement of the microbiota (1).

Adherence to Epithelial Cells

After the potential of *E. albertii* to cause diarrhea was confirmed in animal models (1), its ability to adhere to epithelial cells was the first virulence property identified in this pathogen (1, 2). This property was initially explored in a study that used the type strain and six additional Bangladeshi isolates, obtained from diarrheic children, and the pattern of adherence observed in HeLa cells was referred to as "poor localized adherence (LA/P)," which is now more commonly known as "localized adherence-like (LA-like)." The LA-like pattern is characterized by the formation of loose bacterial microcolonies on the surface of infected epithelial cells in the assays performed with an extended period of bacterial contact with HeLa cells (71). The term "LA-like" was created to discriminate the compact bacterial microcolonies (localized adherence [LA]) which are formed by typical EPEC strains on the surface of epithelial cells after 3 hours of infection (72) from the loose microcolonies formed by atypical EPEC strains after an extended period of incubation (6 hours) (71). The LA phenotype exhibited by typical EPEC strains is mediated by a type IV pilus termed the bundle-forming pilus, and the proteins involved in its biogenesis are encoded by the *bfp* operon located in the EPEC adherence factor plasmid (73, 74). On the other hand, the LA-like phenotype is mainly

mediated by the interaction of the adhesin intimin with its receptor Tir (translocated intimin receptor) ([75]), which is one of the effector proteins translocated to the host cells by the LEE-encoded T3SS ([76], [77]). A recent study demonstrated that half of six *E. albertii* strains isolated from Brazilian children produced the LA-like pattern, while the other three produced more compact bacterial microcolonies (LA) in prolonged assays ([10]). In previous studies performed with strain 1551-2, which is also a Brazilian strain isolated from a diarrheic child, the latter adherence pattern was termed LA_6 to discriminate it from the LA exhibited by typical EPEC strains within 3 hours of bacteria-cell contact ([78], [79]). It is also important to mention that strain 1551-2 can effectively adhere to polarized and differentiated intestinal Caco-2 ([9], [80]) and T84 cells ([81]).

To identify the bacterial structure(s) involved in the LA_6 phenotype, strain 1551-2 has been analyzed in detail. This strain was also initially classified as atypical EPEC ([82]) and was later shown to be devoid of the various adhesins identified in the EPEC and Shiga toxin-producing *E. coli* pathotypes ([78], [82]). Since this strain harbors LEE and, thus, the *eae* gene, the contribution of intimin to the establishment of the LA_6 phenotype was first investigated, and the *eae* mutant of strain 1551-2 was found to lack the ability to form compact bacterial microcolonies, and it adhered to HeLa cells in a diffuse adherence (DA) pattern ([79]). Quantitative adherence assays performed with HeLa cells demonstrated that the adhesiveness of the *eae* mutant was reduced by 33% compared with the wild-type strain ([83]). Moreover, the diffuse adherence exhibited by the *eae* mutant was found to be dependent on the T3SS-translocon since the 1551-2 strain with a mutation in the *escN* gene (encoding an ATPase for T3SS biogenesis) showed a 99% reduction in adhesion to HeLa cells compared with the wild-type strain ([83]). As the T3SS-translocon filament encoded by the LEE is composed by EspA ([84]), the observation that an *eae/espA* double mutant of strain 1551-2 displayed a reduction of 99% in its adherence capacity compared with the *eae* single mutant further confirmed the importance of the T3SS-translocon in epithelial cell colonization by this strain ([83]). However, further studies are still necessary to better understand whether this adhesion phenotype mediated by the T3SS-translocon is specific to strain 1551-2 or could be inferred to other *E. albertii* strains.

Regarding the environmental factors that could affect the adherence of the 1551-2 strain to HeLa cells, the effect of different glucose (5 and 25 mM) and CO_2 (0.03 and 5%) concentrations has been evaluated. The data suggested that a CO_2-enriched atmosphere favors the adhesion of strain 1551-2 since the number of adherent bacteria was significantly higher in the higher concentration of CO_2 (5%). In addition, a high glucose concentration changed the original LA_6 pattern to a mixed LA_6 and aggregative adherence-like (AA-like) pattern. The AA pattern, which is characterized by the formation of bacterial aggregates that resemble stacked bricks on both HeLa cells and surrounding glass surfaces, was originally described in *E. coli* strains of the EAEC pathotype ([85]). The AA-like pattern produced by strain 1551-2 in a high glucose concentration was attributable to the enhanced production of type 1 pilus (T1P), which could allow bacteria to access more extensive intestinal colonization sites in the host during the infectious process ([86]).

Besides the above-mentioned studies performed with cultured epithelial cells, the adhesiveness of strain 1551-2 has been examined by using an *in vitro* organ culture (IVOC). In this assay, the strain was found to colonize ileal fragments of Wistar rats when infected with 10^{10} CFU ([9]). Moreover, it was demonstrated that the interaction of *E. albertii* with epithelial cells in the IVOC model is dependent on a functional T3SS, since the ability of the *escN* mutant to colonize ileal fragments was drastically reduced ([9]).

Formation of AE Lesions

Moon and coworkers demonstrated that three human EPEC strains were able to attach intimately to intestinal epithelial cells and efface microvilli in both the rabbit and pig intestines ([87]). In that study, the formation of pedestal-like structures was observed by electron microscopy on the surface epithelium of cecum from a gnotobiotic pig infected with a human EPEC strain. These characteristic histopathological lesions are nowadays known as attaching and effacing, or AE, lesions. As already mentioned, the ability of the *E. albertii* type strain to induce AE lesions was demonstrated by using an *in vivo* rabbit ileal loop assay ([1]).

One of the most characteristic features of AE lesions in both intestinal mucosa and tissue culture cells infected with EPEC and EHEC strains is the presence of dense microfilaments beneath the intimately attached bacteria ([87]). Knutton and coworkers first suggested that these microfilaments are mainly composed of F-actin, which

results from the reorganization of the brush border cytoskeleton during AE lesion formation (88). The hypothesis was confirmed with an assay using fluorescein-labeled phalloidin to detect F-actin accumulation underneath adherent bacteria (89). This assay, termed the fluorescence-actin staining (FAS) test, provides a highly sensitive test for *in vitro* detection of AE pathogens. In the FAS assay, the type strain and other six Bangladeshi strains showed spots of intense fluorescence underneath adherent bacteria (FAS-positive), thus indicating their ability to accumulate F-actin at the site of adherence, which indirectly indicated their potential to induce AE lesion formation on the infected epithelial cells. The ability of all seven isolates to produce these lesions was subsequently confirmed *in vivo* by using the rabbit ileal loop model (2). Moreover, it was recently observed that all six Brazilian *E. albertii* strains evaluated, including

strain 1551-2, are positive in the FAS assay (10), and the ability of strain 1551-2 to induce AE lesions was evidenced *in vivo* by using the rabbit ileal loop model (Fig. 5; Mônica A. M. Vieira and Tânia A. T. Gomes, unpublished data) and IVOC (9).

Similar to the human *E. albertii* strains, at least four strains isolated from dead birds have been shown to be FAS-positive in HEp-2 cells, one of which (EC370-98) induced AE lesions as observed by transmission electron microscopy (TEM) (90). However, postinfection tissues recovered from 1-day-old specific-pathogen-free chicks inoculated with 1×10^9 CFU of two bird isolates (including EC370-98) demonstrated mild hyperplasia of Peyer's patch but not classical AE lesion formation, even though sparse bacterial microcolonies were found attached to the mucosa in the colon (90). These findings

Figure 5 Transmission electron microscopy (TEM) image of rabbit ileal loop inoculated with the *E. albertii* 1551-2 strain. After 8 hours of inoculation, microvilli effacement and pedestal-like structures (asterisk) underneath adherent bacteria were demonstrated, thus confirming the ability of strain 1551-2 to induce AE lesion formation *in vivo*.

suggest that the chick model does not faithfully reproduce the disease seen in birds from different species or different ages or that other disease mechanisms need to be considered for bird *E. albertii* strains ([90]).

Invasion

TEM analysis of HeLa cells infected with strain 1551-2 that produced the LA_6 pattern revealed the occurrence of bacteria-containing vacuoles ([79]), suggesting that some *E. albertii* isolates could invade epithelial cells. Interestingly, strain 1551-2 in the intracellular compartment promoted actin accumulation around the vacuoles ([79]), and the TEM images of Caco-2 cells infected with strain 1551-2 revealed that bacteria in individual vacuoles formed pedestals ([80]). Quantitative invasion assays have shown that this strain is almost three times more invasive in HeLa cells than strain JPN15, a spontaneous EPEC adherence factor plasmid-deletion mutant of strain E2348/69, the prototype of typical EPEC ([79]). Moreover, in the same study, it was demonstrated that an *eae*-mutant of strain 1551-2 remained adherent to HeLa cells but was no longer invasive. Subsequent studies demonstrated that this strain was able to invade polarized and differentiated intestinal T84 ([81]) and Caco-2 cells ([80]) as well and that its ability to invade Caco-2 cells was dependent on the intimin-Tir interaction ([9]).

Bacterial infections can induce a transitory epithelial barrier destabilization to allow neutrophil migration and exposition of receptors at the basolateral side of epithelial cells, which may facilitate enterocyte invasion ([91]). It has been demonstrated that disruption of tight junctions by ethylene glycol-bis(β-aminoethyl)-*N,N,N′,N′*-tetraacetic acid (EGTA) treatment enhances the ability of the *E. albertii* strain 1551-2 to invade T84 cells, suggesting that the basolateral side of infected cells is a preferential invasion pathway of strain 1551-2 ([81]). The type strain was also shown to decrease transepithelial electrical resistance in polarized intestinal T84 cells by redistributing the tight junction's protein, zona occludens-1, leading to enhanced cell permeability ([92]). Besides the 1551-2 strain, an invasive phenotype was also observed in three other Brazilian *E. albertii* strains, two of which were able to persist inside enterocytes for up to 24 hours ([10]). Although the invasion ability of more strains from other sources needs to be investigated, an intracellular location may protect bacteria from clearance from the intestine and the immune system, which can prolong the diarrheal disease caused by invasive *E. albertii* strains.

Translocation across Intestinal Mucosa

The passage of bacteria and their products from the intestinal lumen to the mesenteric lymph nodes and occasionally to other extraintestinal sites is defined as bacterial translocation ([93]). The first evidence of the ability of *E. albertii* to translocate to extraintestinal sites was provided in an *in vivo* 1-day-old specific-pathogen-free chick model with inoculation of two bird strains, which were originally misclassified as *E. coli* serotype O86:K61 ([11], [90]). The two strains were recovered from the liver and spleen of the animals at 24 hours of oral inoculation ([90]). To investigate if human *E. albertii* strains were able to translocate the intestinal mucosa, the 1551-2 strain was used to inoculate Wistar-EPM rats by oroduodenal catheterization, with the bacteria being confined between the duodenum and ileum with ligatures ([9]). After 2 hours of incubation, the strain was recovered from the mesenteric lymph nodes and liver, though not from the spleen, indicating its ability to translocate the intestinal barrier. Furthermore, the 1551-2 *escN* mutant, which is T3SS-deficient, failed to cross the intestinal barrier, probably due to its reduced capability to adhere to the intestinal mucosa, as observed in the IVOC model ([9]) and/or the inability to secrete T3SS-dependent effector proteins, such as EspF, Map, and NleA, which have been associated with the disruption of tight junctions in the intestinal barrier ([94]–[96]).

The facts that the 1551-2 strain invades through the basolateral side more effectively than through the apical side of polarized and differentiated T84 cells *in vitro* ([81]) and that it can translocate the mucosal barrier of rats *in vivo* ([9]) have raised the idea that *E. albertii* might be able to cross M cells. By using an *in vitro* M cell model ([97]), it was found that the transcytosis of strain 1551-2 occurred more readily in M-like cells than in Caco-2 cells, and this phenotype was partially dependent on a functional T3SS ([9]). Conversely, an isogenic T1P-deficient mutant strain retained its ability to cross M-like cells ([9]), in contrast to the observation that the transcytosis of *E. coli* K-12 through M cells is dependent on this fimbrial adhesin ([98]). Additionally, it has been described that a mutation in the *ibeA* (invasion of the brain endothelium protein A) gene in the adherent-invasive *E. coli* strain NRG857c significantly reduced its transcytosis across M-like cells ([99]). Considering that the 1551-2 strain is devoid of *ibeA*, these data suggest that bacterial transcytosis through M cells is at least partially linked to the T3SS in this strain ([9]).

Toxin Production

To date, CDT and Stx are the only types of toxins that have been reported in *E. albertii* strains. CDTs are genotoxins that induce DNA double-strand breaks in both proliferating and nonproliferating cells and are produced by several Gram-negative bacteria, such as *E. coli* and *Shigella* spp. ([100](#)), *Campylobacter jejuni* ([101](#)), *Haemophilus ducreyi* ([102](#)), *Actinobacillus actinomycetemcomitans* ([103](#)), and *Helicobacter* spp. ([104](#)). CDT induces nuclear distension in infected cells due to an irreversible cell cycle arrest at the G1 or G2 phase and can kill target cells. CDT consists of three subunits (CdtA, CdtB, and CdtC), with CdtB corresponding to the active subunit, while CdtA and CdtC form a heterodimeric subunit required for binding and intracellular delivery of CdtB to the target cells ([105](#)–[108](#)). So far, the contribution of CDT to the pathogenesis of *E. albertii* has been demonstrated only *in vitro*. HeLa cells exposed to protein extracts of five distinct Bangladeshi *E. albertii* strains exhibited typical cytoplasmic distension and nuclear fragmentation, which are genotoxic features associated with CDT ([13](#)). Additionally, a recent study demonstrated that 88% (15/17) of *E. albertii* strains that were examined by the cytotoxicity assay in Chinese hamster ovary cells induced a typical CDT-associated cytotoxic effect, confirming CDT production by these strains ([109](#)).

Stx inhibits protein synthesis in eukaryotic cells, and its production was originally identified in *Shigella dysenteriae* type 1 ([110](#)) and later in Shiga toxin-producing *E. coli* strains ([111](#)). Certain *E. albertii* strains produce Stx2f, as demonstrated by a cytotoxicity assay using Vero (African green monkey kidney) cells ([8](#), [57](#), [59](#), [60](#)). In addition, one *E. albertii* strain producing the Stx2a subtype was obtained from a 48-year-old patient with bloody diarrhea ([61](#)). It has been reported that the production level of Stx2f by *E. albertii* strain AKT5 was similar to that of a prototype EHEC O157:H7 strain (Sakai) by a cytotoxicity assay, suggesting that the AKT5 strain may have the potential to cause severe diseases in infected patients ([109](#)).

Adherence to Abiotic Surface (Biofilm Formation)

Biofilms are complex communities of microorganisms that adhere to biotic or abiotic surfaces and are confined in an extracellular matrix ([112](#), [113](#)). Microbes living in biofilms may resist the action of antibacterial agents such as antibiotics, antibodies, and phagocytic cells and the mechanical movements exerted by intestinal peristalsis ([112](#), [114](#)). In recent studies analyzing Brazilian *E. albertii* strains, four of the six strains tested, including strain 1551-2, were shown to form biofilm on polystyrene plates ([10](#), [83](#)). Analysis of several mutants derived from strain 1551-2 revealed that a mutant of the *fimA* gene, which encodes the major subunit of T1P, showed a significantly reduced efficacy in biofilm formation on abiotic surfaces ([83](#)), indicating the involvement of T1P in this phenotype. However, additional components or structures may also be required for effective biofilm formation on abiotic surfaces. Biofilm formation may help *E. albertii* to persist in the environment and foods and may even contribute to prolonging diarrhea caused by *E. albertii*, although further investigations are required.

CONCLUSIONS

Even though *E. albertii* strains continue to be misidentified, many reports have shown the relevance of the species in human and animal (mainly avian) diseases. Furthermore, the apparent zoonotic character of the disease suggests the importance and requirement of stricter *E. albertii* transmission control measures. To this end, a deeper understanding of the mechanisms of the pathogenicity and the virulence potential of *E. albertii* is necessary, but unfortunately, very few data are available regarding these aspects, as most studies have been conducted with very few strains from limited geographical areas.

On the other hand, increasing amounts of sequence data on the complete or draft genomes of dozens of strains are becoming available, which have provided excellent information about the virulence potential of the species and their diversity. These data have revealed that *E. albertii* carries a large collection of virulence-related genes, many of which are shared with other AE pathogens, mainly EPEC and EHEC. The functionalities of these genes and their roles in pathogenicity *in vivo* are largely yet to be investigated in *E. albertii*. However, in addition to LEE, the *cdtB* gene belonging to the *cdtB*-II/III/V subtype group and the *paa* gene are common traits in most *E. albertii* strains isolated in different geographic regions. Importantly, the presence of large self-transmissible plasmids, although not very common, could contribute to the diversification of *E. albertii* and, perhaps, further adaptation to different hosts. Also, virulence genes that

allow certain *E. coli* strains to survive in the extraintestinal milieu have been identified in some *E. albertii* strains, which could contribute to their survival, which has been demonstrated for at least one *E. albertii* strain *in vivo*.

Much remains to be studied regarding the virulence mechanisms and potentials of *E. albertii*, and more studies analyzing the mechanisms of colonization, survival, and dissemination within and between hosts are needed. Studies focusing on the mechanisms that regulate the expression and accumulation of virulence- and host adaptation-related genes are also lacking and need to be considered. Accordingly, large collections of strains from diverse regions, isolated from animals, humans, and various environments, need to be analyzed to comply with the important concept of "one health" and to have more general knowledge of *E. albertii* biology.

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