RECENT ADVANCES IN VIRULENCE FACTORS OF EMERGING ENTEROPATHOGEN *Escherichia alberii*: A REVIEW

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ABSTRACT

Diarrhea is the leading cause of infant mortality worldwide, especially in the developing world. Among various etiologic agents *within the Escherichia genus, Escherichia albertii is becoming recognized as a significant human enteropathogen*. There has been little knowledge about the colonization and infection of *E. albertii* in comparison to its close relatives, *E. coli*. This bacteria is considered food- and water-borne by birds and is a natural reservoir. Attaching and effacing lesions, which are encoded by a type III secretion system (T3SS) on the locus of enterocyte effacement (LEE), are the primary cause of *E. albertii*'s pathogenicity. Although *E. albertii* contains the gene for Shiga toxin type ecoded by subtype *stx2f* and cytolethal distening toxin (*cdt*), the molecular mechanism of toxin production during infection is still unclear. The hierarchy and the relationship of adhesion and effectors during the invasion of intestinal epithelial cells share similar characteristics to those of gastrointestinal pathogenic *E. coli* bacteria. The review information is essential heplful for investigation to develop effective prevention and treatment for this diarrheal pathogen.

TỔNG QUAN CẬP NHẬT CÁC YẾU TỐ ĐỘC LỰC CỦA VI KHUẨN GÂY BỆNH ĐƯỜNG RUỘT MỚI NỔI *Escherichia alberii*

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TỪ KHÓA

Escherichia albertii
Hệ thống tiết tuýp III
Độc tố Shiga
Nhiễm trùng ruột
Mầm bệnh từ thực phẩm

TÓM TẮT

Tiêu chảy là nguyên nhân hàng đầu gây tử vong ở trẻ em, đặc biệt là ở các nước đang phát triển. Trong số rất nhiều các tác nhân gây bệnh, *Escherichia albertii* là vi khuẩn mới được nhận biết gần đây trong chi *Escherichia* gây bệnh tiêu chảy ở người. So với vi khuẩn *E. coli*, những nghiên cứu và hiểu biết về quá trình xâm nhiễm và gây bệnh của *E. albertii* còn rất hạn chế. Vi khuẩn này được cho là lây nhiễm theo thức ăn và nước uống nhiễm khuẩn và trong tự nhiên thường được phân lập từ gia cầm và chim hoang dã. Nhân tố độc lực chính của *E. albertii* là khả năng tạo tổn thương bám dính AE được quy định bởi hệ thống tiết tuýp III. Mặc dù *E. albertii* có chứa gene quy định độc tố Shiga do gene *stx2f* quy định nhưng cơ chế phân tử của quá trình sản sinh độc tố trong lây nhiễm còn chưa rõ ràng. Thứ tự hoạt động cũng như mối liên quan của các yếu tố bám dính và xâm nhiễm trong quá trình nhân lên ở tế bào biểu mô ruột có nhiều đặc điểm giống với vi khuẩn *E. coli* gây tiêu chảy. Những nội dung cập nhật trong bài viết này là cơ sở hữu ích cho các nghiên cứu tiếp theo nhằm tìm ra giải pháp ngăn ngừa và điều trị hiệu quả mầm bệnh này.

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1. Introduction

Escherichia albertii was first isolated from a 9-month-old Bangladeshi pediatric patient. However, the strain was misclassified as *Hafnia alvei* [1], [2]. Some *A. alvei* strains subsequently isolated from children with diarrhea in Bangladesh shared similar characteristics to pathogenic *E. coli* due to the capability to induce attaching and effacing lesions on rabbit epithelial cells and carry the *eae* gene encoding the intimin protein responsible for this attachment of bacteria [2]. The intimin-positive (*eae*+) *H. alvei* strains were reclassified as new species within the genus *Escherichia* based on biochemical and genetic characteristics [3], [4] and named *E. albertii* for recognition acknowledged by M. John Albert, who described and isolated the first strain of the species [5].

The clinical importance of *E. albertii* as an emerging agent of diarrhea has been underappreciated due to the difficulty of distinguishing it from other *Enterobacteriaceae* species. Closely related genetic and biochemical characteristics between *E. albertii* and *E. coli* hampered the differentiation [6]–[10]. In some outbreaks, *E. albertii* was initially identified as enteropathogenic *E. coli* (EPEC) or enterohemorrhagic *E. coli* (EHEC) [11]–[13]. Thus far, *E. albertii* has been identified in both sporadic infections and outbreaks [12], [14]–[16], and the common clinical symptom is watery diarrhea [1], [16]–[18]. It is assumed that the *E. albertii* infection is associated with food and water contamination [19]. Though the bacteria have been isolated from numerous animal sources [20]–[22], the main natural reservoir is still unclear.

The ability to be an enteropathogen requires the use of adhesin proteins that bring bacteria closer to the epithelia and allow them to colonize the gastrointestine. The hallmark of *E. albertii* infection is the formation of an attaching and effacing (A/E) lesion in the human intestinal mucosa that is similar to other A/E lesion-producing *E. coli* and murine intestinal pathogens (*Citrobacter rodentium*). After that, the toxins and/or effectors are secreted into the host cell through the secretory systems, disrupting bio-physiological processes in the cell and ultimately causing clinical symptoms [23]. The main virulence arsenal of *E. albertii* includes the type three secretion system (T3SS), porcine attaching and effacing lesion (*Paa*), Shiga toxin (*Stx*), and cytolethal distending (*CDT*) [24]. Clarity of the mechanism of how adhesins and toxins work is critical to understanding the pathogenicity of the bacteria as well as solutions for therapeutic intervention.

2. Clinical significance and epidemiology

E. albertii was considered a potentially food- and water-borne pathogen [18]. This species has been found in many types of raw food, such as pork and mutton meat [25], oysters [26], poultry meat [27], milk and cheese [28], and lettuce [29]. In most sporadic cases of infection, the transmission vehicle is commonly not identified. However, there are some outbreaks with confirmed sources of contamination such as salad, water, restaurant food, and lunch boxes [12, 16, 18, 30]. *E. albertii* strains were also isolated from diverse water bodies worldwide, including the water distribution systems [10], [13]. This led to concern about the contamination of seafood and the irrigation system for fresh produce. Despite a strong correlation between E. *albertii* and disease outbreaks in humans, the clinical relevance and transmission cycle remain unexplored.

E. alberti has been isolated from various sources of domestic and wild animals. A high frequency of E. albertii originated from birds and poultry, suggesting a risk for bacterial exposure. *E. albertii* was found in clinically healthy wild birds in Australia [31], Korea [32], and Antarctica [22], dead birds in Scotland [33], Alaska [9], and wild and poultry flocks in Switzerland [34]. Poultry meat might be an important infection vehicle since *E. albertii* was recovered from raw chicken in the USA, Japan, and China [25], [35], [36]. Beside birds, *E. albertii* was also isolated from cattle, pigs, dogs, cats, martens, rabbits, and bats [21], [37], [38]. The occurrence of *E. albertii* in farm and companion animals poses a significant threat for people in close contact since animal- and human-*E. albertii isolates* sometimes are not host-specific [39].

Though the clinical significance of *E. albertii* has not been clearly understood, this bacteria has caused sporadic cases and outbreaks worldwide [15], [39]–[42]. Clinical symptoms caused by *E. albertii* are similar to typical bacterial enteric infections with watery diarrhea, vomiting, fever, abdominal distension, and dehydration [1], [43]. Some strains can produce Shigatoxin, which induces bowel diarrhea [44]. This bacteria was isolated from the blood of a patient with bacteremia [45], [46] and suggested a capability to be a very serious etiologent. *E. albertii* has been first isolated from pediatric patients; the *E. albertii* infection has also been reported in adults of middle and elderly ages [15], [45]. The average incubation period for the E. *albertii* infection was about 12–24 hours [18].

3. Virulence factors

3.1. Type III Secretion System

In *the Escherichia* genus, enterohemorrhagic *E. coli* (EHEC) and enteropathogenic *E. coli (EPEC)* typically employ attaching and effacing (A/E) lesions in intestinal colonization [23]. The (A/E) lesions are characterized by the intimate attachment of bacteria to the epithelial microvilli and form an actin-rich pedestal right beneath the adherence and efface of the brush border microvilli [47]. Similar to *E. coli*, *E. albertii* has a 35–37 kb pathogenicity island (PAI) that is called the locus of enterocyte effacement (LEE) [24], [48]. LEE in *E. albertii* is highly conserved and null in a few strains [14], [48]. Thus far, *PheU* tRNA was the only insertion site of LEE in *E. albertii,* whereas LEE in EHEC and EPEC was present either in *PheU* tRNA, *PheV* tRNA, or *SelC* tRNA. Since LEE is a horizontally acquired pathogenicity island, a single insertion site of LEE in E. albertii suggests a single acquisition in evolutionary history. The typical structure of LEE contains forty-one genes distributed in five polycistronic operons (LEE1–LEE5), a bicistronic operon (*grlAB*), and single gene units [47], [49]. The genomic analysis revealed that the gene composition of LEE in *E. albertii* was highly conserved, with a range of 91–100% prevalence for each gene [48].

The *eae* gene-encoded intimin protein is used as a genetic marker for LEE. Intimin is an outer membrane protein that attaches to the host cell through its translocator, the Tir protein. In EHEC and EPEC, *eae* showed a highly variable sequence in the C-terminal, where the cellular binding site is, and were classified into more than 30 subtypes [50], [51]. There is a prevalent association between *eae*-subtypes, serotypes, and hosts in *E. coli* [50], [52], [53]. In *E. albertii*, many subtypes of *eae* have been recognized, including α8, β3, ε1, ε3, ε4, ξ, z3, τ, ι2, ν, υ, ρ, ρ2, and the novel ones were N1.2, N1.3, N2, N3, N4, N5, α9, α10, β4, γ3, ς3, λ2, o1 [11], [48]. Though some eae-subtypes in *E. albertii* were rare in EPEC and EHEC and even novel subtypes, a limited number of *E. albertii* strains have been isolated and investigated compared to *E. coli*, so the diversity of intimin in *E. albertii* requires more systematic analysis.

LEE encodes for the type three secretion systems that function as molecular machinery to secrete the effector proteins (both LEE-encoded and non-LEE-encoded proteins) into the cytoplasm of the host cells [47]. LEE-encoded translocators (EspA, EspB, and EspD) and effectors (Tir, Map, EspF, EspG, EspH, and EspZ) were also present in 100% of *E. albertii*genomes thus far [14], [47], [48]. However, they exhibit a diverse sequence from EPEC to EHEC. In contrast to LEE-encoded effectors, non-LEE-encoded effectors were found in varying distributions in *E. albertii* genomes [48], [54]. In addition, most non-LEE-effectors were encoded on prophages (PP) and integrative elements (IE) in EHEC [55], whereas *E. albertii* carried fewer PPs and IEs, and some non-LEE-effectors were encoded on chromosomal loci [14]. The non-LEE-enoded effectors had essential functions in forming A/E lesions in EPEC [56]. From these observations, investigation into the regulation and structure-function of genes in LEE in *E. albertii* is essential to understanding the mechanism of initial attachment to intestinal microvilli.

Besides *eae*, LEE5 contains *tir,* which encodes for the intimin translocated receptor Tir protein [47]. Intimin-Tir binding induces the reorganization of the host cell skeleton with F-actin

accumulation underneath the adhering bacteria, resulting in the formation of pedestal-like structures. The in *vivo* investigations confirmed the A/E lesion process in *E. alberii*depended on intimin and its translocator, Tir [57], [58]. The deletion of *eae* and *tir* caused a significant decrease in the invasion index in Caco-2 cells compared to the wild-type *E. albertii* strain 1551-2 [57].

Inducement of F-actin for pedestal formation used two distinct parthways: Tir-Nck dependent and/or Tir-Nck independent [59]–[61]. The F-actin accumulation was observed through the fluorescence-actin staining (FAS) test for Bangladeshi *E. albertii* strains by using the rabbit ileal loop model [2]. The other six Brazilian *E. albertii* strains were also FAS-positive in infected HeLa cells [40]. The A/E lession with pedestal structure caused by *E. albertii* strain 1551-2 on rat intestinal mucosa was observed under scanning electron microscopy [57]. The strain was also able to cross the intestine to reach the liver in *vivo* [57]. A recent study on *E. albertii* strain 1552-1 demonstrated that F-actin polymerization was triggered through a Nck-independent pathway [62]. A new protein, TccP3, colocalized with Tir during the polymerization of F-actin, though it was not found in the Nck-independent pathway previously [62]. The *tccP2* gene is presented in 161 out of 482 *E. albertii* genomes. It would be worth clarifying the function of TccP2 in A/E lesion formation.

3.2. Shiga toxin

Shiga toxin (Stx) was first identified in *Shigella dysenteriae a*nd is present in a subset of EHEC and STEC that is able to produce the toxin. Stxs belong to the group of AB_5 enterotoxins that consists of a single enzymatic A subunit (*N*-glycosidase activity) and five structural B subunits [63]. Shiga toxin inhibits protein synthesis in eukaryotic cells by removing an adenine residue from the 28S rRNA of the 60S ribosome [63]. The five identical B subunits mediate toxin binding to the Gb3 (globotriaosylceramide) receptor of host cells [63]. There are two subtypes of Stxs, including Stx1 and Stx2, that are similar in mechanism but antigenically distinct [64]. The Stx encoding genes are also classified into two types with several subtypes: stx_1 (stx_{1a}, stx_{1c}, stx_{1d}, and stx_{1e}) and fifteen stx_2 subtypes (from $stx2_a$ to $stx2_o$) [65].

The $stx2_f$ variant was first detected in *E. coli* isolated from pigeons, and that study identified 12.5% of pigeon fecal drops as $stx2_f$ positive [66]. Other studies confirmed such a high prevalence of STEC in pigeon-carried $stx2_f$ [67]–[69]. Thus far, all *stx*-positive *E. albertii have* carried $stx2_f$[11], [24], [48], [70]–[73]. Most of the genomic analysis showed $stx2_f$-positive *E. albertii* originated from birds and poultry, suggesting a host-specific pattern. In a genomic analysis of 482 *E. albertii*genomes, a sporadic distribution of $stx2_f$ across phylogenetic tree linages suggested a horizontal transfer of phage-carrying $stx2_f$. There is only one strain $stx2_a$ positive [74]. Genomic location, structure, and regulatory controls of phage-carrying $stx2_f$ in *E. albertii* have not been investigated.

In *E. coli*, the STEC carrying T3SS and $stx2_a$ was considered highly virulence-causing hemorrhagic colitis and hemolytic uremic syndrom (HUS) [75]. The only reported $stx2_a$- and *eae*-positive *E. albertii* strain could cause bloody diarrhea, whereas most of the other *eae*-positive *E. albertii* strains caused diarrhea [44]. In STEC with $stx2_f$-induced milder symptoms, no outbreaks have been reported, and only one HUS patient has been reported [76], [77]. That might be associated with weaker binding to Gb3-lipopolysaccharide and less toxicity to vero cells than Stx2a [78]. Few $stx2_f$-*E. albertii* strains were isolated from patients, and the identified symptoms included non-bloody diarrhea [11]. However, Stx2f itself was more stable at low pH and high temperatures than Stx2a [78], suggesting that many other factors might contribute to the differences in toxicity and pathology.

Several *E. albertii strains* carrying $stx2_f$ show biological activities of Stx2f in cytotoxicity assays using vero cells [70], [72], [79]. However, it was unclear about the level of Stx2f expression, and the lethal effect was complicated by the presence of CDT [70].

3.3. Cytolethal distening toxin (CDT)

Cytolethal distening toxin (CDT) is synthesized by more than thirty Gram-negative bacteria, including common gastrointestinal pathogens such as *E. coli, Shigella, Campylobacter, Samonella, Helicobacter, and E. albertii [80–85]. CDT* intoxication is defined by the nuclear and cytoplasmic expansion of mammalian cells, which causes distention and ultimately results in death. An apoptotic response in fibroblast, epithelial, or lymphoblastoid mammalian cells causes CDT-dependent cell death. The AB_2-type genotoxin CDT is a heterotrimeric complex made up of CdtA, CdtB, and CdtC subunits that are encoded by the eponym genes [86], [87]. The catalytic component CdtB exhibits phosphatase and damages host cell DNA through DNase-like activity in vitro [86], [88]. Subunits CdtA and CdtC are responsible for binding to the receptor on susceptible cells and transporting the active subunit into the cytoplasm (CdtB) [89]. CdtB introduces double-stranded breaks that lead to DNA fragments [90].

The toxin is encoded by the *cdtABC* operon that was initially identified in *E. coli* and classified into five subtypes based on the variation of the *cdtB* gene (I to V) [91]. Such a high frequency of *E. albertii* isolate genomes carry the cdtABC locus, mostly belonging to the subgroups *cdtB-II, III, and V*, whereas subgroup *cdtB-I* was less frequent [92]. A new subtype, *cdtB-VI*, has been newly proposed in *E. albertii* [48]. An observed CdtB-II was the dominant type in *E. albertii* at 68% [48], and a reidentification of 20 *cdt-II* gene-positive *E. coli* as *E. alberti suggests* that *CdtB*-II might be a reliable genetic marker.

There is a limitation to *in vivo* investigation of CDT cytotoxicity in *E. albertii* infection. The Hela cells that were exposed to the protein extracted from *E. albertii* cell lysates exhibited the cytoplasmic distension and nuclear fragmentation that are typical of CDT [8]. However, the expression of *cdtB* needed to be confirmed to exclude the contribution of other factors. The biological activity of CDT-I and CDT-II was detected in the distension of vero cells that had been infected by *E. albertii* strain P2660 [70] by specific antibody reactions.

3.4. Other adhesins and effectors

The *paa* gene that is associated with porcine attaching and effacing lesions was presented in a subset of EPEC and enterotoxigenic *E. coli* (ETEC) [93], [94], but was highly prevalent in *E. albertii* [17], [48], [71]. In ETEC, *paa*located plasmids suggested this virulent gene is a mobile genetic element. The paa-negative but eae-positive porcire EPEC O45 showed a positive A/E lesion, suggesting that Paa contributed to the early stage of adherence in the A/E lesion [95]. The functional characteristics of this putative colonization factor in *E. albertii* have not been investigated.

The bundle-forming pilus (BFP) is a large rope-like structure and an important adherence factor of EPEC and is encoded on the AEF plasmid [96]. A subset of 18 *E. albertii* strains carried *bfpA* [48]. In a single *E. albertii* strain, 1552-1 exhibits localized adherence in HeLa/Hep-2 cells by BFP and forms compact microcolonies [58].

In EPEC, a large protein (366 kDa) called LifA is able to inhibit lymphocyte activation [97]. This protein in EHEC, called Efa1, encoded by efa1, is required for adhesive properties in vitro [98]. The screening of *efa1* shows the gene is variably present in *E. albertii* genomes [48].

In addition to T3SS, *E. coli* type III secretion system 2 was found to be a highly conserved virulence factor of *E. albertii*[92]. Using *the eivG* gene as a marker, Ooka et al. (2005) found that ETT2 was commonly distributed in *E. albertii.* This locus in *E. albertii* was apparently intact [14], [48] in comparison with the highly degraded one in *E. coli [99]*. However, ETT2 in some *E. albertii* linages was mostly deleted, with few remaining genes [48]. Although ETT2 is linked to *E. coli*serum resistance, its role in *E. albertii* pathogenicity and the effectors secreted by ETT2 have yet to be determined.

3.5. Biofilm formation

An assembly of microorganisms living in a community and frequently found adhered to solid surfaces in damp environments is called a biofilm [100]. Microbes in a biofilm secrete a variety of protective substances called extracellular polymeric substances (EPS) that immobilize biofilm cells, keep them close, and enhance their survival efficiency [101]. Microbes residing in biofilms have the ability to withstand the effects of mechanical movements caused by intestinal peristalsis as well as antibacterial drugs such as antibiotics, antibodies, and phagocytic cells [102], [103].

The capacity to form biofilm on an abiotic surface was shown in some strains isolated in Brazil [40]. All six tested strains show biofilm on glass surfaces; however, three of the five show biofilm on polystyrene at a higher density. The efficiency of biofilm formation was significantly lower than that of the EAEC 042 strain (positive control). Among the six strains, strain 1551-2, which was mistyped as atypical EPEC, was reclassified as *E. albertii* [40], [58]. An *eae* mutant 1551-2 strain can adhere to HeLa cells in the absence of intimin [104]. The protein TP1-major pili subunit fimA may play a role in biofilm formation since a *fimA* mutant significantly reduces biofilm formation [104]. However, many other surficial proteins of fimbriae, pili, and autotransporters may be involved in biofilm formation [105], [106]. Although further research is needed, biofilm production may aid the bacteria in surviving in the environment and on food, and it may even help to prolong the diarrhea that *E. albertii* causes.

4. Conclusion

This review confirmed the apparent zoonotic characters of the frequently misidentified enteropathogenic *E. albertii*. A deeper understanding of the pathogenesis mechanisms and virulent potentials of *E. albertii* is essential however, the data is still limited in comparison to other pathogenic species in *the essential;ia* genus. However, as more and more sequencing data on the full or draft genomes of several strains becomes accessible, it has become possible to learn a great deal about the diversity and potential virulence of the species. According to these findings, *E. albertii* possesses a sizable number of virulence-related genes, the majority of which are shared by EPEC, EHEC, and other AE pathogens. The common gene-encoded pathogenic traits are *LEE, cdtB, stx2f,* and *paa*. However, more investigation of the bioactivities of these genes is required.

Further understanding of the pathobiology of *E. albertii* and the mechanisms underlying colonization, survival, and dissemination both inside and between hosts requires further research. Studies that address the mechanisms controlling the expression and accumulation of genes linked to virulence and host adaptation should be considered to better gain general knowledge of *E. albertii*.

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