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First report of *Escherichia albertii* in Iran: a case study highlighting diagnostic challenges in pediatric gastroenteritis

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ABSTRACT

Background *Escherichia albertii* is a gram-negative, facultative anaerobic bacillus in the order Enterobacterales, family Enterobacteriaceae, increasingly recognized as an emerging enteropathogen. Initially isolated in 1991 from a child with diarrhea in Bangladesh and misclassified as *Hafnia alvei*, it was reclassified in 2003 and is now acknowledged as the second pathogenic species of the *Escherichia* genus, following *Escherichia coli*.

Case Summary This article reports the first documented case of *E. albertii* in Iran, infecting a 5-year-old girl who presented with profuse watery diarrhea. During a 7-month surveillance study at Akbar Children's Hospital in Mashhad, stool samples from 231 children with diarrhea were analyzed, with this single case demonstrating infection by *E. albertii*. The strain was identified through pentaplex PCR but exhibited phenotypic traits highly similar to other enteropathogens and showed resistance to antibiotics, such as ampicillin and amoxicillin-clavulanate.

Conclusion The study highlights the diagnostic challenges associated with the discrimination between *E. coli* and *E. albertii*, which is often misidentified when solely biochemical tests are used. Our findings and literature data suggest that *E. albertii* should be considered a significant cause of pediatric gastroenteritis, akin to *Shigella* and enteroinvasive *E. coli*. These observations emphasize the need for adequate diagnostic protocols, including PCR testing, to accurately identify *E. albertii* and inform appropriate clinical management strategies. Further research is necessary to deepen our understanding of this emerging pathogen and its implications for human health.

KEYWORDS *Escherichia albertii*, *Escherichia coli*, Iran, children, PCR, antibiotic

Escherichia albertii is a gram-negative, facultative anaerobic bacillus within the Enterobacterales, family Enterobacteriaceae, recognized as an emerging enteropathogen. Initially isolated in 1991 from a 9-month-old child with diarrhea in Bangladesh as *Hafnia alvei* based on biochemical assays (1, 2), subsequent molecular analysis including DNA-DNA hybridization reclassified a group of D-sorbitol- and lactose-negative isolates as a novel species within the *Escherichia* genus in 2003. *E. albertii* has been named in honor of M. John Albert, who described its first isolate (1). This reclassification had demonstrated again the limitation to base taxonomy solely on biochemical tests and highlights the still evolving accuracy in bacterial taxonomy by the application of molecular methodologies such as PCR and whole genome sequence analyses including its significance for accurate identification of isolates in clinical settings.

E. albertii is now acknowledged as the second pathogenic species of the *Escherichia* genus, following *Escherichia coli* (3, 4). Its genomic complexity and close evolutionary

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relationship with both *E. coli* and *Shigella* (which actually belongs to the species *E. coli*) underscore its emerging status as a human and animal enteropathogen (5). With *E. albertii* to be traditionally linked to diarrhea in humans (6), experimental evidence has confirmed its potential to cause gastrointestinal disease, with adherence to epithelial cells identified as a primary virulence trait (7). Infected individuals often present with symptoms typical of gastroenteritis, including watery diarrhea, dehydration, abdominal pain, and, in some cases, fever (8, 9). Besides causing infection in humans, the foodborne pathogen *E. albertii* can also colonize wild and livestock animals, although it was initially not considered a zoonotic pathogen.

The diagnostic challenges posed by *E. albertii* stem from its genetic and phenotypic similarities to other *Escherichia* spp., in particular *E. coli* and *Escherichia fergusonii* (10). However, recently, the presence of a transcriptional activator (EAKF1_ch4033) has been found to be indicative of *E. albertii* (11). The detection of the *eae* gene, also associated with enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *Escherichia coli* (EHEC) strains of *E. coli*, can lead to misidentification of *E. albertii* in routine laboratory tests (12). As a result, *E. albertii* has previously been underreported concomitantly with a lack of awareness regarding its public health implications. This is particularly concerning, given the potential of some *E. albertii* strains to produce Shiga toxin and its association with causing intestinal lesions, which can result in significant morbidity (13–15). The genome of *E. albertii* KF1 is composed of a chromosome of 4,701,875 bp, exhibiting a G + C content of 49.7%, along with four plasmids. The application of multiplex PCR approaches for the identification of various gastrointestinal pathogens, including diarrheagenic *E. coli*, combined with whole genome sequencing for precise identification and typing, has established a more effective and dependable method for the detection and characterization of *E. albertii* (16, 17).

This article reports the first isolation of *E. albertii* from a 5-year-old child in Iran, highlighting its significance as an emerging public health concern. By investigating the prevalence of *E. albertii* in pediatric diarrhea cases, this study seeks to enhance understanding about the occurrence of diarrheal disease caused by *E. albertii* in Iran, the virulence factors involved, and the antimicrobial resistance profiles. The findings, therefore, contribute valuable insights into the epidemiology of this underestimated pathogen, filling a critical gap in knowledge about the pathogenic potential and informing better strategies for prevention and control of related infections in the region.

CASE PRESENTATION

The Akbar Children's Hospital, a public facility in Mashhad, Iran, serves patients from across Khorasan and has an active surveillance program for diarrheal pathogens, including *Salmonella*, *Shigella*, *Vibrio* spp., and *Campylobacter*. Diagnosis and management of acute diarrhea in children follow the guidelines set by the Iranian Ministry of Health and the World Health Organization.

The patient, a 5-year-old girl from Mashhad, was previously healthy until May 1, 2022, when she developed profuse watery diarrhea without blood. She was admitted to the Akbar Children's Hospital on the same day. She had not taken medications prior to disease development and had not been traveling outside Mashhad. Upon admission, tests for *Salmonella* and *Shigella* returned negative. The patient was stable and was discharged after one day in the hospital. However, her stool sample was subsequently sent to the microbiology department for further analysis in the context of a larger study on infectious agents.

Identification

During a 7-month study period, stool samples from 231 children with diarrhea were collected at the Akbar Children's Hospital, but none of the samples tested positive for *E. albertii* aside from this case. The strain isolated from the patient's stool at the hospital produced clear, colorless, circular colonies on MacConkey agar and has subsequently been identified as *E. albertii* using an improved multiplex PCR reaction targeting genes,

the presence of which is discriminatory between relevant *E. coli*, relevant pathovars and *Escherichia* spp., namely *E. coli*, *E. albertii*, *E. fergusonii*, *Shigella* spp., and enteroinvasive *E. coli* (EIEC). This PCR approach includes detection of the gene for the cyclic di-GMP regulator CdgR (discriminatory for *E. coli*), for the DNA-binding transcriptional activator of cysteine biosynthesis EAKF1_ch4033 (specific for *E. albertii*), and for the palmitoleoyl-acyl carrier protein-dependent acyltransferase EFER_0790 (specific for *E. fergusonii*). By targeting *ipaH* and *lacY* the PCR assay additionally discriminated between *Shigella* spp. and EIEC (11, 18).

Upon successful amplification of the PCR product specific for *E. albertii*, the gene for the transcriptional activator EAKF1_ch4033 has been sequenced using the BigDye Terminator Cycle Sequencing Kit on an ABI PRISM 3130 Genetic analyzer (Thermo Fisher Scientific) according to the manufacturer's instruction. EAKF1_ch4033 showed 99% nucleotide sequence identity to the equivalent gene of verified *E. albertii* isolates. The nucleotide sequence has been submitted to GenBank with accession number PV185715.

Moreover, the results of phenotypic and biochemical tests—negative for motility, oxidase, and lactose utilization and positive for catalase, indole, and utilization of mannitol and sorbitol—indicated the isolate being *E. albertii* (Table 1). Some biochemical and even behavioral traits, including sorbitol, lactose, indole, and motility, can, however, vary in *E. albertii*, with some isolates testing positive and others testing negative (19, 20). This variability highlights the diversity within the species. Therefore, these traits should be interpreted with caution when identifying *E. albertii* based on phenotypic tests.

Antimicrobial susceptibility was assessed using the Clinical and Laboratory Standards Institute disk diffusion method, with *E. coli* ATCC 25922 and *Klebsiella pneumoniae* K6 (ATCC 700603) serving as the quality control strain. The *E. albertii* strain was sensitive to trimethoprim-sulfamethoxazole, cefotaxime, meropenem, tetracycline, gentamicin, amikacin, ciprofloxacin, and azithromycin but resistant to ampicillin and amoxicillin-clavulanate. Additionally, we assessed the strain for the presence of 14 resistance-associated genes, including *mcr1-5*, bla_{TEM}, bla_{SHV}, bla_{OXA}, and various *qnr* genes [*qnrA-qnrD*, *qnrS*, and *aac(69)-Ib-cr*] (Table 2). The amplification conditions were similar to previous studies, with minor adjustments to annealing temperatures as needed (21–23). Positive controls were included to confirm the accuracy of the PCR results, using *Klebsiella pneumoniae* (ATCC 700603) and *E. coli* (ATCC 35218), which carry bla_{TEM} and bla_{SHV}, respectively. A number of validated clinical isolates served as the positive controls for bla_{OXA}, *mcr1-5*, and *qnr* genes. Only the bla_{TEM} gene was detected in this strain, while all other resistance genes were absent. Demonstrating consistency between our phenotypic and PCR results, research has shown that the bla_{TEM} gene is commonly found in ampicillin-resistant *E. coli* samples from humans (24, 25).

TABLE 1 Biochemical properties of the identified *E. albertii* strain

Test	Reaction[a]	Test	Reaction
Oxidase	–	Lactose fermentation	–
Catalase	+	Mucate utilization	–
Indole	+	Nitrate reduction	+
Motility	–	Acetate utilization	–
Voges-Proskauer	–	Gelatin hydrolysis	–
Methyl red	+	Eosin methylene blue agar	–
Urease	–	Mannitol salt agar	–
Ortho-nitrophenyl-β-galactoside	+	Xylose fermentation	–
Citrate utilization	–	Deoxyribonuclease	–
Triple sugar iron agar	K/A	Adonitol fermentation	–
Hydrogen sulfide production	–	Mannitol fermentation	+
Lysine decarboxylase	+	D-sorbitol fermentation	+
Bile esculin hydrolysis	–	Salicin fermentation	–
Raffinose fermentation	–		

[a]+, positive reaction; –, negative reaction; K/A, alkaline over acid.

TABLE 2 Primers used for the identification of *E. albertii* AD1 and for detecting the presence of virulence and resistance genes in this study

Target gene	Sequence (5′–3′)[b]	Primer	Amplicon size (bp)	Reference
Species-specific gene				
EAKF1_ch4033	GTAAATAATGCTGGTCAGACGTTA	Ealb_fw	393	(18)
	AGTGTAGAGTATATTGGCAACTTC	Ealb_rev		
Virulence genes				
eae	GACCCGGCACAAGCATAAGC	Eae_fw	384	(26)
	CCACCTGCAGCAACAAGAGG	Eae_rev		
stx1	ATAAATCGCCATTCGTTGACTAC	Stx1_fw	180	(26)
	AGAACGCCCACTGAGATCATC	Stx1_rev		
stx2	GGCACTGTCTGAAACTGCTCC	Stx2_fw	255	(26)
	TCGCCAGTTATCTGACATTCTG	Stx2_rev		
stx2a	GCGATACTG**R**G**B**ACTGTGGCC	Stx2a_fw		(27)
	CCG**K**CAACCTTCACTGTAAATGTG	Stx2a rev1	349	
	GCCACCTTCACTGTGAATGTG	Stx2a rev2	347	
stx2f	AGATTGGGCGTCATTCACTGGTTG	Stx2f fw	428	(14)
	TACTTTAATGGCCGCCCTGTCTCC	Stx2f_rev		
ehly	GCATCATCAAGCGTACGTTCC	ehly_fw	534	(14)
	AATGAGCCAAGCTGGTTAAGCT	ehly_rev		
cnf1,2	TCGTTATAAAATCAAACAGTC	CNF_fw	450	(28)
	CTTTACAATATTGACATGCTG	CNF_rev		
Est	GCTAATGTTGGCAATTTTTATTTCTGTA	Sta_fw	190	(28)
	AGGATTACAACAAAGTTCACAGCAGTAA	Sta_rev		
Let	ATTTACGGCGTTACTATCCTG	LT_fw	280	(28)
	TTTTGGTCTCGGTCAGATATG	LT_rev		
cdtB	TCGCGGAAGAAGCTATATAC	Cdt-B fw	407	This study[a]
	TGGAATATAAATGTCCGGCA	Cdt-B_rev		
iroN	AATCCGGCAAAGAGACGAACCGCCT	iroN_fw	553	(29)
	GTTCGGGCAACCCCTGCTTTGACTTT	ironN_rev		
ompT	GTTCGGGCAACCCCTGCTTTGACTTT	ompT_fw	496	(29)
	TCATCCCGGAAGCCTCCCTCACTACTAT	ompT_rev		
Paa	CGTTGCTGGATGCAGCTAATCTTT	Paa_fw	561	This study[a]
	CGGCAATATTCATATCACGCCAGA	Paa_rev		
hlyF	GGCCACAGTCGTTTAGGGTGCTTACC	hlyF_fw	450	(29)
	GGCGGTTTAGGCATTCCGATACTCAG	hlyF_rev		
iss	CAGCAACCCGAACCACTTGATG	Iss_fw	323	(29)
	AGCATTGCCAGAGCGGCAGAA	Iss_rev		
iucA	CGTCATGACTGGACCGCCCTGC	iucA_fw	422	This study[a]
		iucA_rev		
	GAAGAGGTGGTCGGCAGCCACG	iucA_rev		
iutA	GGCTGGACATCATGGGAACTGG	iutA_fw	302	(29)
	CGTCGGGAACGGGTAGAATCG	iutA_rev		
Resistance genes				
bla^{TEM}	CATTTCCGTGTCGCCCTTATTC	MultiTSO-T_fw	800	(21)
	CGTTCATCCATAGTTGCCTGAC	MultiTSO-T_rev		
bla^{OXA}	GGCACCAGATTCAACTTTCAAG	MultiTSO-O_fw	564	(21)
	GACCCCAAGTTTCCTGTAAGTG	MultiTSO-O_rev		
bla^{SHV}	AGCCGCTTGAGCAAATTAAAC	MultiTSO-S_fw	713	(21)
	ATCCCGCAGATAAATCACCAC	MultiTSO-S_rev		
mcr-1	AGTCCGTTTGTTCTTGTGGC	mcr1_320bp_fw mcr1_320bp_rev	320	(22)
	AGATCCTTGGTCTCGGCTTG			
mcr-2	CAAGTGTGTTGGTCGCAGTT	mcr2_700bp_fw mcr2_700bp_rev	715	(22)
	TCTAGCCCGACAAGCATACC			
mcr-3	AAATAAAAATTGTTCCGCTTATG	mcr3_900bp_fw mcr3_900bp_rev	929	(22)

(*Continued on next page*)

TABLE 2 Primers used for the identification of *E. albertii* AD1 and for detecting the presence of virulence and resistance genes in this study (*Continued*)

Target gene	Sequence (5′–3′)[b]	Primer	Amplicon size (bp)	Reference
	AATGGAGATCCCCGTTTTT			
mcr-4	TCACTTTCATCACTGCGTTG	mcr4_1100bp_fw mcr4_1100bp_rev	1,116	(22)
	TTGGTCCATGACTACCAATG			
mcr-5	ATGCGGTTGTCTGCATTTATC	MCR5_fw	1,644	(22)
	TCATTGTGGTTGTCCTTTTCTG	MCR5_rev		
qnrA	CAGCAAGAGGATTTCTCACG	qnrA_fw	630	(23)
	AATCCGGCAGCACTATTACTC	qnrA_rev		
qnrB	GGCTGTCAGTTCTATGATCG	qnrB_fw	488	(23)
	GAGCAACGATGCCTGGTAG	qnrB_rev		
qnrS	GCAAGTTCATTGAACAGGGT	qnrS_fw	428	(23)
	TCTAAACCGTCGAGTTCGGCG	qnrS_rev		
qnrD	CGAGATCAATTTACGGGGAATA	qnrD_fw qnrD_rev	581	(23)
	AACAAGCTGAAGCGCCTG			
qnrC	GCAGAATTCAGGGGTGTGAT	qnrC_fw qnrC_rev	118	(23)
	AACTGCTCCAAAAGCTGCTC			
aac(69)-Ib-cr	TTGGAAGCGGGGACGGAM	aac(69)-Ib-cr_fw aac(69)-Ib-cr_rev	260	(23)
	ACACGGCTGGACCATA			

[a]The annealing temperatures were 57°C for *cdtB*, 54°C for *Paa*, and 63°C for *iucA*.
[b]R=A,G; B=C,G,T; K=G,T; M=A,C.

Recognizing that certain *E. albertii* strains may encode virulence factors similar to *E. coli*, we conducted single and multiplex PCR analyses to investigate the presence of a fraction of those virulence-associated genes. The genes examined included attachment gene *eae* and *Paa*; toxin genes *stx1*, *stx2*, *stx2a*, *stx2f*, *hlyF*, *elt*, *est*, *cdt*, *iss*, and *ompT*; enterohemolysin *ehlY*; and siderophore genes *iucA*, *iutA*, *and iroN*, all of which contribute to the pathogenicity of *E. coli* (Table 2). *Paa* (porcine attaching and effacing-associated gene coding for the Paa protein) and *cdt* (cytolethal distending toxin gene with its CdtB catalytic subunit) have previously been found in nearly 100% of *E. albertii* isolates (30). Multiple single and multiplex PCR assays were employed, adhering to established protocols (14, 26, 27, 29). *E. coli* O157 strain ATCC 35218 and *E. coli* O157 strain Sakai ATCC BAA-460 were used as positive controls for all these PCR reactions targeting virulence genes. Analysis of the results of the PCR reaction using agarose gel electrophoresis showed the presence of a specific-sized band for the *eae*, *cdtB*, *Paa*, *iucA*, and *iutA* genes (data not shown). The strain, designated *E. albertii* AD1, tested negative for all the other intestinal and extraintestinal virulence genes commonly found in *E. coli*.

DISCUSSION

Escherichia albertii is a gram-negative species increasingly recognized as a cause of human gastroenteritis (31). Historically, biochemical tests have led to misidentifications of *E. albertii* as *E. coli*, *Shigella*, *Yersinia ruckeri*, or *Hafnia alvei* (32). Infections can occur through the consumption of tainted water, vegetables, and meat or contact with contaminated animals (33). This case report marks the first documented occurrence of *E. albertii* in Iran, presenting a rare instance of diarrhea and hospitalization in a 5-year-old girl.

Mild diarrhea was a prominent symptom associated with *E. albertii*-AD1-related gastroenteritis in the patient. More severe pathogenesis may be attributed to virulence factors such as the *stx1* and *stx2* genes, along with the *eae* gene, which are known to interact with intestinal cells, leading to fluid accumulation and symptoms like diarrhea and abdominal pain. These virulence factors can also contribute to damage of the intestinal villi (34).

The biochemical properties of *E. albertii* closely resemble those of *E. coli*, with a few notable differences such as variable motility capacity, depending also on experimental conditions and in the fermentation of distinct sugars. For instance, *E. albertii* has

conventionally been considered to be non-motile and unable to ferment certain sugars, including adonitol, xylose, and lactose, within 48 hours (2, 35, 36), while other biochemical traits can be variable. These biochemical and phenotypic similarities and variabilities often lead to its misidentification as *E. coli* and other species in routine biochemical tests (18, 35). In this epidemiological study, *E. albertii* AD1 was accurately identified as *E. albertii* through subsequent informative genomic analysis by multiplex PCR. Of note, phylogenetic grouping with the established multiplex PCR protocol (37) has not been successful (data not shown), providing additional indication for the isolate not belonging to the *E. coli* spp. As a specific molecular approach to identify *E. albertii,* to be generally applicable, the recently established identification of *E. albertii* by matrix-assisted laser desorption/ionization time-of-flight would require the inclusion of a specific database based on verified *E. albertii* isolates.

E. albertii AD1 carried a limited number of virulence genes, among them the *eae* adhesion initially thought to be an EPEC and the *Paa* adhesin gene, prevalent in strains of EHEC and ETEC pathovars and previously shown to be present in *E. albertii* (30). The *cdtB* gene was also detected in this strain; the *cdtB* gene in *E. albertii* encodes the active subunit of the cytolethal distending toxin, a tripartite genotoxin being encoded by *cdtA*, *cdtB*, and *cdtC* genes (38). In addition, we detected the *iutA* and *iucA* genes, which are part of the *iucABCD-iutA* operon. This operon encodes the high-affinity aerobactin siderophore system that aids in acquiring iron in iron-limited environments, such as within the host, and is considered an important virulence factor (39).

Although the average incubation period for infection by *E. albertii* is estimated to be 12–24 hours, the specific transmission route remained unclear in this case (32). The prevalence of *E. albertii* gastroenteritis appears to decline significantly with age, dropping from nearly 50% in children aged 0–10 years to 0% in adults over 20 (35). While these findings did not show statistically significant associations, they suggest that younger children are at greater risk of infection compared to adults. Previous studies support this observation, indicating that children under 10 and immunosuppressed individuals may be particularly vulnerable, although outbreaks have been reported in otherwise healthy populations (19).

While the source of infection has not been identified in this presented case, studies suggest that *E. albertii* may have zoonotic potential, with possible animal reservoirs serving as a source of infection. Various animal species, including poultry and livestock, have been implicated as carriers of *E. albertii*, which could be transmitted to humans (40, 41). Other factors such as poor hygiene during food preparation, the consumption of raw or undercooked poultry, and drinking untreated water may elevate the risk for *E. albertii* infection (1). The heightened incidence of clinical disease in younger age groups could reflect either a true association or sampling bias, as enteric infections can have more severe consequences for young children. Additionally, in cases of Shiga toxin-producing *E. coli* infections, children under 5 are more likely to develop hemolytic uremic syndrome (HUS). While not definitively confirmed, it is plausible that the risk of HUS in *E. albertii* infections may also be higher among young children, especially in strains that harbor both the *stx2* and *eae* genes (41).

Conclusion

This report marks the first documented occurrence of *E. albertii* in Iran, highlighting a rare case of diarrhea and hospitalization in a 5-year-old girl. The strain exhibited resistance to antibiotics such as ampicillin and amoxicillin-clavulanate, underscoring challenges in treating infections caused by this pathogen. The diagnosis of *E. albertii* can be demanding and is often missed, but this case emphasizes the status of *E. albertii* as an emerging pathogen in both humans and animals. The findings show that *E. albertii* can cause diarrhea in children, warranting further investigation about additional virulence factors. Our understanding of its pathobiology, mechanisms of pathogenesis, virulence factors, and drug resistance remains limited even if recently more in-depth analyses have been performed (16, 17). Therefore, suspected *E. albertii* cases need to be interpreted with

caution and confirmed through diagnostic PCR tests to ensure accurate diagnosis and appropriate management.

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DATA AVAILABILITY

Data supporting the findings of this study are available within the article; raw data, including agarose gel images, are available from the corresponding author upon reasonable request. The nucleotide sequence of the EAKF1_ch4033 gene from the *E. albertii* AD1 isolate has been submitted to GenBank under accession number PV185715.

ETHICS APPROVAL

The study was carried out in accordance with the Iran National Committee for Ethics in Biomedical Research. Accordingly, written informed consent was obtained from all participants for human experimentation. The research committee reviewed and confirmed that all study protocols were conducted in accordance with the related guidelines and regulations (IR.1403.58606).

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