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Research article

First molecular evidence and antibiotic resistance profile of emerging enteropathogen *Escherichia albertii* from faeces of pet dogs from Chattogram, Bangladesh

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A B S T R A C T

Escherichia albertii (*E. albertii*) is an emergent enteropathogen genetically identical to *Escherichia coli* (*E. coli*), often confused as *E. coli* phenotypically during routine diagnostic procedures. This pathogen possesses cytolethal distending toxin (*cdt*) responsible for the invasion and persistent colonization of this bacterium in the gut leading to enteric infections in humans and other animals. The present study attempted to explore the occurrence and antibiotic resistance profile of *E. albertii* derived from faeces of pet dogs and cats in Chattogram, Bangladesh. Faecal samples were collected aseptically from pet dogs (n=31) and cats (n=23) using sterile cotton swabs and stored in sterile buffered peptone water. After overnight enrichment in buffered peptone water, a loopful of the enriched broth was inoculated onto a selective media (XR-MacConkey) to isolate *E. albertii*. XR-MacConkey agar was prepared by supplementing MacConkey agar with D (+) Xylose and L (+) Rhamnose monohydrate. Inoculated samples on XR-MacConkey agar were incubated at 37°C for 24 hours. The visible white colonies were finally verified through polymerase chain reaction (PCR) by amplifying the gene fragments of *E. albertii* specific cytolethal distending toxin (*Eacdt). E. albertii* isolates were then tested for antimicrobial resistance against 12 selected antimicrobials by disk diffusion method. Two *E. albertii* isolates were positively identified in dog samples, while none of the cat samples were positive for *E. albertii*. Both *E. albertii* isolates from dogs showed multidrug resistance (MDR). The present study suggests that pet dogs may harbour *E. albertii*, which might be transmitted to humans in study areas.

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1. INTRODUCTION

Escherichia albertii is a member of the Enterobacteriaceae family closely resembling *E. coli*. Although this bacterium was first identified in sick children with diarrhoea in 1991, it was primarily misidentified as atypical *eae*-positive

Hafnia alvei (Albert et al., 1991). Later, it was further characterized by cultural, biochemical and genotypic studies, while in 2003, this bacterium was proposed as a new species

named *E. albertii* (Huys et al., 2003). Discriminating *E. albertii* from other closely related species of the Enterobacteriaceae family through cultural and molecular procedures is still challenging (Hyma et al., 2005; Oaks et al., 2010). The current method of identifying *E. albertii* is primarily based on the presence of cytolethal distending toxin gene (*cdtB*) found in *E. albertii* (Hyma et al., 2005). Although the actual function and clinical relevance of this toxin are not clear, it is believed to cause persistence and increased severity of the disease. Recently, *E. albertii* has been reported as an emerging enteropathogen linked to several clinical infections and disease outbreaks in humans (Ooka et al., 2012; Inglis et al., 2015). The occurrence of *E. albertii* in humans has not been reported yet. Previous reports indicated that birds and other animal hosts may act as a reservoir of infection in humans. Poultry and wild birds have been identified as potential carriers for *E. albertii* (Oaks et al., 2010; Barmettler et al., 2022). Alongside, the bacterium has been reported from domestic and wild animals, including pigs, cattle, sheep, cats, dogs and deer (Ooka et al., 2012; Duangtathip et al., 2020; Naka et al., 2022). Dogs and cats are the most common pets, and their close relationship with humans indicates a possible transmission of any bacterium and impact on human health.

While antimicrobials are frequently used as therapeutic and prophylactic agents in companion animals, AMR among zoonotic pathogens is common. Alongside domesticated animals, pets and other companion animals are reportedly interrelated to AMR spread due to excessive use and misuse of antibiotics. This may lead to the occurrence and spread of antibiotic-resistant pathogenic bacteria to humans by consuming food contaminated by bacteria and shared environments (Damborg et al., 2016; Pomba et al., 2016). A number of reports have indicated that *E. albertii* exhibits antimicrobial resistance, particularly multidrug resistance to two or more classes of antibiotics (Li et al., 2018). Despite the clinical significance of *E. albertii* in humans, research has yet to be done to know about the role of dogs and cats carrying *E. albertii* and the antimicrobial resistance pattern in Bangladesh. Therefore, this study aimed to identify the occurrence and antibiotic resistance of *E. albertii* in pet dogs and cats from Chattogram, Bangladesh.

2. MATERIALS AND METHODS

Ethical approval

Studies on animals were approved by the Ethics Committee of Chattogram Veterinary and Animal Sciences University (Approval Number: CVASU/Dir (R&E) EC/2020/169/8). All procedures were conducted according to the ethics committee's guidelines.

Sample collection

A total of 54 rectal swab samples were randomly collected from 31 dogs and 23 cats of different ages using sterile cotton swabs. Samples were collected between November 2021 and February 2022 from Chattogram in Bangladesh. Collected samples were placed in a sterile falcon tube containing 5 ml of buffered peptone water, kept at 4°C and transported to the Microbiology Laboratory, Chattogram Veterinary and Animal Sciences University. These samples were stored at 4°C until further analysis.

Culture and isolation of *E. albertii*

Rectal swab samples in buffered peptone water were placed in an incubator and incubated at 37°C for 24 hours for enrichment. *E. albertii* isolates were primarily identified based on their inability to ferment D (+)-Xylose and L-Rhamnose using a selective medium XR-MacConkey agar. This media was prepared using MacConkey agar base powder (Oxoid, UK) and 10% D (+)-Xylose (Merck KGaA, Darmstadt, Germany) and 10% L-Rhamnose monohydrate (MerckKGaA, Darm-stadt, Germany). A loopful broth from each enrichment culture was inoculated on XR-MacConkey agar and incubated for 18 hours at 37°C. *E. coli* utilize xylose and Rhamnose sugars and produce pink-colored colonies. However, *E. albertii* isolates cannot ferment these added sugars and sucrose and lactose of Mac-Conkey agar, hence produce white colonies. Isolates that only produced white colonies on XR-MacConkey agar were culturally identified as *E. albertii*. Presumably, identified well-isolated white colonies of *E. albertii* were sub-cultured into Trypticase soy broth (TSB) (Oxoid, United

Kingdom) by incubating at 37°C for 24 hours. Until further study, pure TSB cultures with 15% glycerol were preserved at -80°C freezer.

DNA extraction

Two to three colonies from blood agar media were inoculated in 5 mL of TSB and incubated overnight at 37°C to get pure culture materials. Genomic DNA was extracted using commercial DNA extraction kit (Favorgen®, Taiwan). Extracted total DNA samples were quantified and stored at -20°C before being used as amplicon for PCR assay.

PCR assay

Phenotypically characterized *E. albertii* isolates were further confirmed by PCR using previously described method through amplification of *cdt* gene (Hinenoya et al., 2019). PCR run conditions and primers are listed in Table 1.

Antimicrobial susceptibility testing

Antimicrobial susceptibility of *E. albertii* isolates were tested using Bauer-Kirby disk diffusion method utilizing breakpoints from the Clinical Laboratory Standards Institute (CLSI, 2021). Disc for 12 antimicrobials: sulfametho-xazole-trimethoprim (1.25/23.75 µg), erythro-mycin (15 µg), gentamicin (10 µg), cefalexin (30 µg), chloramphenicol (30 µg), ceftriaxone (30 µg), tetracycline (30 µg), ciprofloxacin (5 µg), amoxicillin (10 µg), ampicillin (10 µg), cefepime (30 µg), nalidixicacid (30 µg) (Oxoid ltd). Interpretation of results was carried out based on zone of inhibition size according to the standard in the CLSI M100-S26 for the Enterobaceriaceae family. We characterized isolates as "resistant (R)", "intermediately resistant (I)", and "sensitive (S)" against the tested antimicrobials (Table 2). Isolates that were non-susceptible to at least one agent in three or more antibiotic groups were identified as MDR isolates (CDC, 2022).

Table 1. Primers and conditions used in the PCR amplification (30 cycles)

Primer		**PCR conditions**			**Amplicon size (bp)**	**Ref.**
Name	**Sequence (5′-3′)**	**Denaturing**	**Annealing**	**Extension**		
EaCDTsp-F	GCTTAACTGGATGATTCTTG	Initial denaturation at 95°C for 2 min, followed by 95°C for 30s	50°C for 30s	72°C for 60s	449	Hinenoya et al. (2019)
EaCDTsp-R	CTATTTCCCATCCAATAGTCT					

Table 2. Interpretive breakpoints used for the enterobacteriaceae (CLSI, 2021).

Group of Antimicrobial agents	**Antimicrobial agents**	**Zone diameter**		
		R	**I**	**S**
Cephems	Ceftriaxone (CRO)	≤19	20-22	≥ 23
	Cefepime (FEP)	≤18	19-24	≥ 25
	Cefalexin (CL)	≤14	15-17	≥ 18
Tetracycline	Tetracycline	≤11	12-14	≥ 15
Penicillin	Ampicillin (AMP)	≤13	14-16	≥ 17
β–lactamase inhibitor	Amoxicillin (AML)	≤13	14-17	≥ 18
Amino glycosides	Gentamicin (CN)	≤12	13-14	≥ 15
Macrolids	Erythromycin (E)	≤13	14-22	≥ 23
Fluoroquinolones	Ciprofloxacin (CIP)	≤21	22-25	≥ 26
Quinolones	Nalidixic acid (NA)	≤13	14-18	≥ 19
Folate pathway inhibitor	Trimethoprim-Sulfamethoxazole (SXT)	≤10	11-15	≥ 16
Phenicoles	Chloramphenicol (C)	≤12	13-17	≥ 18

Statistical analysis

The proportion of animals carrying *E. albertii* was calculated employing a modified 'Wald method' using Graph Pad software (http://www.graphpad.com/quickcalcs/confInterval2/). A 95% confidence interval (CI) was used. The significant differences in the proportions for

E. albertii for health status and previous antibiotic treatment were tested by Fisher's exact test (Bengtsson et al., 2023).

3. RESULTS

Cultural identification of *E. albertii*

We have used complementary approaches to confirm the isolates. Results showed that out of 54 swab samples tested, only two (3.7%) produced white colonies on XR-MacConkey agar plates (Figure 1A) as it produced characteristics large pink colour colonies that can easily be differentiated from the white colonies produced by *E. albertii*. For further verification, these culturally positive isolates were again sub-cultured on to XR-MacConkey agar plates and both the isolates showed characteristic white colonies.

Proportion of dogs and cats carrying *E. albertii*

Following phenotypic characterization using culture methods, *E. albertii* isolates from dogs were further validated using PCR assays. The PCR results showed that two isolates were having 449bp bands indicating amplification of *cdt* gene frgments from *E. albertii*. (Fig. 2). The overall proportion of animal in this study harbouring *E. albertii* was 3.7% (95% CI 0.3-13.2). These *E. albertii* isolates were identified among 4.45% of the dogs. No *E. albertii* was isolated from cats. The distribution of *E. albertii* in dogs based on health status and previous antibiotic uses are also assessed (Table 3).

A

B

Figure 1. Differential colonial morphologies on XR-MacConkey agar. A. Colonial morphology of *E. albertii* on XR-MacConkey agar (white) B. Typical colonial morphology of *E. coli* displayed on XR-MacConkey agar (pink) plate.

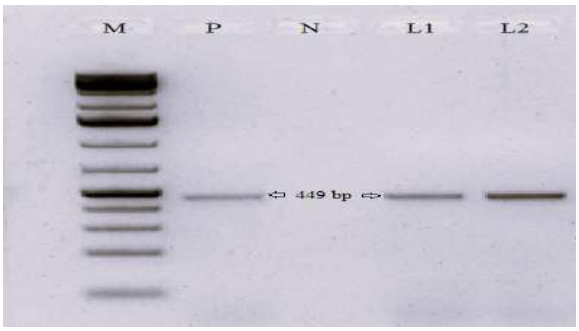

Figure 2. 2% Gel electrophoresis PCR results for the *cdt* gene of *E. albertii*. M=DNA ladder; P= Positive control, and N = Negative control for *Eacdt* gene of *E. albertii*, L1 and L2= *Eacdt* gene amplicon sized 449 bp.

The differences in the proportion of *E. albertii* isolates between different variables (health status and previous uses of any antibiotics) were not statistically significant.

Antibiotic resistance profile of *E. albertii*

The results showed that two dog samples tested positive were resistant to tetracycline, trimethoprim/sulfamethoxazole, erythromycin, ceftriaxone and nalidixic acid. One isolate exhibited resistance to cefalexin, ciprofloxacin, ampicillin, amoxicillin, and gentamicin. Both tested isolates showed sensitivity to cefepime and ceftriaxone (Figure 3). Both *E. albertii* isolates were MDR.

4. DISCUSSION

E. albertii is a recently identified entero-pathogenic species of the genus *Escherichia,* causing diarrhoea in humans. This bacterium has previously been confused with *E. coli* or related species of genus *Escherichia* owing to insufficient cultural, biochemical and molecular identification tools (Huys et al., 2003; Gomes et al., 2020).

Table 3. Distribution of *Escherichia albertii* in pet dogs in Chattogram, Bangladesh.

Species	Variable	Categories of animals (n)	Total number positive for *E. albertii*	Proportion of animals carrying *E. albertii* (95% CI)	P-value
Dogs (31)	Health status	Healthy (20)	1	5.0 (0.01 to 25.41)	1
		Sick (11)	1	9.09 (0.01 to 39.91)	
	Antibiotic Use	Used previously (15)	2	13.3 (2.48 to 39.14)	0.22
		Never used (16)	0	0	

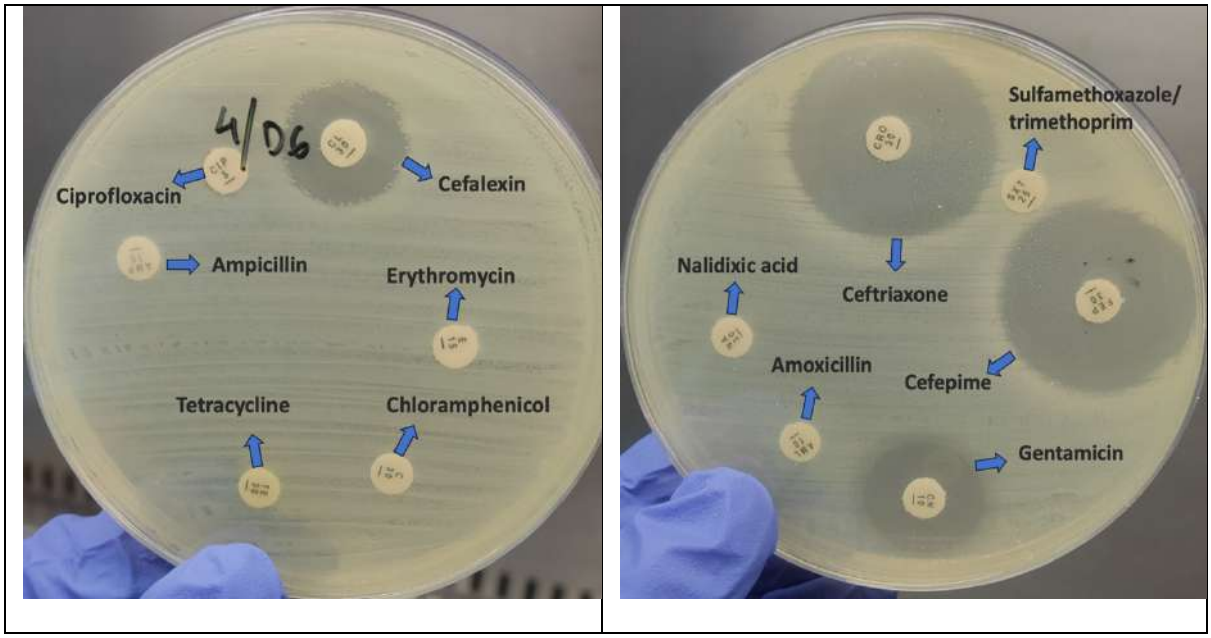

Figure 3. Antimicrobial resistance of *Escherichia albertii* isolates through disk diffusion method. Isolates were resistant to Sulfamethoxazole/trimethoprim (SXT), Erythromycin (E), Gentamicin (CN); Cefalexin (CL), Chloramphenicol (C), Tetracycline (TE), Ciprofloxacin (CIP), Amoxicillin (AML), Nalidixic acid (NA), and Ampicillin (AMP), and sensitive to Cefepime (FEP) and Ceftriaxone (CRO).

After recognition as a novel organism, the *cdtB* gene fragments have primarily been used to detect *E. albertii*. Recently, a PCR assay targeting the *cdt* gene in *E. albertii* has been developed that could distinguish *E. albertii* from other species with high sensitivity and specificity (Hinenoya et al., 2019). During this study, the cultural characterization based on xylose and rhamnose sugar fermentation were followed by PCR assay to validate the occurrence of *E. albertii*. The epidemiological study to identify the animal reservoir of this human pathogen is very scanty compared to other well-known enteric pathogens like *E. coli*, *Campylobacter* and *Salmonella*. Few studies have shown that *E. albertii* was recovered from cattle, pigs, dogs, and cats ((Barmettler et al., 2023; Duangtathip et al., 2020; Naka et al., 2022). However, no previous study has explored the occurrence of *E. albertii* in dogs and cats in Bangladesh. Therefore, the present study intended to report this emerging pathogen in dogs and cats which

can be a potential public health risk in Bangladesh. This study is the first report of *E. albertii* in fecal samples of dogs and cats in the country. Since these animals are reared as pets and live close to humans, this organism could easily transmit to humans and cause enteric diseases. The current study showed that out of the 54 samples, two were reliably identified as *E. albertii* based on the cultural properties in a selective media recommended for *E. albertii* isolation (XR-MacConkey). Based on the PCR result, 4.45% of the dogs were found to be the reservoir of *E. albertii*. Though none of the cat samples were found to harbour *E. albertii*, using large number of cat samples could ensure the actual scenario. One of our previous studies identified that 3.5% of backyard poultry samples from Chattogram, Bangladesh, were positive for *E. albertii* (Das Gupta, 2023). Another report from Australia indicated a higher prevalence of *E. albertii*, from 6.7 to 33% in chickens and 14.3 to 18% in magpies (Gordon, 2011; Oh et al., 2011). However, a smaller proportion of *E. albertii*, as low as 1.6%, is also found in rinse samples from chicken carcasses in the US (Lindsey et al., 2015). In a previous study done by our group, we found a lower proportion of *E. albertii* in backyard chickens (3.5%). This is the preliminary evidence that pet dogs in Bangladesh harbouring *E. albertii*, hence, a larger sample size from several locations in Bangladesh can give us a better picture about the epidemiology and actual distribution around the nation. This baseline study suggests that dogs are worthy of continued investigation for this organism to check the clinical severity and exposure risks to humans. In addition, MDR organisms add further burden to public health due to the difficulties in treating the diseases caused by them. The MDR rate in *E. albertii* isolates from dogs was surprisingly high (100%). The rate of MDR in our study might be overestimated due to the smaller sample size. However, this result corroborates the previous study's finding that MDR Chinese *E. albertii* isolates were highly resistant to 10 to 14 different antimicrobial classes (Li et al., 2018). Another previous study has observed a higher MDR rate of *E. albertii* isolates (85.9%) from poultry sources in China (Luo et al., 2021). Similar higher resistance (100%) was observed against sulfamethoxazole-trimethoprim, tetra-cycline, nalidixic acid and erythromycin. Another report showed 62.7% of *E. albertii* isolates were tetracycline resistant, and 56.9% were nalidixic acid resistant (Li et al., 2018). Pets associated with MDR *E. albertii may* transmit to humans, raising a great concern for human health. Therefore, proper investigation of the occurrence of MDR isolates in pets may help to plan control strategies that prevent the spread of MDR *E. albertii* to humans.

5. CONCLUSION

This is the first report of the occurrence of *E. albertii* in pet dogs in Chattogram, Bangladesh, indicating that pet dogs may act as a potential source of a zoonotic pathogen, *E. albertii.* Systematic surveillance plans covering a broader area and one health approach in Bangladesh are required for the future study of *E. albertii* to better understand its public health significance.

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