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Occurrence and antibiogram of *Escherichia albertii* in backyard poultry and pigeons in Bangladesh

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Abstract

Background and Aim: *Escherichia albertii* is an emerging enteric pathogen that causes mass avian mortality events in the Northern Hemisphere, as well as mortality in captive birds and poultry. This study aims to investigate the occurrence and antibiotic resistance pattern of *E. albertii* in backyard poultry and pigeons in Bangladesh.

Materials and Methods: A total of 200 cloacal swabs were collected from backyard poultry and pigeons from Chattogram and Cox's Bazar districts in Bangladesh. *Escherichia albertii* isolates were isolated and identified by culturing on selective growth media. Polymerase chain reaction was used to confirm the presence of cytolethal distending toxin gene (*Eacdt*). Antimicrobial resistance patterns of *E. albertii* isolates were investigated using the Bauer-Kirby disk diffusion method. In addition, the *E. albertii* isolates were screened for sulphonamide-resistant *sul1* and *sul2*.

Results: In total, 7/200 (3.5%) isolates from backyard poultry tested positive for *Eacdt.* The isolation rate of *E. albertii* was highest in chicken (4.76%), followed by turkey (2.78%) and duck (2.2%). Pigeon samples were negative for *E. albertii*. Based on the disk diffusion test, all seven *E. albertii* isolates were resistant to ≥3 antimicrobials. Resistance was highest against tetracycline (86%), followed by trimethoprim/sulfamethoxazole (71%). In addition, 6/7 isolates tested positive for *sul1* and *sul2*.

Conclusion: To the best of our knowledge, this study is the first to show that backyard poultry and pigeons in Bangladesh may be reservoirs of *E. albertii*, indicating the importance of determining the transmission and pathogenicity of *E. albertii* to humans.

Keywords: antimicrobial resistance, Bangladesh, pathogenicity, tetracycline.

Introduction

Escherichia albertii is an emerging enteropathogen, first described by Huys *et al*. [1], based on isolates identified from children with diarrhea in Bangladesh. *Escherichia albertii* was initially believed to be atypical *eae*-positive *Hafnia alvei* [2, 3], but was later identified as a new species in Bangladesh [1]. *Escherichia albertii* produces the virulence factors cytolethal distending toxin (CDT) and intimin [4], which are associated with the disruption of tight intercellular junctions in intestinal epithelial cells, leading to diarrhea [5]. Identifying *E. albertii* strains based on phenotypic characteristics is difficult due to their poorly defined properties and close similarities with *Escherichia coli*. The biochemical properties of confirmed *E. albertii* isolates, such as inability to ferment D-xylose, L-rhamnose, and lactose, have been used to differentiate *E. albertii* from *E. coli* [1, 6, 7]. A polymerase chain reaction (PCR) assay targeting *Eacdt* specific for *E. albertii* was used to differentiate *E. albertii* from other closely related *Enterobacteriaceae* strains, including *E. coli.*

In humans, pathogenic *E. albertii* was reported in a restaurant-associated outbreak in Japan [8, 9]. Foodborne transmissions of *E. albertii* have been reported in other studies [10, 11]. *Escherichia albertii* was identified as a cause of epidemic mortality in birds in the northern hemisphere [4]. Some studies have suggested that birds are the source of *E. albertii* infection in humans [4, 12–14]. *Escherichia albertii* can be transmitted between birds and humans if bird populations harbor this zoonotic pathogen.

Escherichia albertii is a zoonotic pathogen that causes diarrhea in humans, antibiotic therapy has been used to manage severe symptoms and reduce illness duration. Antibiotic treatment is the main control method for most bacterial diseases of domestic and wild captive birds in Bangladesh due to the lack

of efficient commercial vaccines. However, continuous use of antibiotics at suboptimal doses may lead to bacterial resistance against antimicrobial drugs. The emergence of antimicrobial resistance (AMR) in pathogenic bacteria, comprehensive surveillance of AMR in *E. albertii* in birds has become important to understand the risk of *E. albertii,* poses to public health. Birds that harbor antibiotic-resistant *E. albertii* may act as vehicles for transmitting *E. albertii* to humans.

Although it was first identified as a pathogen in humans, no study has examined the presence and antibiotic resistance of *E. albertii* in backyard poultry and pigeons in Bangladesh. *Escherichia albertii* can be misidentified as *E. coli*. Therefore, it is necessary to conduct a baseline study on the occurrence and antibiotic resistance of *E. albertii* in domestic bird populations before advocating approaches to captive bird farmers in Bangladesh.

Farmers, especially women and children, are involved in managing domestic and captive wild birds. Therefore, this study investigated the occurrence and antibiotic resistance of *E. albertii* in domestic birds and captive wild birds in Bangladesh to identify the potential risk of *E. albertii* to people in close contact. To the best of our knowledge, this study is the first to report the presence of *E. albertii* in domestic and captive birds in Bangladesh.

Materials and Methods

Ethical approval

Approval for this study was obtained from the Animal Experimentation Ethics Committee at 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 and requirements.

Study period and location

The study was conducted from November 2021 to February 2022. The samples were collected from Chattogram and Cox' Bazar districts in Bangladesh. The samples were processed at the Microbiology Laboratory, Chattogram Veterinary and Animal Sciences University, Bangladesh.

Study population and sample collection

Cloacal swabs were collected using sterile cotton from 200 apparently healthy and diseased birds, including household chickens, deshi and Muscovy ducks, pigeons, and turkeys. The collected swabs were placed in a Falcon tube containing 5 mL of buffered peptone water (HiMedia, India) and immediately transported to the Microbiology Laboratory, Chattogram Veterinary and Animal Sciences University, Bangladesh. In addition, questionnaire-based demographic information of sampled birds was collected for the epidemiological study.

Culture and isolation of *E. albertii*

Escherichia albertii isolates were primarily detected based on their inability to utilize and ferment D (+)-xylose and L-rhamnose (colorless colonies) using selective media (XR-MacConkey agar), which was prepared using MacConkey agar base powder (Oxoid, UK), 10% (w/v) D (+)-xylose (Merck KGaA, Damstadt, Germany), and 10% (w/v) L-rhamnose monohydrate (Merck KGaA). Cloacal swab samples in buffered peptone water were placed in an incubator and incubated at 37°C for 24 h for enrichment. XR-MacConkey agar plates were streaked with a loopful of each enrichment culture and incubated overnight at 37°C. Isolates with colorless colonies on XR- MacConkey agar were phenotypically identified as *E. albertii.* Presumptively identified well-isolated colorless colonies of *E. albertii* were subcultured into Tryptone Soya Broth (TSB) (Himedia) at 37°C for 24 h. Bacterial cultures in TSB (Oxoid) were stored at −80°C with 15% glycerol, for further use.

DNA extraction

Genomic DNA was extracted from overnight-grown bacterial cultures (1000 µL) in TSB using FavorPrep Tissue Genomic DNA Extraction Mini Kit (Favorgen Biotech Corporation, Taiwan). Extracted DNA was stored at −20°C until PCR testing.

Detection of *Eacdt*

All isolates were screened for *Eacdt* through PCR assay using primers listed in Table-1 [15]. Polymerase chain reaction was performed in a 25-µL reaction volume, containing 1 µL each of 20 pmol primer, 1 µL of DNA template, and 22 µL DreamTaq Green PCR Master Mix (Thermo Fisher Scientific Inc., USA). Polymerase chain reaction amplification was performed using a Thermal (2720 Thermal cycler, Applied Biosystems, USA) under the following conditions: Denaturation at 95°C for 2 min; 30 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 60 s; and final extension at 72°C for 8 min. An *E. albertii* isolate from a recently completed project by our research group (unpublished data) was used as a positive control. For negative control, only PCR reaction mixture without DNA template was used.

Antimicrobial sensitivity testing

Escherichia albertii isolates were tested against 12 selected antimicrobials (Oxoid Ltd.): 25 µg amoxicillin, 10 µg ampicillin, 30 µg cefepime, 30 µg chloramphenicol, 30 µg ceftriaxone, 5 µg ciprofloxacin, 30 µg cephalexin, 15 µg erythromycin, 10 µg gentamycin, 30 µg nalidixic acid, 10 µg tetracycline, and 25 µg trimethoprim-sulfamethoxazole. The Bauer-Kirby disk diffusion method was used to test the antimicrobial susceptibility of 7 *E. albertii* isolates [16]. We characterized the isolates based on the guidelines of Clinical Laboratory Standards Institute (CLSI) for *Enterobacteriaceae* [17]. We prepared Mueller-Hinton agar according to the manufacturer's instructions (Oxoid). The 0.5 McFarland standard was prepared by mixing 0.5 mL of 1% $BaCl_2.2H_2O$ with 99.5 mL of 1% H_2SO_4. The bacterial suspension was

prepared using sterile saline. The final dilution of bacteria was determined by comparing the turbidity of the bacterial suspension with the McFarland standard. After bacterial inoculation was complete, commercial disks (Oxoid) for antimicrobials were inserted on agar plates and incubated at 37°C for 24 h. Isolates were considered "resistant (R)," "intermediately resistant (I)," and "sensitive (S)" against the tested antimicrobials by comparing the size of the zone of inhibition with the standard from CLSI.

Polymerase chain reaction for AMR genes

Two AMR genes (*sul1* and *sul2*) were identified in *E. albertii* isolates using uniplex PCR. The primers and cycle conditions used for PCR amplification are described in Table-2 [18, 19]. Polymerase chain reaction products were analyzed by electrophoresis using 2% agarose gel (MP Bio-Medicals, USA) and visualized with ultraviolet light after staining with 3 μL of SYBR Safe DNA Gel Stain (Invitrogen, Thermo Fisher, USA).

Statistical analysis

The proportion of birds carrying *E. albertii* was calculated using "95% confidence interval of a proportion by modified Wald method" in GraphPad Prism 9.5.0 (http://www.graphpad.com/quickcalcs/confInterval2/). Significant differences in the proportion of *E. albertii* based on health status, previous antibiotic treatment, and location from where samples were collected were assessed by Fisher's exact test using GraphPad software. $p < 0.05$ was considered statistically significant.

Results

Characteristics of *E. albertii* cultured on XR-MacConkey agar

In total, seven isolates from 200 cloacal swabs produced colorless colonies on XR-MacConkey agar and were appeared as Gram-negative rods with Gram staining. *Escherichia coli* produced characteristically large, pink colonies that were clearly differentiated from colorless colonies produced by *E. albertii*. The seven isolates produced characteristic colorless colonies when subcultured 2 times on XR-MacConkey agar. Differentiating growth features between *E. coli* and *E. albertii* on XR-MacConkey agar are shown in Figure-1.

Proportion of backyard poultry carrying *E. albertii*

Polymerase chain reaction results showed that all seven isolates were positive for *Eacdt* (Figure-2); hence, the proportion of birds harboring *E. albertii* was 3.5% (95% CI: 1.5–7.1). Species-wise isolation rates were 4.76% for chicken (5/105), 2.78% (1/36) for turkey, and 2.2% for duck (1/44) (Table-3). No isolate was identified in 15 samples from pigeons. The differences in the proportion of *E. albertii* isolates between variables (location, health status, and history of antibiotics use) were not statistically significant (Supplementary Table-1).

Antibiotic resistance pattern of *E. albertii* isolates

The AMR pattern of *E. albertii* isolates is presented in Table-4. All 7 (100%) isolates were

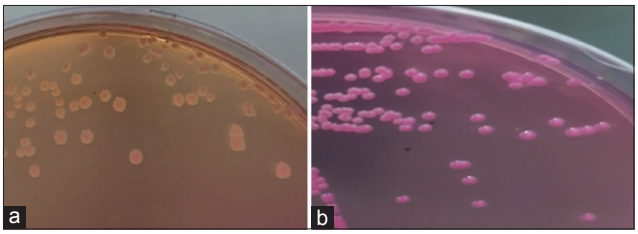

Figure-1: Comparative colonial morphologies of *Escherichia albertii* and *Escherichia coli* displayed on XR-MacConkey agar; (a) Colonial morphology of *E. albertii* (colourless) (b) Typical colonial morphology of *E. coli* (pink).

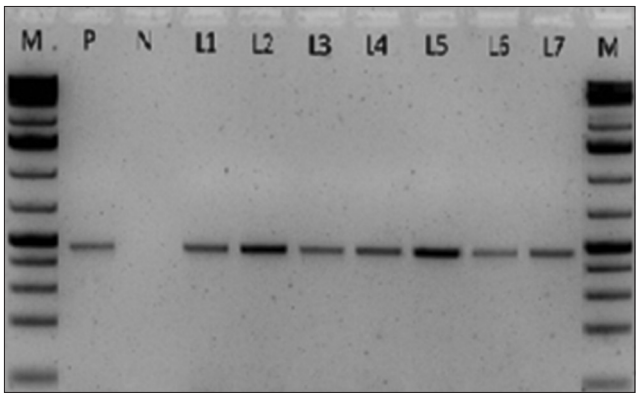

Figure-2: Polymerase chain reaction results for *Eacdt* gene of *Escherichia albertii*; Lane M=1kb DNA marker; Lane P = positive control for *Eacdt* gene of *E. albertii*; Lane N= negative control for *Eacdt*, L1-L7= *Eacdt* gene-amplicon of 449 bp.

Table-1: Oligonucleotide primer sequences and amplicon size used in *Eacdt* gene amplification.

Primer	Sequence (5'–3')	Target gene	Amplicon size (bp)	Reference
EaCDTsp-F	GCTTAACTGGATGATTCTTG	*Eacdt*	449	[15]
EaCDTsp-R	CTATTTCCCATCCAATAGTCT			

Table-2: Oligonucleotide primer sequences, annealing temperature and amplicon size used in amplification of *sul1* and *sul2* genes.

Primer	Primer sequence (5'– 3')	Target gene	Annealing temp.(°C)	Amplicon size (bp)	References
Sul1 F	CGG CGT GGG CTA CCT GAA CG	*Sul1*	51	433	[18]
Sul1 R	GCC GAT CGC GTG AAG TTC CG				
Sul 2 F	CCT GTT TCG TCC GAC ACA GA	*Sul2*	59	435	[19]
Sul 2 R	GAA GCG CAG CCG CAA TTC AT				

multidrug-resistant (MDR), with resistance to ≥3 antimicrobial agents. Six (86%) isolates were resistant to tetracycline and trimethoprim/sulfamethoxazole, 5 (71%) to nalidixic acid, and 4 (57%) to erythromycin. Some *E. albertii* isolates (29%–43%) were resistant to chloramphenicol, ciprofloxacin, ceftriaxone, cefalexin, and cefepime; 1 (14%) isolate was ampicillin resistant.

Of the 7 *E. albertii* isolates tested for the presence of sulfonamide resistance genes *sul1* and *sul2*, all six isolates showed phenotypic resistance to sulfamethoxazole and were also positive for *sul1* and *sul2* (Figure-3).

Discussion

The prevalence and epidemiology of *E. albertii* must be correctly defined, because *E. albertii* is misidentified by commonly used biochemical tests. *Escherichia albertii* is a close relative of *E. coli* and was initially classified as *eae*-positive *H. alvei*. However, based on available genetic and biochemical data, *E. albertii* was classified as a novel bacterium in 2003 [1, 7]. Biochemical properties that help distinguish between *E. albertii* and *E. coli* include inability of *E. albertii* to ferment D-xylose, L-rhamnose, and melibiose [20]. In addition, *E. albertii* harbors *cdtB*, which encodes CDT [4, 21]. Based on these biochemical and molecular properties, our study investigated the prevalence of *E. albertii* in backyard poultry in Bangladesh. *Escherichia albertii* has been reported in domestic and wild birds and found to be associated with epidemics globally [4, 14, 22–25]. Several

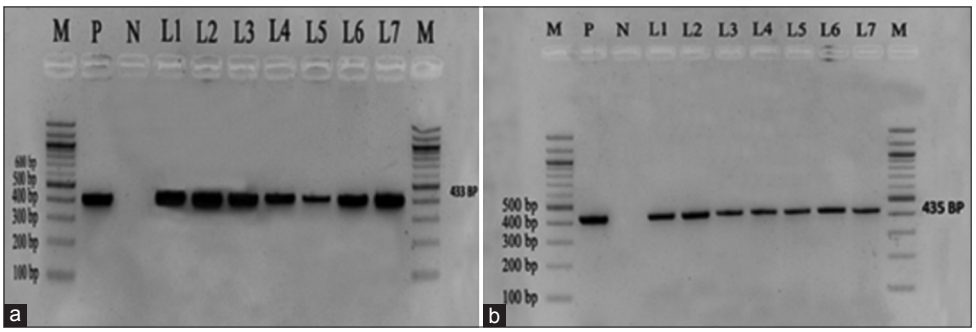

Figure-3: Polymerase chain reaction results for two antimicrobial resistance genes: sul1 and sul2. (a) sul1 gene (433 bp) amplicon: Lane M = 100bp DNA ladder; lane P, Positive control, N=Negative control, lanes L1–L6, sul1-positive isolates; (b) sul2 gene (435 bp) amplicon: Lane M = 1 kb plus DNA ladder; lane P, Positive control, N = Negative control, lanes L1–L6, sul2-positive isolates.

Table-3: Proportion of *E. albertii* in different species of backward poultry.

Species of birds (n)	Total number positive for *E. albertii*	Proportion of birds carrying *E. albertii* (95% CI)
Chicken (105)	5	4.76 (1.7–10.9)
Duck (44)	1	2.2 (0.01–12.8)
Turkey (36)	1	2.78 (0.01–15.42)

E. albertii=Escherichia albertii, CI=Confidence interval

Supplementary Table-1: Variable-wise distribution of *E. albertii* in backward poultry in Bangladesh.

Species of birds	Variables	Categories of birds (n)	Total number positive for *E. albertii*	Proportion of birds carrying *E. albertii* (95% CI)	p-value
Chicken	Health status	Healthy (85)	2	2.3 (0.14–8.5)	0.053
		Sick (20)	3	15.7 (4.6–38.4)	
	Antibiotic use	Used previously (30)	2	6.6 (0.8–22.3)	0.62
		Never used (75)	3	4.0 (0.9–11.5)	
	Location	Chattogram (65)	2	3.0 (0.2–11.1)	0.36
		Cox's bazar (40)	3	7.5 (1.8–20.5)	
Duck	Health status	Healthy (34)	0	0 (0–12.0)	0.22
		Sick (10)	1	10 (0.01–42.6)	
	Antibiotic use	Used previously (15)	1	6.6 (0.01–31.8)	0.34
		Never used (29)	0	0 (0–13.8)	
	Location	Chattogram (24)	0	0 (0–16.3)	0.45
		Cox's bazar (20)	1	5 (0.01–25.4)	
Turkey	Health status	Healthy (30)	1	3 (0.01–18.1)	1.0
		Sick (6)	0	0 (0–44.2)	
	Antibiotic use	Used previously (10)	1	10 (0.01–42.6)	0.27
		Never used (26)	0	0 (0–15.2)	
	Location	Chattogram (21)	1	4.7 (0.01–24.4)	1.00
		Cox's Bazar (15)	0	0 (0–23.8)	

E. albertii=Escherichia albertii

Table-4: Multidrug resistance pattern of *Escherichia albertii* in backyard poultry.

Isolate number (species)	Resistance pattern
C9 (chicken)	STX, CN, CL, NA, C, CRO
C25 (chicken)	STX, CN, TE, CIP, NA
C76 (chicken)	STX, E, FEP, TE, AML, AMP, NA
C84 (chicken)	E, CN, FEP, CL, TE, NA, CRO
C98 (chicken)	STX, E, TE, CIP, CRO
D7 (Duck)	STX, E, C, TE, CIP, NA
T11 (Turkey)	STX, CL, TE

STX=Sulfamethoxazole/trimethoprim, E=Erythromycin, CN=Gentamicin, FEP=Cefepime, CL=Cefalexin, C=Chloramphenicol, TE=Tetracycline, CIP=Ciprofloxacin, AML=Amoxicillin, NA=Nalidixic acid, CRO=Ceftriaxone, AMP=Ampicillin

studies have identified a higher isolation rate of *E. albertii* in poultry and their meat [13, 26, 27]. For example, studies have shown that the prevalence of *E. albertii* ranges from 6.7% to 33% in chickens and 14.3%–18% in magpies in Australia [24], as well as 23.8% in wild birds in Switzerland [25]. In addition, *E. albertii* was recently been identified in 29.6% of migratory birds in China [28]. Consequently, poultry and its meat could be a potential reservoir, posing increased risk for humans [14, 22]. *Escherichia albertii* has been isolated from cats [12], cattle [29], pigs [29], and dogs [30]. However, despite having zoonotic significance, no study has examined the presence of *E. albertii* in bird species in Bangladesh. To the best of our knowledge, this is the first study on *E. albertii* in backyard poultry in Bangladesh. The results of this study revealed 7/200 (3.5%) samples were positive for *E. albertii*, as evidenced by the production of colorless colonies on XR-MacConkey agar (a selective medium recommended for *E. albertii*) and the presence of *Eacdt*. This prevalence rate was within the range of those reported in other studies. In a study in China, *eae*-positive and lactose non-fermenting *E. albertii* strains were observed in 6.73% of retailed raw meat [27]. The samples included duck intestines, duck meat, chicken intestines, chicken meat, mutton meat, and pork meat. Recently, *E. albertii* strains were isolated from broiler farms, with isolation rates ranging from 0% to 23.3% [22]. In another study, only 2/104 (~2%) retail chicken products were positive for *E. albertii*, which is lower than our overall rate (3.5%). Thus, the prevalence of *E. albertii* varies in different studies. Recent studies from China, Japan, and USA have highlighted the potential for foodborne transmission of *E. albertii* to humans through poultry consumption [14, 22, 31]. Our study identified backyard poultry-associated *E. albertii* isolates, suggesting domestically acquired *E. albertii* infections in humans from birds in Bangladesh.

Increasing AMR in *E. albertii* is posing a threat to global health. Antimicrobials are the most commonly used method to treat human infectious diseases. However, studies in China have demonstrated resistance of some *E. albertii* isolates to important antimicrobials used to treat human infections [31, 32]. Li *et al*. [32] reported higher resistance to tetracycline (62.7%) and nalidixic acid (56.9%). Consistently, we observed a high rate of resistance to sulfonamides (86%), tetracyclines (86%), and nalidixic acid (71%) in our *E. albertii* isolates.

In this study, the rate of multidrug resistance in *E. albertii* isolates was surprisingly high (100%). Consistently, a study in China found that 85.9% of the Chinese *E. albertii* isolates were predicted phenotypically to be MDR, and 35.9% harbored genes that confer resistance against 10–14 classes of antibiotics [31]. Moreover, MDR *E. albertii* isolates were mainly found in poultry [31]. The risk of antibiotic resistance is high in countries where antibiotic control strategies are missing and antibiotics use is unregulated [33].

Conclusion

To the best of our knowledge, this study is the first to report the occurrence and antibiotic resistance of *E. albertii* in backyard poultry in Bangladesh, suggesting that these birds might be a potential vehicle for *E. albertii* transmission to humans. If sanitary regulations are not adequately maintained, antibiotic-resistant zoonotic *E. albertii* strains may be transmitted to humans.

Authors' Contributions

MDG and AD: Conceptualization, project administration, and supervision. MDG and MS: Data curation. MDG and AD: Formal analysis and funding acquisition. MDG, MS, AL, KG, and TAL: Investigation. MDG, MS, and AL: Methodology. MDG: Resources. MDG: Writing-original draft. AD: Writing, reviewing, and editing. All authors have read, reviewed, and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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