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Occurrence and cross contamination of *Escherichia albertii* in retail chicken outlets in Bangladesh

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ABSTRACT

Escherichia albertii is an emerging zoonotic pathogen linked to human gastrointestinal illnesses, with poultry meats being considered as a key source of human infections. However, there is little information regarding the distribution and characteristics of this bacterium in Bangladesh. This study investigated the occurrence, antimicrobial resistance, and virulence of *E. albertii* in chicken meats from retail outlets in Bangladesh. We collected samples from 61 dressed chickens across 17 retail shops from 4 upazilas, along with swabs from cloaca, processing utensils, and worker hands. Detection of *E. albertii* by species-specific PCR revealed substantial occurrence of *E. albertii* in retail chicken meat (63.9 %), cloaca (71.4 %), human hand (45.5 %), bleeding cone (13.3 %) and blade (10 %). Almost all the *E. albertii* isolates (94.4 %) exhibited resistance to at least one of the tested antimicrobials, among which 50 % were multidrug resistant, including resistance to clinically relevant antimicrobials such as tetracycline, ampicillin, gentamicin, kanamycin, nalidixic acid and ciprofloxacin. Whole genome sequencing analysis identified the presence of corresponding antimicrobial resistance genes and critical virulence genes (*eae*, *Eacdt*). Notably, although wgSNP-based phylogenetic analysis showed the genomic diversity of the isolates, some of the isolates from the same shop displayed clonal relationships among meats, cloacal swabs, and human hand swabs, indicating contamination during processing. These findings highlight the public health risk posed by *E. albertii* in retail poultry, underlining the poultry's role as a potential vector for zoonotic transmission and the need for improved biosecurity and antimicrobial management practices in poultry production.

1. Introduction

Escherichia albertii is an emerging zoonotic enteropathogen causing gastrointestinal diseases in humans, such as watery diarrhea, abdominal pain, vomiting and fever (Bhatt et al., 2019; Masuda et al., 2020; Oaks et al., 2010). This bacterium was first isolated from a diarrheic child in Bangladesh (Albert et al., 1991), but was initially identified as *Hafnia alvei* using the API 20E biochemical identification strip. Subsequent extensive analyses of this *H. alvei* isolate proposed a new member of Genus *Escherichia*, *E. albertii* (Huys et al., 2003). In addition to an increase in clinical reports worldwide, several foodborne outbreaks caused by *E. albertii* have occurred, especially in Japan (Kashio et al., 2020; Masuda et al., 2020; Ooka et al., 2013; Tokoi et al., 2018), and consequently, this bacterium has garnered increasing attention with respect to public health globally. *E. albertii* is a Gram-negative, non-spore forming, non-motile (in conventional bacteriological tests), facultative anaerobic bacillus. *E. albertii* carries the locus of enterocyte effacement (LEE) pathogenicity island encoding type three secretion system and *eae* gene-encoded adhesin called intimin, by which this bacterium can form attaching and effacing lesion on intestinal epithelium. *E. albertii* also express CDTs encoded by *Eacdt* (almost ubiquitously present) and *Eccdt-I* (less frequently present), which could be associated with virulence and persistent colonization (Scuron et al., 2016). Certain strains also produce Shiga toxin 2 (Stx2a or Stx2f) (Brandal et al., 2015; Hinenoya et al.,

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2017a; Murakami et al., 2014), which is a primary virulence factor of enterohemorrhagic *E. coli*. Due to such similar phenotypic and genotypic feature with other bacteria belonging to the order Enterobacterales, *E. albertii* has often been misidentified as *H. alvei*, *Shigella boydii*, enteropathogenic *E. coli*, EHEC and CDT-II-producing *E. coli* (Hinenoya et al., 2019a; Hinenoya et al., 2017b; Hyma et al., 2005; Ooka et al., 2012), suggesting the underestimation of *E. albertii* infections in humans.

Many epidemiological studies have explored the environmental distribution of *E. albertii*, identifying animal reservoirs and potential human infection routes. Wildlife surveys reported this bacterium in several animals, such as raccoons, badgers, martens, foxes, bats, dogs and cats (Gordon, 2011; Hinenoya et al., 2020a; Hinenoya et al., 2022b; Naka et al., 2024; Naka et al., 2022; Ooka et al., 2012), suggesting that these animals may serve as reservoirs or carriers. Notably, raccoons are considered as the significant natural reservoir of *E. albertii* (Hinenoya et al., 2020a; Xu et al., 2023). Additionally, wild birds have also been shown to frequently carry this bacterium in several countries (Barmettler et al., 2022; Hinenoya et al., 2022a; Liu et al., 2022b; Naka et al., 2022). Moreover, among livestock, *E. albertii* has been predominantly detected in poultry (Barmettler et al., 2023; Gordon, 2011; Hinenoya et al., 2021; Oaks et al., 2010; Wang et al., 2022) and their meats (Asoshima et al., 2015; Gupta et al., 2023; Konno et al., 2021; Lindsey et al., 2015; Maeda et al., 2015), with a higher occurrence than in other meats (beef, mutton and pork) (Wang et al., 2016). Thus, poultries are considered as an important reservoir for human *E. albertii* infections.

The poultry industry is a major contributor to human nutrition across the world, providing affordable and accessible protein sources. However, the poultry supply chain faces significant challenges, particularly regarding the emergence and spreading of antimicrobial resistance (AMR) in bacteria, including the zoonotic pathogens (Ferdous et al., 2023; Samad et al., 2023). AMR and multidrug-resistant *E. albertii* have also been identified in isolates from chickens (Hinenoya et al., 2021; Wang et al., 2022). Overcrowded and unhygienic conditions on farms facilitate the spread of infectious diseases among birds, often necessitating the extensive use of antimicrobials as prophylactics and growth promoters (Ferdous et al., 2023; Imam et al., 2023). This indiscriminate use of antimicrobials accelerates the development and selection of antimicrobial-resistant bacteria, which can enter the human food chain via contaminated meats. In developing countries, where the food cold chain is not well developed, live poultry markets and shops are common to keep meats fresh. Poultry is slaughtered just before the products are provided to consumers. Processing generally lacks strict sanitation protocols and is carried out in areas not isolated from the live birds, suggesting the high risk of microbial contamination among poultry products, food handlers, and processing tools (Chowdhury et al., 2020; Uddin et al., 2019). In Bangladesh, chickens are the most ubiquitous and abundant species of domestic livestock. A recent study has shown the presence and AMR profiles of *E. albertii* in backyard chickens (Gupta et al., 2023), but the situation of *E. albertii* in retail chicken meats from the poultry industry remains unknown. Thus, epidemiological studies are warranted to assess the occurrence, virulence, and AMR of *E. albertii* in retail chickens in Bangladesh. Such studies would help to evaluate the potential role of chicken meats as a source of human infections.

Thus, this study aimed to investigate the occurrence of *E. albertii* in chicken meats sold at the retail chicken shops in Bangladesh and characterize the isolates through their genotypic and phenotypic analyses, including whole-genome sequencing. This study will provide critical insights into the role of poultry meats in the transmission of *E. albertii* to humans and the public health risks associated with the pathogen's AMR in Bangladesh.

2. Materials and methods

2.1. Ethical approval

Verbal approval was taken from the retail shop owners and associated personnels from whom the samples were collected. The study design and methodologies were approved by the Animal Welfare and Experimentation Ethics Committee, Bangladesh Agricultural University [Approval no. AWEEC/BAU/2021 (48)].

2.2. Sample collection and processing

Whole dressed broiler chickens without skin ($n = 61$), along with their giblets (liver, proventriculus-gizzard), were purchased from 17 retail outlets in 4 upazila (Sadar, Tarakanda, Trishal and Muktagacha) in Mymensingh district, Bangladesh between October 2021 and March 2023 (Supplementary Fig. 1). The 3 markets (K. R. market, Bangladesh Agricultural University; Kewatkhali Bazar, Ketwatkhali; Mechua Bazar, Mechua) are managed by Mymensingh city corporation, and others including Tarakanda Bazar, Trishal Bazar and Muktagacha Bazar are managed by their own municipalities. The chickens were slaughtered and dressed (removed skin and separated internal organs) at the outlets by the owners or workers according to their regular practices. The meat, liver and proventriculus-gizzard (P-G) from each chicken were put separately in plastic bags, labeled accordingly, and transported to the laboratory in ice box. At the laboratory, thigh and breast muscles were separated aseptically. Afterward, four different types of samples - thigh, breast, liver, P-G - from each chicken were subjected to microbiological analysis for *E. albetii*. Twenty-five gram of each sample was homogenized in 225 mL buffered peptone water (HiMedia Laboratories Private Ltd., Mumbai, Maharashtra, India), and incubated overnight (~20 h) at 37 °C for enrichment. After November 2022, cloacal swabs ($n = 35$) from the chickens before slaughtering, hand swabs ($n = 11$) of shop owners and/or outlet workers, slaughtering blade swab ($n = 10$), and bleeding cone swab ($n = 15$) were also collected in 3 mL of LB broth (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) using sterile cotton buds, and were transported to the laboratory under cold condition. Likewise, swab samples collected in LB broth were incubated overnight (~20 h) at 37 °C for enrichment.

2.3. Detection and isolation of E. albertii

After enrichment, DNA was extracted from 100 μL of the culture by alkaline heat-extraction method. Briefly, bacterial cells were pelleted by centrifuging 100 μL of the enrichment culture at ca. 10,000 *g* for 3 min. The pellet was then resuspended in 85 μL of 50 mM NaOH, boiled for 10 min, and cooled on ice. Afterward 15 μL of 1 M Tris-HCl buffer (pH 7.8) was added, and the mixture was centrifuged at 16,000 *g* for 5 min at 4 °C. The resulting supernatant was collected and used as the DNA template for PCR. DNA template from enrichment cultures of the swabs was prepared by a boiling method (Bag et al., 2021). The DNA templates were subjected to an *E. albertii*-specific PCR assay targeting *Eacdt* genes for the detection of *E. albertii* (Hinenoya et al., 2019b).

When the *Eacdt* gene was detected by the PCR, the corresponding enrichment culture was spread onto XRM-MacConkey (XRM-MAC) agar plates for isolating *E. albertii* (Hinenoya et al., 2020b), and the plates were incubated at 37 °C overnight (~ 20 h). Potential *E. albertii* colorless colonies (2–12 colonies per sample) were screened for the *Eacdt* gene and the gene-positive colonies were streaked onto XRM-MAC plates for single isolation. Obtained colonies were confirmed as *E. albertii* by another PCR assay which can differentiate 3 *Escherichia* species including *E. albertii*, *E. coli* and *E. fergusonii* (Lindsey et al., 2017). The *E. albertii* isolates were cultured in 3 mL of LB-broth at 37 °C overnight (~ 20 h) and kept with glycerol (final conc. 20 %) at −80 °C until use.

2.4. Enterobacterial repetitive intergenic consensus (ERIC)-PCR

ERIC-PCR was applied for molecular typing of *E. albertii* isolates (Versalovic et al., 1991) with minor modifications. The reaction mixture had a final volume of 20 μL consisting of 10 μL GoTaq® Green 2× master mix (Promega Corp., Madision, WI, USA), 10 pmol of each primer, 1 μL of DNA template. The PCR was performed on a thermal cycler (TurboCycler Lite; BLUE-RAY Biotech, Zhongshan, Taipei, Taiwan) with an initial denaturation of 95 °C for 2 min, followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 52 °C for 1 min, and extension at 72 °C for 2.5 min, with a final extension at 72 °C for 3 min.

2.5. Pulsed field gel electrophoresis (PFGE)

DNA fingerprint of the bacterium was analyzed by PFGE according to the protocol of pulse Net USA with slight modifications. Briefly, freshly cultured bacterial isolates were embedded into 0.5 % SeaKem Gold agarose (Lonza Rockland, Inc., Rockland, ME, USA) followed by in situ lysis in cell lysis buffer (50 mM Tris-HCl [pH 8.0], 50 mM EDTA) containing 0.5 mg/mL Proteinase K (P8044-5G, Sigma-Aldrich Co. LLC, St. Louis, MO, USA) and 1.0 % N-laurylsarcosine (Sigma-Aldrich) at 54 °C for 2 h. The agarose-embedded genomic DNA was digested with 20 U of *Xba*I (Takara Bio., Shiga, Japan) at 37 °C for 2 h. The digested DNA was electrophoresed on a 1.0 % pulsed-field certified agarose gel (Bio-Rad laboratories Inc., Hercules, CA, USA) in 0.5× TBE buffer under auto-algorithm with a molecular weight ranging from 30 to 600 kb using a CHEF Mapper (Bio-Rad laboratories Inc.) *Xba*I-digested *S.* Braenderup strain H9812 was used as a molecular size marker.

2.6. Antimicrobial susceptibility test

Selected *E. albertii* isolates were subjected to antimicrobial susceptibility testing. The antimicrobial susceptibility of the isolated *E. albertii* was determined by the Kirby Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute guidelines (M100-Ed30) (CLSI, 2020) and interpreted based on the diameter size of inhibition zone as susceptible, intermediate and resistant. A total of 15 antimicrobials including ampicillin (AMP, 10 μg), cefotaxime (CTX, 30 μg), ceftazidime (CAZ, 30 μg), cefoxitin (FOX, 30 μg), fosfomycin (FOF, 50 μg), meropenem (MEM, 10 μg), imipenem (IPM, 10 μg), gentamicin (GEN, 10 μg), kanamycin (KAN, 30 μg), streptomycin (STR, 10 μg), tetracycline (TET, 30 μg), chloramphenicol (CHL, 30 μg), nalidixic acid (NAL, 30 μg), ciprofloxacin (CIP, 5 μg), sulfamethoxazole-trimethoprim (SXT, 23.75/1.25 μg) were tested for each isolate. The antimicrobial discs were purchased from Becton, Dickinson and Company. *E. coli* strain ATCC25922 was used as the control strain in each experiment. Isolates showing resistance to three or more classes of antimicrobials are considered multidrug resistant (MDR) (Magiorakos et al., 2012).

2.7. Detection of virulence genes

E. albertii isolates were screened for the presence of *eae* and *stx* genes by a PCR assay which can specifically detect *eae*, *stx* (both *stx1* and *stx2*) and *cdt* genes (Hassan et al., 2019).

2.8. Whole genome sequencing (WGS) and analysis

Short-read sequencing was performed on NextSeq 1000 sequencing system. *E. albertii* isolates were cultured in 3 mL of LB broth (Becton, Dickinson and Company) and genomic DNA was extracted using DNeasy Blood & Tissue Kits following the protocols for Gram-negative bacteria (Qiagen, Hilden, Germany). DNA library was prepared by dual indexing using QIAseq FX DNA Library CDI Kit (QIAGEN). DNA concentration of the library was measured by Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). A 150 bp pair-end sequencing was done on NextSeq 1000 sequencer using a NextSeq 1000/2000 P1 reagent cartridge (300 cycles). Fastp v0.24.0 was used for trimming and filtering of raw read sequences (Chen, 2023), and SKESA v2.5.1 was used for de novo assembling with default parameters (Souvorov et al., 2018). The draft sequences were applied as the draft genomes to the following analyses. Virulence gene profiling was done by uploading the draft genomes to Virulence Factor Database (VFDB; http://www.mgc.ac.cn/VFs/) using the Genus *Escherichia* database (Liu et al., 2022a). AMR gene, plasmid profiling and cgMLST were done on the online platform of Center for Genomic Epidemiology (https://www.genomicepidemiology.org/services/) using the ResFinder v4.6.0 (Bortolaia et al., 2020; Camacho et al., 2009), PlasmidFinder v2.1 with the Enterobacteriales database (Camacho et al., 2009; Carattoli et al., 2014), and cgMLSTFinder v1.0.1 (Clausen et al., 2018; Jolley and Maiden, 2010), respectively. EAO and EAH genotypes were determined by in silico analysis. Sequencing reads of each isolate were mapped to *wzx/wzy* gene sequences of the 40 EAOgs and *fliC* gene sequences of the 4 EAHgs from each reference strain, respectively (Nakae et al., 2021; Ooka et al., 2019) on CLC Genomics Workbench 24 (Qiagen). For phylogenetic analysis, Snippy v3.1 was used to identify single nucleotide polymorphisms (SNPs) in the genomes of the *E. albertii* strains and generate the SNP alignment using the genome of *E. albertii* strain CB9786 (Accession number AP014856.1) as reference. Prophage regions were identified using PHASTEST (https://phastest.ca/) and masked from the alignment. Recombinant regions were identified and removed using Gubbins v3.4 (Croucher et al., 2014). The final core alignment of 45,375 bases was extracted with SNP-sites (Page et al., 2016). A maximum likelihood phylogenetic tree was inferred under the best-fit model of TVM + G with 1000 bootstrap replicates using RAxML Next Generation v1.2.2 (Kozlov et al., 2019). For pairwise SNP distance calculation, core genome alignment was performed through Roary Pangenome pipeline (Page et al., 2015), and SNP distance was calculated from the core genome alignment using snp-dists (https://github.com/tseemann/snp-dists) and visualized with plot_matrix_heatmap.sh (https://eead-csic-compbio.github.io/get_homologues/manual/). The mid-point rooted tree was visualized using iTOL ver. 6 (Letunic and Bork, 2024) together with other characteristics and sample information of each isolate.

3. Results

3.1. Occurrence of E. albertii in retail chickens, handlers and equipment

To know the distribution of *E. albertii* in retail chicken meats in Bangladesh, initially chicken meats were purchased by the whole body 2 times at 2 outlets (A and B) in K. R. market of Sadar upazila, and 4 parts of meats including breast, thigh, P-G and liver from each chicken were individually tested for the presence of *E. albertii* after enrichment (Table 1). Although *E. albertii* was not detected in the meats from all the 4 chickens of outlet A by an *E. albertii*-specific PCR assay, those from the chickens ($n = 5$) of outlet B were constantly 100 % PCR-positive, suggesting that occurrence of *E. albertii* could be different by outlets. Then, chicken samples were extensively collected from outlets (H to Q; 15 from 4 upazila) in different locations. From November 2022, swab was also collected from cloaca of chickens, hands of handlers, and blades and exsanguination cones for slaughtering, respectively. While no meats were PCR-positive at 3 outlets (H, J and Q), any of the meats were PCR-positive in all the tested chickens at the remaining 12 outlets. Consistent with the *E. albertii* detection in the meats, corresponding cloacal swabs were also positive by the PCR assay except for the chickens of outlet Q, suggesting the contamination of meats by intestinal contents containing *E. albertii* during processing. Accordingly, when the meats were PCR-positive, any of the surrounding environmental samples including hands, cones and blades were also positive for *E. albertii* detection. *E. albertii* was detected in the meats of 60.7 % thigh, 59.0 % breast, 57.4 % liver and 50.8 % P-G (Table 1), where no statistical difference was found in the occurrence of *E. albertii* among the parts ($p = 0.804$ by Fisher's exact test). Using an *E. albertii*-selective isolation medium, XRM-

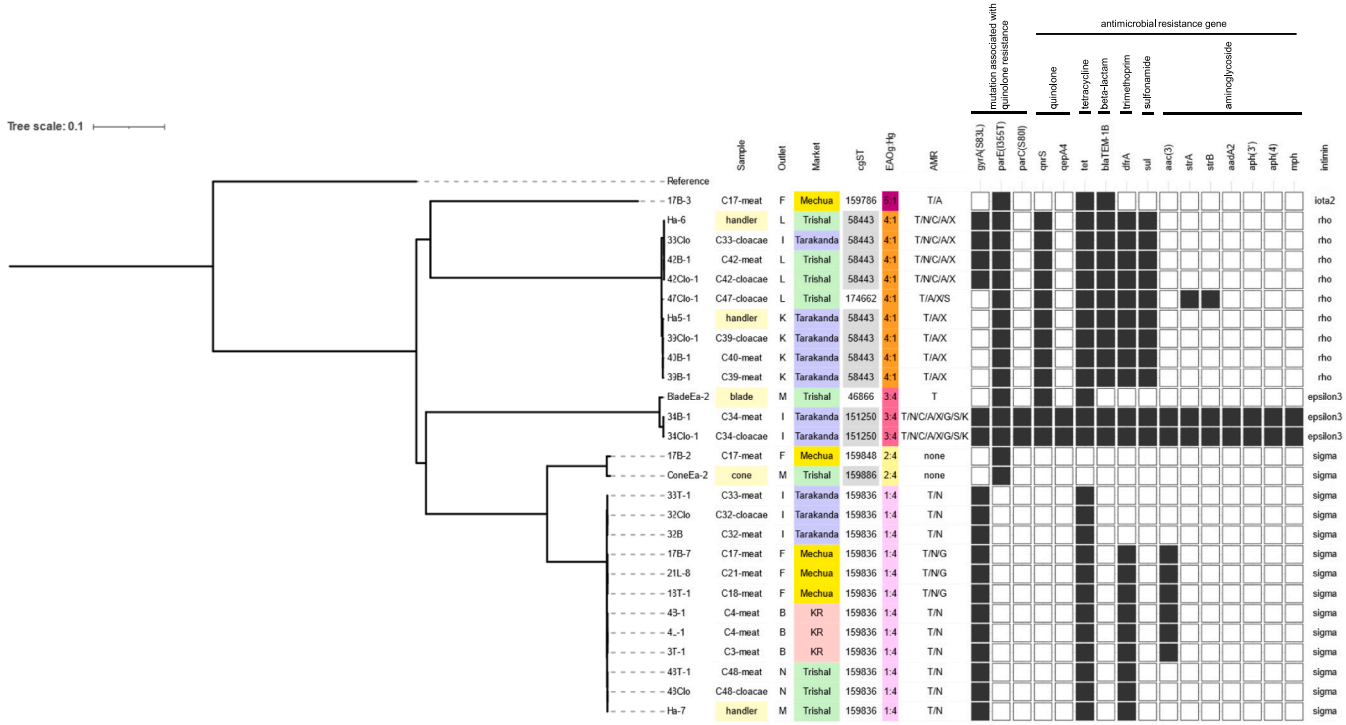

Fig. 1. Phenotypic and genotypic characteristics, and phylogenetic relatedness of *Escherichia albertii* isolates from chickens, their handlers and equipment in retail outlets, Bangladesh.
Maximum likelihood phylogenetic tree was constructed based on the 45,375 core genome SNPs of 27 *E. albertii* strains. The mid-point rooted tree is shown with strain names, their sampling information and characteristics. *E. albertii* strain CB9786 was used as a reference strain (Accession number AP014856.1). cgST of the isolates was determined by cgMLST Finder v1.0.1 with *E. coli* database. O- and H-antigen genotypes were determined by in silico analysis using whole genome sequences of the isolates. Antimicrobial resistance of the isolates were included: T, tetracycline; N, nalidixic acid; C, ciprofloxacin; A, ampicillin; X, sulfamethoxazole-trimethoprim; G, gentamicin; S, streptomycin; K, kanamycin. Intimin subtypes were determined by using the putative amino acid sequences from *eae* genes. Presence and absence of antimicrobial resistance genes and mutations are shown by closed and open squares, respectively. Chicken ID is shown by number with prefix 'C' in sample column.

MacConkey agar, a total of 69 *E. albertii* isolates were obtained from the 36 PCR-positive samples including 8 thighs, 2 P-G, and 7 each of breasts, livers and cloaca, 3 hands and 1 each of cone and blade (Table 1, Supplementary Table 1). By virulence gene profiling using a multiplex PCR assay, all the 69 isolates were *eae* gene-positive and *stx* gene-negative. By ERIC-PCR based genotyping to assess the diversity of the isolates within the samples, it was found that the colonies from a breast 17B exhibited 3 different ERIC genotypes, and the isolates from other samples exhibited single ERIC-patterns, respectively (Supplementary Table 1). Inter-sample heterogeneity of the *E. albertii* strains from each chicken body was not observed excluding the chicken ID 17. PFGE typing of the selected isolates obtained from the samples collected by June 18th in 2022 discriminated them equally to the ERIC-PCR typing (Supplementary Fig. 2), indicating that the ERIC-PCR based genotyping was reliable at least to examine the intrasample diversity of the *E. albertii* isolates. Finally, 1 isolate per ERIC genotype was selected from each sample, yielding 38 *E. albertii* isolates in total. However, since 2 isolates (22L-1 and 23L-1) unfortunately could not be revived from the stocks, 36 *E. albertii* isolates were subjected to the following characterizations.

3.2. Antimicrobial resistance

Antimicrobial susceptibility of the 36 *E. albertii* isolates were screened against 15 antimicrobials by disc diffusion method. TET resistance was predominant (94.4 %) among the isolates, followed by the resistances to NAL (69.4 %), AMP (38.9 %), SXT (36.1 %), GEN (19.4 %), CIP (16.7 %), STR (8.3 %) and KAN (5.6 %) (Supplementary Table 1). No isolate showed resistance to CTX, CAZ, FOX, IMP, MEM, FOF and CHL. As shown in Table 2, 18 isolates (50.0 %) were found to be MDR, showing resistances to three and more classes of antimicrobials (Table 2, Supplementary Table 1).

3.3. Genomic characteristics of the E. albertii isolates

Based on the ERIC-PCR genotyping and AMR profiling of the isolates, 27 *E. albertii* isolates were further selected and subjected to genomic characterization through WGS using Illumina short read platform. WGS-based analyses revealed the bacteriological characteristics and genomic diversity of the 27 *E. albertii* isolates (Fig. 1, Supplementary Table 1). In silico O and H genotyping divided the isolates into 5 genotypes including 1:4 (12 isolates), 2:4 (2), 3:4 (3), 4:1 (9) and 5:1 (1). The core-genome multilocus sequencing typing (cgMLST) using the *E. coli* database separated them into 9 diverse cgSTs, and some of the isolates belonging to same O:H genotypes showed the diversity of cgSTs, such as 46866 and 151250 in 3:4. Virulence gene profiling of the strains using the software (Virulence Factor Database) confirmed the presence of *eae* gene and the absence of *stx* genes in all 27 isolates. No other *cdt* genes than *Eacdt* were present in the isolates. Intimin subtyping using the putative amino acid sequences from *eae* gene revealed 4 subtypes including epsilon3, iota2, rho and sigma with sigma being the prominent, and no variation of the subtypes was found among the isolates of each O:H genotype. wgSNP-based phylogenetic analysis separated the isolates into 5 diverse clades consisting of respective single O:H genotype. Although the phylogenetic diversity of the isolates was shown, the isolates with clonal relationship were also identified, in which the isolates from the same chicken, outlets and/or market were located in same clusters, respectively (Fig. 1). For example, in chicken ID 32 (32Clo and 32B), 34 (34Clo-1 and 34B-1), 39 (39Clo-1 and 39B-1) and 42 (42Clo-1 and 42B-

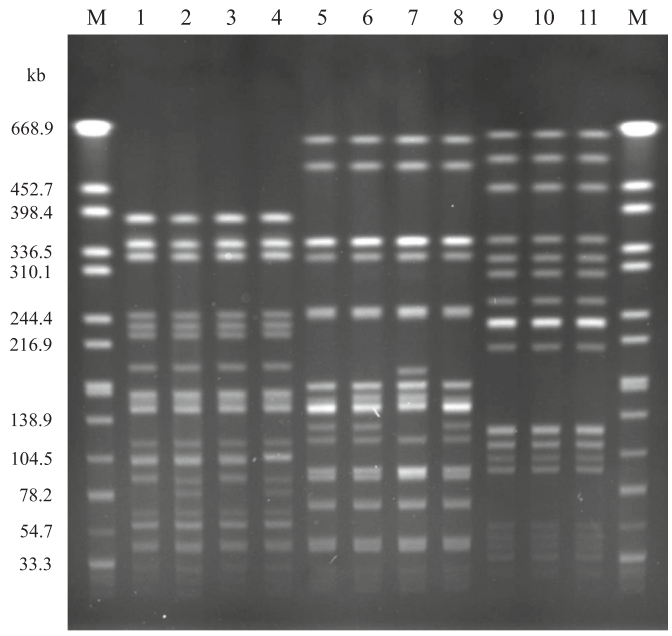

Fig. 2. Genetic relatedness of the *Escherichia albertii* isolates from chickens and handlers.
Genomic DNA was digested with *Xba*I and separated by PFGE. Lanes 1, Ha5-1 (hand); 2, 39Clo-1 (cloaca); 3, 39B-1 (breast); 4, 40B-1 (breast); 5, Ha-6 (hand); 6, 42Clo-1 (cloaca); 7, 42B-1 (breast); 8, 33Clo (cloaca); 9, Ha-7 (hand); 10, 48Clo (cloaca); 11, 48T-1 (thigh). M, *Xba*I-digested genomic DNA of *Salmonella* Braenderup strain H9812 used as a molecular size marker.

1), the isolates from their cloacae and meats were clonally related with 0 to 3 pairwise SNP distances (Supplementary Fig. 3). The handler's isolate Ha-6 was also clonally related to the isolates from chicken ID 42 that the handler processed although the handler's isolate Ha-5 showed 33 to 40 SNP distances with the isolates from the chickens that the handler processed. Furthermore, clonal relationships were found between the meat isolates from different chicken individuals in same sampling batch (17B-7 vs 18T-1 vs 21L-8 from outlet F, and 3T-1 vs 4L-1 vs 4B-1 from outlet B) although cloacal and handler's samplings were not done at that time. Interestingly, the handler's isolate Ha-7 from outlet M was also clustered together with the chicken isolates from another outlet N (48Clo and 48T-1). These data indicate a possible cross contamination among handlers and chickens. The genomic similarity of the isolates was further confirmed by PFGE (Fig. 2). Isolates from the chicken meat, cloacal and human hand swab revealed identical PFGE patterns. Some of them showed one band difference each other, but they can be interpreted as clonal considering the Tenover's criteria (Tenover et al., 1995). Moreover, genetic relatedness was also observed between isolates from different Market (33Clo vs Ha-6 vs 42Clo vs 42B).

Analysis of AMR genes revealed the presence of genes against respective AMR-phenotypes (Fig. 1, Supplementary Table 1), while the isolates susceptible to all the antimicrobials tested didn't carry any known AMR genes. Among the 34 TET resistant isolates, 88 % carried *tetA* and the rest (12 %) carried *tetB* gene. On the other hand, quinolone resistant isolates showed different combinations of chromosomal gene mutations as well as plasmid-mediated quinolone resistance (PMQR) genes. All the 25 quinolone resistant isolates carried mutation in at least one of chromosomal resistance genes (*gyrA*, *parE*, *parC*), and/or harbored PMQRGs (*qnrS*, *qepA*). S83L mutation in *gyrA* gene, *gyrA* (S83L) was found in all the 25 isolates, but not in the isolates with no resistance to quinolones. *parE* (I355L) mutation was found in 7 quinolone-resistant isolates while the mutation was also found in 6 quinolone-sensitive isolates. Two quinolone resistant isolates additionally harbored *parC* (S80I) mutation. *qnrS* (*qnrS1* or *qnrS13*) gene was found in 11 resistant and 1 susceptible isolate to quinolones, respectively. *qepA3* gene was also detected in 2 quinolone resistant isolates.

All the ampicillin resistant isolates carried $bla_{\text{TEM-1B}}$, and the SXT resistant isolates carried *sul2* and *dfrA* (*dfrA1* or *dfrA14*) genes, indicating their association with the respective antimicrobial resistances. Besides, aminoglycosides resistance genes *aph(4)Ia*, *aph(6)-Id*, *aph(3′)-Ia*, *aph(3″)-Ib* and *aac(3)-IV* were detected in 2 isolates showing resistance to respective antimicrobials (Fig. 1, Supplementary Table 1). The 2 isolates additionally carried hygromycin-resistance gene, *aph(4)-Ia*, and macrolide-resistance gene, *mph(A)* gene although the phenotypes was not tested.

Plasmid typing of the isolates was further performed using a Plasmid Finder software, and various plasmid replicons were identified in the isolates, such as Col156, IncFIA, IncFIB, IncFII, IncX1, IncX4, IncI1, IncHI2, InHI2A and IncX1 (Supplementary Table 1). Interestingly, *qnrS* and *tetA* genes were located on the same contigs with the replicon IncI1 in all the nine isolates of cgST 58,443 and an isolate of cgST46866. The three AMR genes ($bla_{\text{TEM-1B}}$, *qnrS1* and *tetA*) of isolate 17B-3 shared the contig with the replicon IncX1. Furthermore, six AMR genes ($bla_{\text{TEM-1B}}$, *strA*, *strB*, *qnrS1*, *sul2* and *tetA*) shared the contig with the replicon IncFII in the isolate 47Clo-1.

4. Discussion

This study highlights the significant occurrence of *E. albertii* in retail chicken outlets in Bangladesh, with 63.9 % of chickens testing positive in their meats. The detection of *E. albertii* in multiple anatomical sites, including thigh muscle, breast, liver, and P-G, suggests widespread contamination across the meat products examined. Notably, cross-contamination was evident, as isolates from chicken meat, cloacal swabs, and human hand swabs from the same retail outlets were genetically identical, underscoring the importance of hygienic practices during meat processing and handling (Ferdous et al., 2023). To the best of our knowledge, this is the first report of *E. albertii* from retail chicken meats in Bangladesh, which raises concerns about the potential risks to public health.

The prevalence of *E. albertii* in our study is considerably higher than that reported in similar studies globally. For instance, previous studies have identified *E. albertii* as an emerging pathogen in wild animals, birds, poultry, poultry carcass washings and occasionally in humans, but its detection in retail meat, especially at such high rates, is uncommon (Hinenoya et al., 2021; Hinenoya et al., 2020a; Hinenoya et al., 2022b; Lindsey et al., 2015; Maeda et al., 2015; Ooka et al., 2013). The occurrence of *E. albertii* in chicken meats and giblets was reported as 2.83–2.88 % in Japan (Konno et al., 2021; Maeda et al., 2015). In addition to meats, this study detected 71.4 % *E. albertii* from cloacal swab which is much higher than those reported in cloacal swab of broiler chicken in the USA (15.92 to 30 %) (Hinenoya et al., 2021; Wang et al., 2022), and backyard poultry (3.5 %) in Bangladesh (Gupta et al., 2023). The high level of difference might be associated with the sample size or geographical location, however, association of poor hygiene and production practices at pre-harvest level cannot be overlooked. In addition, we encountered a significant difference in the *E. albertii* occurrence at different locations. The significant differences in occurrence between sampling locations also suggest potential variations in local handling practices or environmental factors that may influence the spread of this pathogen. These findings are a crucial addition to the growing body of literature on the role of foodborne pathogens in disease transmission.

A concerning finding from our study is the high level of MDR exhibited by *E. albertii* isolates, with 50.0 % of the strains being classified as MDR. Resistance to antimicrobials such as TET, NAL, AMP, CIP and GEN is alarming, particularly in the context of Bangladesh, where use of these antimicrobials is widespread and officially approved for use in animal production (DGDA, 2023; Foysal et al., 2024; Hoque et al., 2020;

Table 1
Occurrence of *Escherichia albertii* in broiler chickens and the retail outlets in Mymensingh, Bangladesh.

Location	Retail markets	Outlet	Collection date (YYYY/MM/DD)	No. of chickens		No. of PCR-positive samples/No. of *E. albertii* isolation-positive samples					No. of PCR-positive/No. of samples (%) /No. of *E. albertii* isolation-positive		
				Tested	*E. albertii*-positive in meats	Breast	Thigh	P-G	Liver	Cloaca	Hand	Cone	Blade
Sadar	K. R. market	A	2021/10/06	2	0 (0)	0/NA	0/NA	0/NA	0/NA	ND	ND	ND	ND
			2022/01/01	2	0 (0)	0/NA	0/NA	0/NA	0/NA	ND	ND	ND	ND
		B	2021/11/08	2	2 (100)	2/1	2/2	2/1	2/1	ND	ND	ND	ND
			2022/01/07	3	3 (100)	3/0	3/0	3/0	3/0	ND	ND	ND	ND
		Subtotal		10	5 (50.0)	5/1	5/2	5/1	5/1	ND	ND	ND	ND
	Kewatkhali market	C	2022/01/21	3	2 (66.7)	1/0	1/0	1/0	0/NA	ND	ND	ND	ND
		D	2022/02/11	3	3 (100)	2/0	3/0	2/0	3/0	ND	ND	ND	ND
		E	2022/09/22	3	1 (33.3)	0/NA	1/0	0/NA	0/NA	ND	ND	ND	ND
		Subtotal		9	6 (66.7)	3/0	5/0	3/0	3/0	ND	ND	ND	ND
	Mechua Bazar	F	2022/06/18	5	5 (100)	5/1	5/2	5/0	5/2	ND	ND	ND	ND
		G	2022/09/22	2	2 (100)	2/0	2/0	2/0	2/2	ND	ND	ND	ND
		H	2022/11/20	5	0 (0)/NA	0/NA	0/NA	0/NA	0/NA	0/NA	0/2 (0)/NA	0/3 (0)/NA	0/1 (0)/NA
		Subtotal		12	7 (58.3)	7/1	7/2	7/0	7/4	0/NA	0/2 (0)/NA	0/3 (0)/NA	0/1 (0)/NA
Tarakanda	Tarakanda Bazar	I	2022/12/06	3	3 (100)	3/2	3/2	3/1	3/1	3/3	0/1 (0)/NA	0/3 (0)/NA	0/1 (0)/NA
		J		4	0 (0)	0/NA	0/NA	0/NA	0/NA	0/NA	0/1 (0)/NA	0/2 (0)/NA	0/1 (0)/NA
		K		3	3 (100)	3/2	3/1	3/0	3/1	3/1	1/1 (100)/1	1/1 (100)/0	0/1 (0)/NA
		Subtotal		10	6 (60.0)	6/4	6/3	6/1	6/2	6/4	1/3 (33.3)/1	1/6 (16.7)/0	0/3 (0)/NA
Trishal	Trishal Bazar	L	2023/01/08	3	3 (100)	3/1	3/0	3/0	3/0	3/1	1/1 (100)/1	0/1 (0)/NA	0/1 (0)/NA
		M		3	3 (100)	3/0	3/0	3/0	3/0	3/1	1/1 (100)/1	1/1 (100)/1	1/1 (100)/1
		N		4	4 (100)	4/0	4/1	4/0	4/0	3/1	0/1 (0)/NA	0/1 (0)/NA	0/1 (0)/NA
		Subtotal		10	10 (100)	10/1	10/1	10/0	10/0	9/3	2/3 (66.7)/2	1/3 (33.3)/1	1/3 (33.3)/1
Muktagacha	Muktagachha Bazar	O	2023/03/02	3	3 (100)	3/ND	2/ND	0/ND	2/ND	3/ND	1/1 (100)/ND	0/1 (0)/NA	0/1 (0)/NA
		P		3	2 (66.7)	2/ND	2/ND	0/NA	2/ND	3/ND	1/1 (100)/ND	0/1 (0)/NA	0/1 (0)/NA
		Q		4	0 (0)	0/NA	0/NA	0/NA	0/NA	4/ND	0/1 (0)/NA	0/1 (0)/NA	0/1 (0)/NA
		Subtotal		10	5 (50)	5/ND	4/ND	0/ND	4/ND	10/ND	2/3 (66.7)/ND	0/3 (0)/NA	0/3 (0)/ NA
Total				61	39 (63.9)	36 (59.0)/7	37 (60.7)/8	31 (50.8)/2	35 (57.4)/7	25 (71.4)/7	5/11 (45.5)/3	2/15 (13.3)/1	1/10 (10.0)/1

ND, not done; NA, not applicable (i.e. the samples were negative by *E. albertii*-specific PCR thus not subjected for the isolation of *E. albertii*); P-G, proventriculus gizzard.

Imam et al., 2023). According to Bangladesh National Veterinary Formulary (BDNVF) (DGDA, 2023), several antimicrobials have been routinely used for the growth and production as well as treatments of animals including livestock, in which all the antimicrobials to which the *E. albertii* isolates in this study frequently showed resistances are included. Such resistance patterns are consistent with previous studies on other foodborne pathogens in Bangladesh, reinforcing concerns about the public health implications of antimicrobial use in poultry farming (Ali et al., 2023; Foysal et al., 2024; Imam et al., 2023).

The detection of AMR genes further emphasizes the risk of antimicrobial-resistant *E. albertii* being transmitted through the food chain. Interestingly, we observed multiple avenues of achieving quinolone resistance in the study isolates. The isolates carried PMQR genes alongside chromosomal mutations in *gyrA*, *parE* and *parC*. Although PMQR genes have been identified, such as *qnr*, *qep* and *aac(6′)-Ib-cr* (Jacoby et al., 2014), major mechanism of quinolone resistance is chromosomal gene mutations encoding for DNA gyrase subunits (*gyrA* and *gyrB*) and topoisomerase IV subunits (*parC* and *parE*), particularly in the quinolone resistance determining regions (QRDRs). Various mutations associated with resistance to quinolones have been identified (Boueroy et al., 2023; Sáenz et al., 2003), and accumulation of the mutations makes the resistance level of bacteria higher (Bagel et al., 1999; Huseby et al., 2017). In this study, all the whole genome-sequenced isolates harbored mutations in one or more of the genes associated with quinolone resistance, suggesting that the routine use of antimicrobials has enhanced the selection of the strains with resistance to quinolones. Indeed, 70.3 % and 21.6 % of the *E. albertii* isolates were resistant to NAL and CIP, respectively. While sole mutation of *parE*

Table 2
Antimicrobial resistance pattern of the *Escherichia albertii* isolates.

Resistance pattern	No. of antimicrobial class	No. of isolates
TET, GEN, KAN, STR, AMP, CIP, NAL, SXT	5	2
TET, AMP, CIP, NAL, SXT	4	4
TET, STR, AMP, SXT	4	1
TET, AMP, SXT	3	6
TET, GEN, NAL	3	5
TET, AMP	2	1
TET, NAL	2	14
TET	1	1
None	0	2
Total		36

(I355T) was identified in the isolates with and without resistance to NAL, all the NAL-resistant isolates harbored *gyrA* (S83L) gene mutation. Considering the mutation is the major quinolone resistance mechanism of *E. coli* (Boueroy et al., 2023; Huseby et al., 2017; Sáenz et al., 2003), the *gyrA* (S83L) gene mutation could also be responsible for the NAL resistance of the *E. albertii* isolates in this study. The *E. albertii* isolates with resistance to CIP showed additional common genetic features of harboring a mutation of *parE* (I355T) and carrying *qnrS* gene, suggesting that either or both the genes decreased the susceptibility to quinolones, which lead to CIP resistance of the isolates. To the best of our knowledge, this is the first report showing the mutations in both *gyrA* and *parE* gene mutations in *E. albertii*. However, since this study did not quantitatively analyze the quinolone resistances, it cannot be explained how much they are associated with the quinolone resistance of the *E. albertii* isolates. Thus, further studies are needed to unveil their contribution in resistance by gene-manipulation works. Moreover, although multiple mutations decreasing the susceptibility to quinolones are frequently identified in the chromosomal genes for quinolone resistance in *E. coli* (Boueroy et al., 2023; Sáenz et al., 2003), only single mutation has been identified in those genes of *E. albertii* including the isolates of the present study (Iguchi et al., 2023). However, the information is severely limited in *E. albertii*. Continuous and comprehensive epidemiological investigations are highly warranted to understand the real situation of the quinolone resistances in this bacterium.

In addition to quinolones resistance genes, *tetA*, *tetB*, $bla_{\text{TEM-1B}}$, and *sul2* were detected in the *E. albertii* isolates. The association of *qnrS* and *tetA* genes with IncFII and IncI1 plasmids suggests that these mobile genetic elements play a key role in the horizontal transfer of resistance genes, further emphasizing the risk of transferring these resistances through food chain. Such resistances and associated genotypes were also reported in *E. albertii* isolated from human and animals including chicken (Bengtsson et al., 2023; Li et al., 2018). Tetracycline, beta-lactams and sulfonamides are among the mostly used antimicrobials in the poultry industries of Bangladesh, and occurrence of corresponding resistance genes have been reported from food borne pathogens isolated from cloacal swab, meat and chicken rearing environment in Bangladesh (Ali et al., 2023; Foysal et al., 2024; Parvin et al., 2022), indicating a widespread distribution of these resistance genes in the environment and food borne pathogens. In addition, the presence of plasmid types such as IncX1 and IncX4, which have been frequently linked to the dissemination of AMR genes in other pathogens (Dobiasova and Dolejska, 2016; Juraschek et al., 2021) further highlights the potential for the spread of AMR through the food supply chain.

The genomic characterization of *E. albertii* isolates revealed genetic diversity, as evidenced by the clustering patterns based on cgMLST and EAO:H genotyping. Despite this diversity, isolates from the same retail outlets, and even the same chicken were genetically identical, indicating potential cross-contamination during processing. In the present study, the genetic similarity among same EAOg:Hg type was further confirmed by PFGE analysis, which demonstrated identical PFGE patterns among isolates from chicken meat, cloacal swabs, and human hand swabs. These findings strongly suggest that poor hygienic practices during handling and processing may facilitate the transmission of *E. albertii* from contaminated chicken to human hands, posing a risk of human infections.

The findings of this study have important public health implications, particularly in Bangladesh, where poultry is a major component of the diet and foodborne pathogens remain a significant concern. The high prevalence of *E. albertii* in retail chicken meat, combined with the evidence of cross-contamination during processing, raises serious concerns about food safety. The transmission of MDR *E. albertii* from poultry to humans, as suggested by the identical genomic and phenotypic characteristics of isolates from chicken and human hand swabs, underscores the need for improved hygiene and biosecurity measures in retail meat outlets. Public health interventions, including education on proper handling practices and routine monitoring of foodborne pathogens, are essential to mitigate the risks associated with contaminated poultry products.

5. Conclusions

This study provides compelling evidence of the widespread occurrence of *E. albertii* in retail chicken meat in Bangladesh and the potential for cross-contamination during processing. The high rates of multidrug resistance, coupled with the presence of virulence genes, highlight the need for stringent food safety measures and continued surveillance of emerging pathogens like *E. albertii*. Future research should focus on the sources of contamination at the farm level, as well as the potential human health risks associated with the consumption of contaminated poultry products.

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CRediT authorship contribution statement

Jayedul Hassan: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Kishor Sosmith Utsho:** Investigation, Formal analysis, Data curation. **Susmita Karmakar:** Investigation, Data curation. **Md. Wohab Ali:** Investigation, Data curation. **Sharda Prasad Awasthi:** Investigation, Formal analysis, Data curation. **Chiharu Uyama:** Investigation, Formal analysis. **Noritoshi Hatanaka:** Data curation. **Shinji Yamasaki:** Writing – review & editing, Formal analysis, Data curation. **Atsushi Hinenoya:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All sequence data obtained in this study have been deposited in DDBJ under BioProject accession number PRJDB19619.

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