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RESEARCH ARTICLE

Genomic Characteristics of ETT2 Gene Clusters in Avian Pathogenic *Escherichia coli* Identified by Whole-genome Sequencing

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ABSTRACT

Escherichia coli type three secretion system 2 (ETT2) is the second type of *E. coli* three secretory system. The complete ETT2 locus encodes 35 open reading frames (ORFs), while most *E. coli* have significant deletions and insertions in the ETT2 gene cluster. The intact ETT2 cluster or genes located in the ETT2 cluster contribute to the pathogenicity of avian pathogenic *Escherichia coli* (APEC). However, there are few reports on the genomic information and characteristics of ETT2 gene clusters. Here, we used whole-genome sequencing to analyze the characteristics of the ETT2 cluster and compared the types of ETT2 in this study with those reported previously. Compared with the ETT2 gene cluster in *E. coli* O157:H7, there were five different types of ETT2 in these strains. Strains possessing the complete ETT2 gene cluster maintained a closer affinity to those devoid of any genes from the ETT2 gene cluster. ST117 type predominated among these strains, and O166 was also adept at harboring an ETT2 cluster. However, no discernible correlation was discerned between the presence of the ETT2 gene cluster and virulence genes. This work will help researchers to understand ETT2 more comprehensively.

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INTRODUCTION

The type III secretion system constitutes a fundamental mechanism facilitating direct interaction between bacteria and their hosts, exhibiting an indispensable role in the process of bacteria-host interplay (Bohn *et al.*, 2019). The type III secretion system, prevalent in Gram-negative bacteria, diverges into two distinct variants: type III secretory system 1 (ETT1) and type III secretory system 2 (ETT2) (Zhou *et al.*, 2014). In the voyage of whole-genome sequencing for enterohaemorrhagic *E. coli*, researchers initially unearthed the ETT2 cluster. A comprehensive ETT2 locus encompasses an array of 35 open reading frames (ORFs), including *yqe*, *yge*, *etr*, *epr*, *epa*, and *eiv*. Diverging from alternative secretion systems, not all ETT2-positive *E. coli* strains manifest a complete assembly comprising these 35 genes (Ideses *et al.*, 2005). Only a handful of intact ETT2 sets have been documented, while most strains acquire ETT2 genes harboring deletions and insertions.

Until now, the ETT2 gene cluster has been detected in extraintestinal pathogenic *Escherichia coli* (ExPEC) obtained from chickens, pigs, and humans (Wang *et al.*, 2016a; Shulman *et al.*, 2018; Yuan *et al.*, 2018). The ETT2 gene clusters play a significant role in influencing the virulence of ExPEC. APEC, the causative agent of avian colibacillosis, shares similar virulence genes with NMEC and UPEC, making it a potential reservoir for ExPEC virulence genes in humans (Hu *et al.*, 2022). In APEC, numerous ETT2 genes and ETT2 clusters have been demonstrated to impact virulence, encompassing YqeI, YqeH, YgeG, YgeK, EivC, EtrA, and EivF (Wang *et al.*, 2016b; Wang *et al.*, 2017; Xue *et al.*, 2020; Fu *et al.*, 2021; Yin *et al.*, 2021; Xue *et al.*, 2022; Yin *et al.*, 2022). However, the effects of these genes on virulence exhibit discrepancies. Inactivation of YqeI, YqeH, YgeK, EtrA, or EivC led to diminished flagella formation, biofilm formation, bacterial survival capability and so forth (Wang *et al.*, 2016b; Wang *et al.*, 2017; Xue *et al.*, 2020; Xue *et al.*, 2022; Yin *et al.*, 2022). Conversely,

inactivation of YgeG or EivF resulted in heightened biofilm formation, adherence to and invasion of DF-1 cells, and serum resistance (Fu *et al.*, 2021; Yin *et al.*, 2021). Following the deletion of ETT2 in uropathogenic *E. coli* (UPEC), both the synthesis and secretion of flagellin, along with exercise capacity, were significantly diminished. Simultaneously, there was an enhancement in resistance to hydrophobic antibiotics, whereby ETT2 helped pathogens survive in serum and trigger sepsis (Shulman *et al.*, 2018). Through the elimination of the ETT2 gene cluster in *E. coli* meningitidis EC10, the invasiveness and intracellular viability of EC10 on human brain microvascular endothelial cells (HBMEC) were weakened. Furthermore, when the *eivA* gene within the ETT2 gene cluster was deleted, EC10's hostility towards HBMECs decreased by 50% while its intracellular viability reduced by 80%. This underscores the crucial role played by *eivA* in the pathogenesis of *E. coli* infection in host cells (Yao *et al.*, 2009).

A previous study showed that among the APEC strains analyzed, 57.6% contained the ETT2 gene cluster, presenting five distinct variants. Compared to human ExPEC, APEC exhibited a wider distribution and a greater variety of ETT2 gene clusters. PCR once served as the primary modality for discerning the existence of the ETT2 gene in isolates (Wang *et al.*, 2016a). Sequence analysis revealed that the deletion of the ETT2 gene in the majority of strains stemmed from the presence of a premature stop codon at the ETT2 locus. Nevertheless, the genomic attributes of ETT2 remained obscured. Whole-genome sequencing affords an efficacious avenue for bacteriomes research to garner comprehensive bacterial genome intelligence (Chen *et al*., 2024; Wang *et al.*, 2024). To learn more about the genomic characteristics of ETT2 in APEC, and explore the existence types of ETT2 and the relationships between the existence types, MLST, serotypes, and virulence, in this investigation, we employed the Illumina PE150 sequencing platform to execute whole-genome sequencing on select APEC strains archived in the laboratory. We scrutinized multi-locus sequences, serotypes, virulence factors, and the ETT2 gene clusters, and then analyzed their relationship, which will provide reference for the researchers to next research in ETT2.

MATERIALS AND METHODS

Sequencing library preparation and processing of raw disembarkation data: In this study, we obtained twenty-five APEC strains and extracted bacterial DNA utilizing the High Pure PCR Template Preparation Kit (Roche, USA). Subsequently, we determinated DNA concentration using the Qubit Fluorometer. Beijing Novogene Technology Co., Ltd prepared the Whole Genome Sequencing library. The samples' quality aligned with the prerequisites for library construction and sequencing, while the total quantity fulfilled the requirements for one or more library constructions. The raw data undergone filtration through the Novogene pipeline's quality control to procure valid data, denoted as clean data. The specific steps of filtration encompassed the exclusion of reads with low-quality bases (quality value ≤ 38) surpassing a defined proportion (40bp), elimination of reads with N bases reaching a specific proportion (10bp), and excision of reads with adapter overlap surpassing a designated threshold (15bp).

Genome splicing: Employing SPAdes 3.10.2 and Velvet 1.2.10 software, the preprocessed fragments of sequences underwent the process of splicing and assembly, culminating in contigs encapsulating the comprehensive genomic information of bacteria. Tailoring the optimal Kmer parameters in accordance with the sequencing read length, adjustments were made to other parameters to procure the initial assembly outcomes. Subsequent to the assembly's conclusion, utilize krskgf to refine the preliminary assembly results, sieving out fragments below 500bp, and scrutinize and dissect the assembly results.

Bioinformatics analysis: The genetic sequence of the ETT2 gene cluster in *E. coli* O157:H7 Sakai served as a benchmark for evaluating the prevalence of the ETT2 gene cluster among APEC strains. Employing diverse bioinformatics methodologies crafted by the Center for Genomic Epidemiology (GGE) at the Technical University of Denmark, the data derived from whole-genome sequencing undergo scrutiny to elucidate bacterial typing, prognosticate potential pathogenicity, ascertain evolutionary relationships, and more. The analytical techniques deployed in this experiment included MLST (Multilocus sequence typing, https://cge.cbs.dtu.dk/services/MLST), SerotypeFinder (WGS-based serotyping of *Escherichia coli* https://cge.cbs.dtu.dk/services/serotypefinder), Virulence Finder (Identification of virulence genes in *E. coli* https://cge.cbs.dtu.dk/services/VirulenceFinder).

Multi-locus sequence typing analysis: Seven essential genes pertaining to the cellular functions (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, *recA*) within *E. coli* were juxtaposed to conduct a multi-locus sequence type (MLST) analysis. The scrutinized bacterial strains underwent categorization into distinct sequence types (ST) through a comparative examination of the genetic sequences inherent to the pivotal housekeeping genes.

Serotyping: The serotyping process relies upon the amalgamation of lipopolysaccharide (O antigen) and flagellar (H) antigenic structures. The Serotype Finder O-type database comprehensively encompasses all recognized O antigen types from O1 to O187, excluding O14 and O57. Simultaneously, the Serotype Finder H-type database establishes a repository of flagellin genes for Whole Genome Sequencing (WGS)-based H typing, encompassing 102 distinct flagellin gene variants and accounting for 53 identified H-types.

Virulence factor identification: Virulence Finder constitutes a computational genomics and epidemiology (CGE) online analytical instrument, designed for the automated identification and extraction of virulence genes associated with *E. coli* from whole-genome sequencing (WGS) data. In this scholarly inquiry, Virulence Finder was employed to conduct sequence alignment of *E. coli* virulence determinants. The aligned virulence genes predominantly encompassed. vacuolating autotransporter toxin (*vat*), temperature-sensitive hemagglutinin (*tsh*),

tellurium ion resistance protein (*terC*), siderophore receptor (*fyuA*), siderophore receptor (*ireA*), serine protease autotransporters of *Enterobacteriaceae* (*pic*), *Salmonella* HilA homolog (*eilA*), putative type I secretion outer membrane protein (*estC*), polysialic acid capsule biosynthesis protein (*neuC*), polysialic acid capsule biosynthesis protein (*nucC*), outer membrane usher fimbriae (*papC*), outer membrane protein complement resistance (*traT*), outer membrane protease (*ompT*), outer membrane hemin receptor (*chuA*), microcin H47 part of colicin H (*mchB*), microcin C (*cvaC*), mchC protein (*mchC*), major pilin subunit F11 (*papA_F11*), long polar fimbriae (*lpfA*), iron transport protein (*sitA*), increased serum survival (*iss*), high molecular weight protein 2 non-ribosomal peptide synthetase (*irp2*), hemolysin F (*hlyF*), heat-resistant agglutinin (*hra*), glutamate decarboxylase (*gad*), ferric aerobactin receptor (*iutA*), enterobactin siderophore receptor protein (*iroN*), enteroaggregative immunoglobulin repeat protein (*air*), east-1 heat-stable toxin (*astA*), colicin M (*cma*), colicin ib (*cib*), colicin ia (*cia*), colicin E1 (*cea*), colicin B (*cba*), capsule polysaccharide export inner-membrane protein (*kpsE*), avian *E. coli* haemolysin (*hlyE*), afimbrial adhesion (*afaD*), aerobactin synthetase (*iucC*), adherence protein (*iha*), ABC transporter protein MchF (*mchF*).

RESULTS

Distribution of ETT2 gene clusters in APEC: The high-throughput APEC sequencing data underwent assembly via Megahit, and the sequence compilation of these strains is delineated in Table 1. Upon juxtaposition with the ETT2 gene cluster in *E. coli* O157: H7, it has come to light that five strains conspicuously lack any genetic manifestation within the ETT2 gene cluster. Conversely, the remaining twenty APEC strains exhibit possession of the ETT2 gene cluster, delineated into four discernible categories (Fig. 1-2):

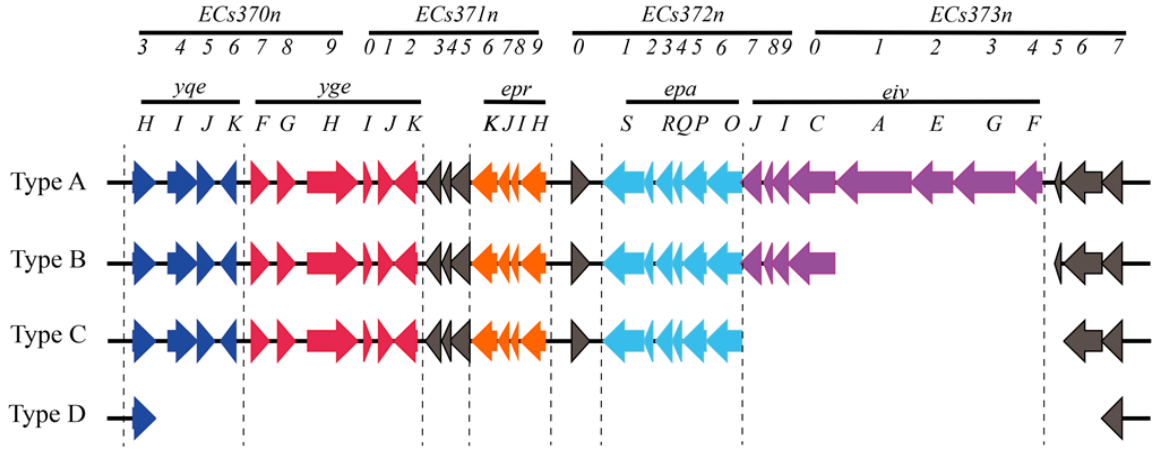

Fig. 1: Four types of ETT2 cluster in APEC in this study.

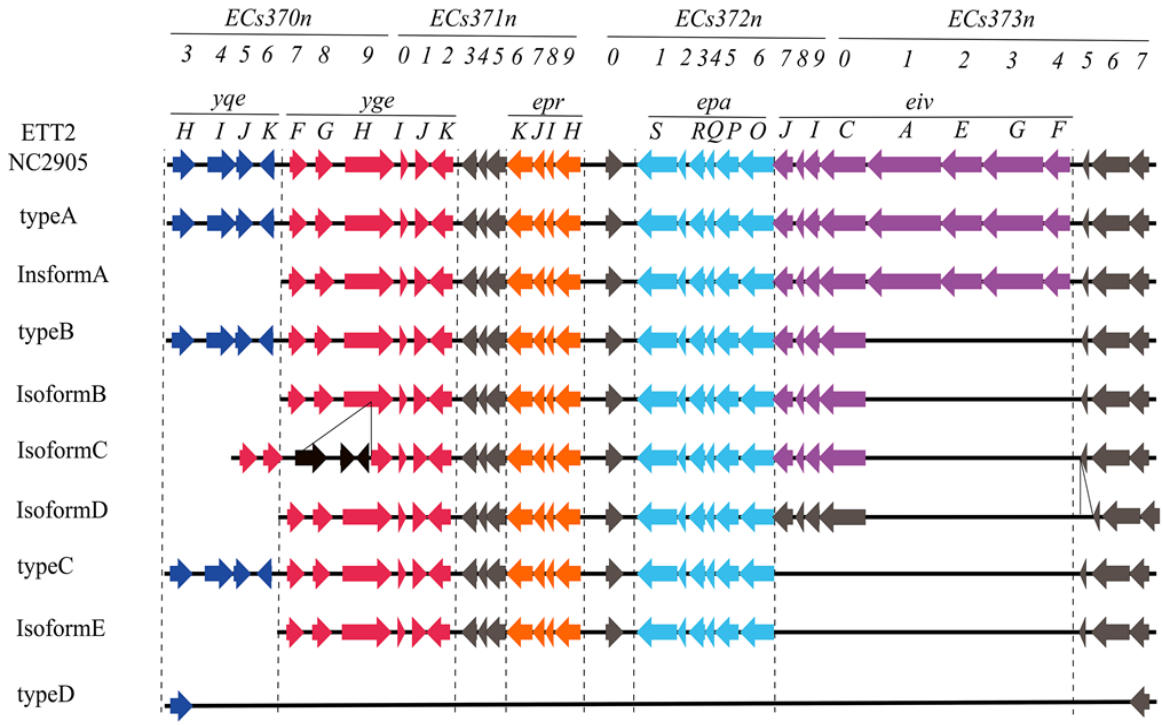

Fig. 2: The comparison of the ETT2 cluster types (type A, type B, type C and type D) in this study and the previously reported ETT2 types by Wang et al., (Isoform A, Isoform B, Isoform C, Isoform D and Isoform E).

Table 1: The sequence assembly information of 25 APEC

Strains	Sequence Size	Number of Conting	GC Content (%)	Maximum Conting (bp)	N50	N90
AE11	4963168	191	51.15%	400509	178636	42735
AE15	5475716	165	57.59%	700174	467674	75166
AE19	5136190	163	50.92%	470186	193090	61051
AE21	5136657	184	51.12%	655697	271005	31020
AE25	5047624	137	50.87%	617366	253121	60789
AE29	5218238	212	51.23%	404709	224474	41562
AE31	5052091	157	50.92%	617362	253123	60789
AE35	5077288	134	50.67%	715586	208976	55615
AE39	4965779	148	50.94%	422429	168951	52506
AE41	5221519	232	51.28%	404858	224474	41562
AE45	4767020	94	50.78%	657591	187667	52818
AE49	5076114	131	50.67%	715458	208976	55615
AE51	5274328	272	51.30%	519950	202100	40785
AE55	4633225	135	50.95%	289772	138196	34240
AE59	4874231	213	51.26%	592856	208689	41626
AE61	4937332	106	50.90%	594718	270450	59989
AE65	4921864	111	50.94%	582064	219608	65309
AE69	5262417	232	51.23%	519950	206386	43794
AE71	5137104	182	51.09%	655697	271005	31020

A. Type A: Comprising the comprehensive ETT2 gene cluster, encompassing all Ecs_3703-Ecs_3737 genes.
B. Type B: Characterized by the incomplete ETT2 gene cluster, encapsulating 31 genes, excluding Ecs_3731-Ecs_3734 genes.
C. Type C: Manifesting as the incomplete ETT2 gene cluster, harboring 27 genes, sans Ecs_3727-Ecs_3734.
D. Type D: Evidencing the incomplete ETT2 gene cluster, confined to solely carrying Ecs_3703 and Ecs_3737.

The findings proffer a discernible pattern in the distribution of ETT2 gene clusters, where deletions exhibit a notable prevalence, albeit in a non-dispersed segmental fashion.

Among the assemblage of 25 APEC strains, AE81 possessed the integral ETT2 gene cluster (Type A), while AE59 housed solely the Ecs_3703 and Ecs_3737 genes (Type D). Notably, AE25 and AE31 showcased the ETT2 gene cluster, excluding Ecs_3731-Ecs_3734 (Type B), whereas the remaining 16 strains bore the ETT2 gene cluster except for Ecs_3727-Ecs_3734 (Type C).

Relationship between APEC evolution and ETT2 cluster: We analyzed the evolutionary interrelation and the ETT2 gene cluster carriage via a meticulous examination of the distribution pattern and evolutionary rapport of the ETT2 gene cluster. We found that strains harboring analogous ETT2 gene cluster types exhibited a proximate connection in the evolutionary hierarchy. The presence of the ETT2 gene cluster was intricately linked to the strains' evolutionary progression. Noteworthy, AE15, AE21, AE29, AE41, AE71, devoid of any genes within the ETT2 gene cluster, were positioned in closer proximity within the evolutionary tree, signifying a heightened kinship. Similarly, strains exclusively carrying the ETT2 gene cluster, albeit lacking the Ecs_3727-Ecs_3734 segment, demonstrated a closer alignment in the evolutionary tree. Furthermore, strains possessing the complete ETT2 gene cluster manifested a closer affinity to those devoid of any genes from the ETT2 gene cluster (Fig. 3).

APEC multi-locus sequence typing: A total of 14 distinct ST classifications manifested within the cohort of 25 APEC strains (Table 2). Specifically, AE21, AE29, AE41, and AE71 were classified as ST117 type (4/25, 16%); AE35, AE49, AE85, and AE91 were classified as ST1582 type (4/25, 16%); AE45 and AE75 were classified as ST1730 type (2/25, 8%); AE25 and AE31 classified the ST23 classification (2/25, 8%); AE51 and AE69 were classified as ST2040 type (2/25, 8%); and AE19 and AE79 were attributed to the ST297 classification (2/25, 8%). Each of the other distinct ST classifications corresponds to a singular APEC strain.

Table 2: Multi-locus sequence typing and serotypes of 25 APEC isolates

Strain	ST	Serotype	Strain	ST	Serotype
AE11	ST10	O8: H17	AE55	ST3014	O9: H4
AE15	ST3014	-	AE59	ST93	O7: H4
AE19	ST297	O??: H37	AE61	ST162	O88: H21
AE21	ST117	O24: H4	AE65	ST1324	O74: H20
AE25	ST23	O78: H9	AE69	ST2040	O159: H20
AE29	ST117	O180: H4	AE71	ST117	O24: H4
AE31	ST23	O78: H9	AE75	ST1730	O??: H21
AE35	ST1582	O8: H21	AE79	ST297	O45: H14
AE39	ST1431	O8: H19	AE81	ST1011	O166: H45
AE41	ST117	O180: H4	AE85	ST1582	O8: H21
AE45	ST1730	O??: H10	AE89	ST101	O15: H10
AE49	ST1582	O8: H21	AE91	ST1582	O8: H21
AE51	ST2040	O159: H20			

APEC serotyping: Among the 25 APEC strains, 17 serotypes were discerned in the remaining 24 APEC strains, barring AE15. Four strains manifest serotype O8:H21 (4/25, 16%), two strains exhibit serotype O24:H4 (2/25, 8%), and an additional pair of strains showcase serotype O78:H9 (2/25, 8%). Furthermore, AE29 and AE41 feature serum O180:H4 (2/25, 8%), while AE51 and AE69 boast serotype O159:H20 (2/25, 8%). Each distinct serotype aligns precisely with a singular strain.

APEC virulence genes: The repertoire of virulence genes harbored by the 25 APEC strains predominantly encompasses adhesion factors (*lpfA*, *iha*, *eilA*, *vat*), toxins (*astA*, *Pic*, *hlyE*), proteases (*tsh*), and invasion factors (*iss*), as well as three categories of secretion system-associated genes (*mchF*, *mchC*, *mchB*, *air*), iron uptake-related genes (*iroN*, *ireA*), among other genes (*gad*, *cma*, *cba*), culminating in a total of 41 virulence genes. The virulence genes exhibiting a prevalence exceeding 80% encompass *terC* (25/25, 100%), *iss* (21/25, 84%), *ompT* (22/25, 88%), *lpfA* (20/25, 80%), while those manifesting a distribution rate below 20% encompass *cea* (5/25, 20%), *pic* (4/25, 16%) , *vat* (4/25, 16%), *tsh* (3/25, 12%), *ireA* (4/25, 16%), *papC* (3/25, 12%), *mchB* (2/25,

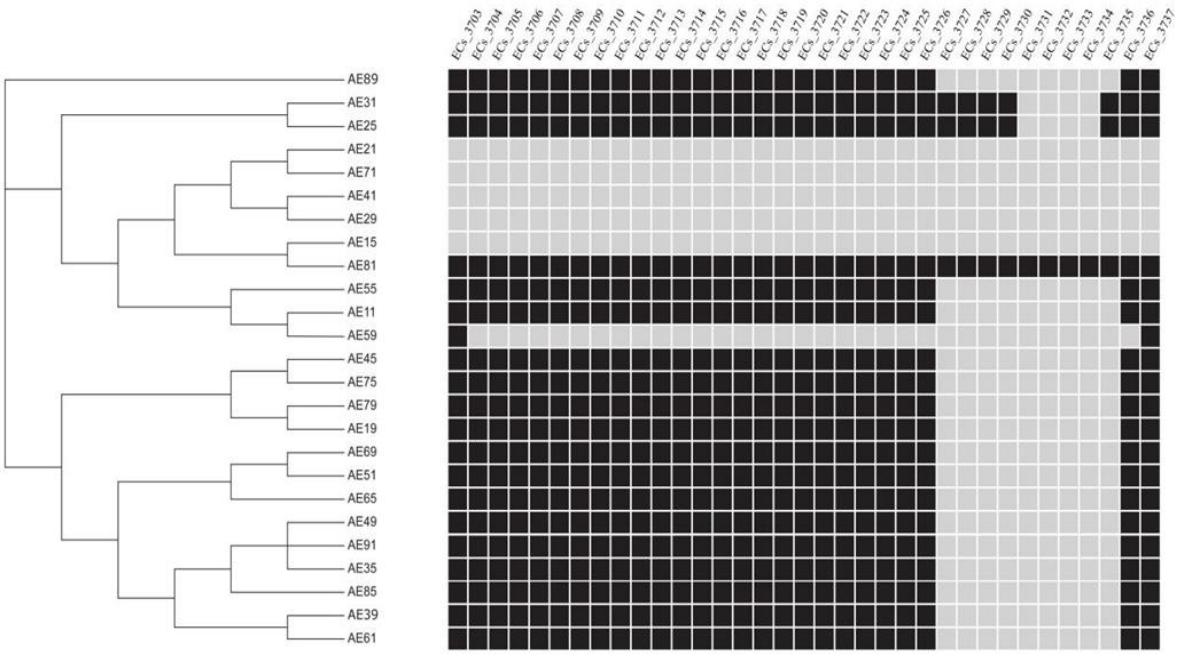

Fig. 3: The ETT2 gene cluster and evolutionary tree of 25 strains.

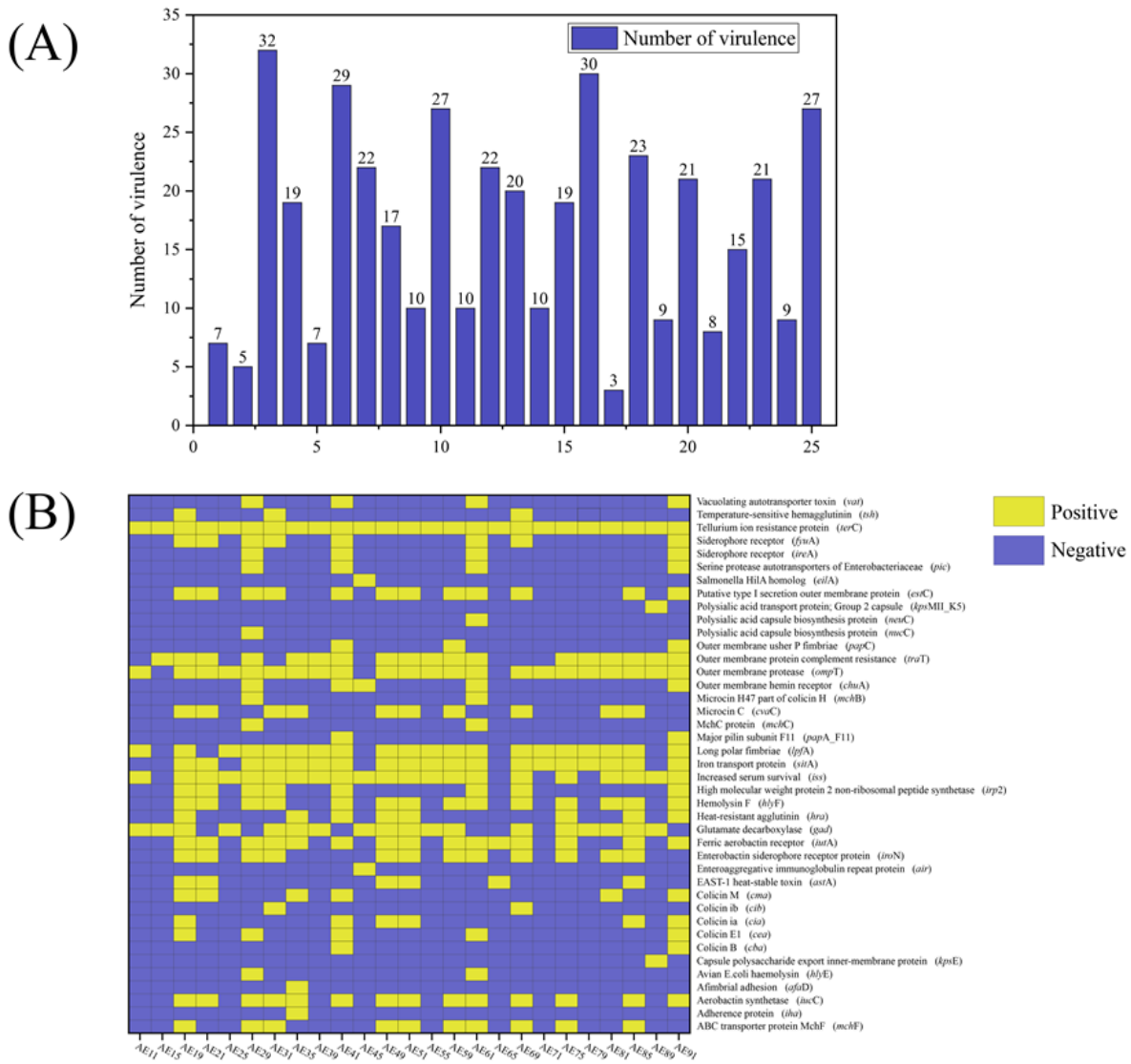

Fig. 4: Number of virulence genes in 25 APEC strains.

8%), *mchC* (2/25, 8%), *neuC* (1/25, 4%), *nucC* (1/25, 4%), *cib* (2/25, 8%), *cba* (2/25, 8%), *papA* (2/25, 8%), *hlyE* (2/25, 8%), *vat* (4/25, 16%), *afaD* (1/25, 4%), *iha* (1/25, 4%), *kpsE* (1/25, 4%), *kpsMII* (1/25, 4%), *air* (1/25, 4%), and *eilA* (1/25, 4%). The precise details of these virulence genes are delineated in Fig. 4.

DISCUSSION

APECs broadly harbor the ETT2 gene cluster. In a preceding investigation, scholars delineated five variants of the ETT2 gene clusters. Within the current inquiry, a total of four ETT2 gene cluster types were discerned during sequence alignment with the ETT2 gene cluster in O157: H7. In comparison to the classifications presented by Wang *et al*. (2016a) barring the comprehensive ETT2 gene cluster (herein denoted as Type A), Type B (entailing the deletion of Ecs3731-Ecs3734) and Type C (entailing the deletion of Ecs3727-Ecs3734) in this examination bear resemblance to Isoform D and Isoform E as reported by Wang *et al*. (2016a).

Within this study, the sequence types (ST), serotypes, evolutionary associations, and virulence factors underwent scrutiny via whole-genome sequencing. Through multi-locus sequence typing, it was ascertained that the ST117 type predominated among these strains. Specifically, AE21, AE29, AE41, and AE71 were classified under ST117, all devoid of the ETT2 cluster. Apart from AE21, AE29, AE41, and AE71, AE15 represented another isolate lacking ETT2 genes, albeit it's multi-locus sequence typing remained unknown.

A survey conducted in 2017 reported the prevalent APEC type in Nordic poultry farms as ST117 O78:H4 (Ronco *et al.,* 2017). Subsequently, in 2019, a survey disclosed that among the 95 APEC strains in Australia carrying the intl1 integrase gene, 22 belonged to ST117 (Cummins *et al.,* 2019). In 2020, a survey revealed that 75 clinically isolated APECs from Pakistan, pertaining to a total of 14 ST types, manifested the largest proportion (16%) as ST117 (Azam *et al.,* 2020). The collective evidence from prior research and the outcomes of this study implies that ST117 APEC may constitute the predominant strain among APEC types.

In antecedent investigations, it was delineated that an undivided ETT2 island predominantly manifested in serotypes O1 and O2 (Wang *et al.,* 2016a). In the present discourse, we assert that APEC O166 is also adept at harboring an intact ETT2 cluster. Herein, we have ascertained a clinical APEC isolate of serotype O166 bearing the entirety of the ETT2 gene cluster. A study in 2019 communicated that solely one among the 79 APEC clinical isolates in South Korea exhibited serotype O166 (Kim *et al.,* 2020), with the O166 serotype representing a diminutive proportion of APEC clinical isolates.

Within the confines of this investigation, a cumulative of 5 APEC strains (AE21, AE29, AE41, AE71, AE15) were devoid of any genes within the ETT2 gene cluster. Furthermore, AE21, AE29, and AE41 were carriers of 23 distinct types of virulence genes, constituting the encoded virulence genes within 25 strains. In another study, all APEC isolates harbored the virulence genes iss and *terC*, and 98% of isolates harbored *ompT*, supporting the high occurrence of *iss*, *terC* and *ompT* (Jonare *et al.,* 2023). No discernible correlation was discerned between the presence of the ETT2 gene cluster and virulence. The strain AE71 eschewed the possession of the ETT2 gene cluster, yet it encoded 22 varieties of virulence genes, thereby establishing an absence of correlation between the ETT2 gene cluster and the virulence genes. Through the mechanism of Polymerase Chain Reaction (PCR) identification, antecedent researchers adjudicated that the existence of the ETT2 gene cluster evinced no connection to the presence of the HPI virulence island, LEE Virulence Island, et cetera. *E. coli* K-12 bears the ETT2 gene cluster. Notwithstanding that some of the cluster genes have been substantiated to be intertwined with the virulence of the strains, it remains undetermined whether they can serve as discerning markers for detecting the virulence of pathogenic bacteria.

Conclusions: In summary, this study analyzed the ETT2 characteristics and obtained the ST type, serotype, and evolutionary relationship and virulence factors of APEC clinical isolates by means of whole genome sequencing, which will contribute to the study of APEC prevention.

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Authors contributions: Mei Xue designed the study. Mei Xue, Zhonghong Li, Ping Zhang, and Weiqiang Lei performed experiments and data analysis. Mei Xue prepared the first manuscript draft. All authors read and approved the final manuscript.

REFERENCES

Azam M, Mohsin M, Johnson TJ, *et al*., 2020. Genomic landscape of multi-drug resistant avian pathogenic *Escherichia coli* recovered from broilers. Vet Microbiol 247: 108766.

Bohn E, Sonnabend M, Klein K, *et al.,* 2019. Bacterial adhesion and host cell factors leading to effector protein injection by type III secretion system. Int J Med Microbiol 309(5): 344-350.

Chen T, Wang Y, Chi X, *et al.,* 2024. Genetic, virulence, and antimicrobial resistance characteristics associated with distinct morphotypes in ST11 carbapenem-resistant *Klebsiella pneumoniae.* Virulence 15(1):2349768.

Cummins ML, Roy Chowdhury P, Marenda MS, *et al.,* 2019. *Salmonella* Genomic Island 1B Variant Found in a Sequence Type 117 Avian Pathogenic *Escherichia coli* Isolate. mSphere 4(3): e00169-19.

Fu D, Li J, Wu J, *et al.,* 2021. The ETT2 transcriptional regulator EivF affects the serum resistance and pathogenicity of avian pathogenic *Escherichia coli*. Microb Pathog 161: 105261.

Hu J, Afayibo DJA, Zhang B, *et al.,* 2022. Characteristics, pathogenic mechanism, zoonotic potential, drug resistance, and prevention of avian pathogenic *Escherichia coli* (APEC). Front Microbiol 13: 1049391.

Ideses D, Gophna U, Paitan Y, *et al.,* 2005. A degenerate type III secretion system from septicemic *Escherichia coli* contributes to pathogenesis. J Bacteriol 187(23): 8164-8171.

Jonare L, Östlund E, Söderlund R, *et al*., 2023. Core genome multilocus sequence typing (cgMLST) confirms systemic spread of avian pathogenic *Escherichia coli* (APEC) in broilers with cellulitis. Vet Microbiol 282:109755.

Kim YB, Yoon MY, Ha JS, *et al.,* 2020. Molecular characterization of avian pathogenic *Escherichia coli* from broiler chickens with colibacillosis. Poult Sci 99(2): 1088-1095.

Ronco T, Stegger M, Olsen RH, *et al.,* 2017. Spread of avian pathogenic *Escherichia coli* ST117 O78:H4 in Nordic broiler production. BMC Genomics 18(1): 13.

Shulman A, Yair Y, Biran D, *et al.,* 2018. The *Escherichia coli* Type III Secretion System 2 Has a Global Effect on Cell Surface. mBio 9(4): e01070-18.

Wang S, Liu X, Xu X, *et al.,* 2016a. *Escherichia coli* type III secretion system 2 (ETT2) is widely distributed in avian pathogenic *Escherichia coli* isolates from Eastern China. Epidemiol Infect 144(13): 2824-2830.

Wang S, Liu X, Xu X, *et al.,* 2016b. *Escherichia coli* Type III Secretion System 2 ATPase EivC Is Involved in the Motility and Virulence of Avian Pathogenic *Escherichia coli*. Front Microbiol 7: 1387.

Wang S, Xu X, Liu X, *et al.,* 2017. *Escherichia coli* type III secretion system 2 regulator EtrA promotes virulence of avian pathogenic *Escherichia coli*. Microbiology (Reading) 163(10): 1515-1524.

Wang Y, Zuo J, Duan C, *et al.,* 2024. Large language models assisted multi-effect variants mining on cerebral cavernous malformation familial whole genome sequencing. Comput Struct Biotechnol J 23:843-858.

Xue M, Fu D, Hu J, *et al.,* 2022. The Transcription Regulator YgeK Affects Biofilm Formation and Environmental Stress Resistance in Avian Pathogenic *Escherichia coli*. Animals (Basel) 12(9): 1160.

Xue M, Xiao Y, Fu D, *et al.,* 2020. Transcriptional Regulator Yqel, Locating at ETT2 Locus, Affects the Pathogenicity of Avian Pathogenic *Escherichia coli.* Animals (Basel) 10(9): 1658.

Yao Y, Xie Y, Perace D, *et al.,* 2009. The type III secretion system is involved in the invasion and intracellular survival of *Escherichia coli* K1 in human brain microvascular endothelial cells. FEMS Microbiol Lett 300(1): 18-24.

Yin L, Li Q, Wang Z, *et al.,* 2021. The role of *Escherichia coli* type III secretion system 2 chaperone protein ygeG in pathogenesis of avian pathogenic *Escherichia coli*. Res Vet Sci 140: 203-211.

Yin L, Cheng B, Tu J, *et al.,* 2022. YqeH contributes to avian pathogenic *Escherichia coli* pathogenicity by regulating motility, biofilm formation and virulence. Vet Res 53(1): 30.

Yuan CW, Liu WX, Hou JL, *et al.,* 2018. Prevalence of pathogenicity island ETT2 in *Escherichia coli* isolated from piglets with diarrhea in northeast of China. Pol J Vet Sci 21(1):5-12.

Zhou M, Guo Z, Duan Q, *et al.,* 2014. *Escherichia coli* type III secretion system 2: a new kind of T3SS? Vet Res 45(1): 32.