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Genomic analysis of the zoonotic ST73 lineage containing avian and human extraintestinal pathogenic *Escherichia coli* (ExPEC)

Andre Becker S. Saidenberg [a,c,g,*], Arnoud H.M. van Vliet [b], Marc Stegger [c], Thor Bech Johannesen [c], Torsten Semmler [d], Marcos Cunha [a], Alessandro C. de O. Silveira [e], Eleine Kuroki Anzai [e], Isabel C.A. Scaletsky [f], Anders Dalsgaard [g], Roberto M. La Ragione [b,h], Terezinha Knöbl [a]

[a] Veterinary Pathology Department, College of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil
[b] Department of Pathology and Infectious Diseases, School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, United Kingdom
[c] Department of Bacteria, Parasites and Fungi, Statens Serum Institut, Copenhagen, Denmark
[d] Department of Microbial Genomics, Robert Koch Institute, Berlin, Germany
[e] Department of Pharmaceutical Sciences, Regional University of Blumenau, Blumenau, Brazil
[f] Microbiology, Immunology and Parasitology Department, Federal University of São Paulo, Escola Paulista de Medicina, São Paulo, Brazil
[g] Section for Food Safety and Zoonoses, Institute for Veterinary and Companion Animal Science, Københavns Universitet, Copenhagen, Denmark
[h] Department of Microbial Sciences, School of Biosciences and Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, Surrey, United Kingdom

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ABSTRACT

Extraintestinal pathogenic *Escherichia coli* (ExPEC) is a globally distributed pathogen, with uropathogenic *E. coli* (UPEC) and sepsis-associated *E. coli* (SEPEC) pathotypes particularly involved in human and companion animal disease, while avian pathogenic pathotype (APEC) severely impact poultry health and production. Similarities between APEC from poultry/meat and human ExPEC suggest that some APEC lineages may have zoonotic potential. ExPEC sequence type 73 (ST73) and its clonal complex (CC) are increasing causes of urinary tract infections and sepsis, but its role in zoonotic disease is less well understood. Here, we analyzed the genome sequences of 25 *E. coli* isolates from Brazil (11 APEC and 14 UPEC) from two time periods, from poultry producing areas and hospitals in the same geographical regions. Isolates were compared to 558 publicly available ST73/CC73 global sequences. Brazilian APEC harbored virulence factors associated with UPEC/SEPEC such as *sfa*, *cnf*1, *vat*, *usp*, *hly*A, iron acquisition and protectins/serum resistance systems, while lacking some common APEC markers and widespread multidrug resistance. Analysis of core genome MLST and SNP phylogenetic trees indicated evolutionary relationships between subgroups of the Brazilian APEC to two contemporary Brazilian UPEC isolates from the same region, and one Brazilian UPEC available from another study. Phylogenies showed a non-host, geographical, or pathotype specificity, with APEC isolates clustering closely with international human UPEC, SEPEC. The remaining Brazilian UPEC grouped within human clusters. Collectively, this suggests a zoonotic potential for subgroups of Brazilian APEC from the ST73 lineage that could contaminate poultry products and subsequently cause human infection.

1. Introduction

Extraintestinal pathogenic *Escherichia coli* (ExPEC) is an important human and animal pathogen, responsible for a wide range of extraintestinal diseases including urinary tract infections and sepsis (Manges et al., 2019). ExPEC also possess a diverse array of virulence factors that facilitate the colonization and dissemination to organs outside the intestinal tract. In recent years pandemic ExPEC lineages have been recognized affecting healthcare and community settings (Riley, 2020). ExPEC can cause urinary tract infections (UTIs), septicemia and neonatal meningitis being classified as specific pathotypes: uropathogenic *E. coli* (UPEC), sepsis-associated *E. coli* (SEPEC), and neonatal

* Corresponding author at: Department of Bacteria, Parasites and Fungi, Statens Serum Institut, Copenhagen, Denmark.
E-mail addresses: adbs@ssi.dk, andresaidenberg@sund.ku.dk (A. Becker S. Saidenberg).

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meningitis *E. coli* (NMEC) (Kathayat et al., 2021).

The increasing incidence of human infections associated with ExPEC in the last decades has led to significant economic burden as well as increased morbidity and mortality rates, particularly when linked to antibiotic resistance (Manges et al., 2019). Besides affecting humans, livestock, and companion animals, ExPEC can also cause infections in poultry which are caused by avian pathogenic *E. coli* (APEC). Growing challenges are being faced by the poultry industry where APEC infections result in severe economic losses while also impacting animal welfare (Christensen et al., 2021).

The phylogenetic and genotypic similarities of certain subsets of APEC with human ExPEC isolates, and the confirmation that some avian strains can cause disease in mouse infection models, as well as certain human strains being able to experimentally infect poultry (Tivendale et al., 2010; Jakobsen et al., 2012; Mortensen et al., 2019), support the hypothesis that poultry could be a source of zoonotic ExPEC infections. Therefore, poultry could serve as a reservoir of pathogenic strains or genes encoding virulence determinants/antibiotic resistance with subsequent dissemination into the human microbiota via contaminated food products (Johnson et al., 2017; Hornsey et al., 2019).

UPEC and SEPEC clinical isolates are frequently of sequence type (ST) ST73 multilocus sequence typing (MLST) within clonal complex 73 (Mellata, 2013). This lineage is now recognized as of worldwide importance causing increasing clinical cases of UTIs and sepsis in humans in Europe (Alhashash et al., 2015; Toval et al., 2014; Rebelo et al., 2017), United States (Adams-Sapper et al., 2013), Australia (Bogema et al., 2020), and Brazil (Silva et al., 2017). The ST73 belongs to the B2 phylogroup where many of the ExPEC lineages are found (Manges and Johnson, 2012).

Whole-genome sequencing (WGS) has been increasingly used as an epidemiological tool to investigate disease outbreaks, and to understand the evolutionary history and diversity of pathogens (Allard et al., 2017). WGS has also been used to compare *E. coli* from diverse sources, and it has demonstrated that some APEC isolates have significant genomic similarities with human ExPEC strains (Jørgensen et al., 2019).

Previously, we have described the genotypic similarities of a collection of Brazilian O6-B2-ST73 APEC isolates to human ExPEC, in particular UPEC isolates (Cunha et al., 2017). Here we have aimed to expand the information on overlapping characteristics of ST73 avian, animal, and human isolates by using WGS of 10 ST73 and one CC73 APEC (10 from a historical collection and one recent isolate) and 14 human ST73 UPEC (9 from a historical collection and five more recently obtained), to further determine the genotypic and phylogenetic characteristics of these isolates.

2. Materials and methods

2.1. Origin of isolates

The 25 isolates analyzed in this study were collected over two separate periods of time and consisted of isolates from colibacillosis cases in Brazilian broilers in different poultry farms, and UTI cases in Brazilian patients. One group originated from a 2006 sampling (10 poultry and 9 human isolates), and the other group (one poultry and 5 human isolates) were collected between 2019/2020 (Table S1). Both groups represent the main poultry producing regions and corresponding hospitals at main cities in the South and Southeast Brazil. The sequences were selected for the study based on previous screening with specific PCR primers for the ST73/CC73 group (Doumith et al., 2015) and performing whole genome sequencing of the isolates belonging to this group.

2.2. DNA preparation and sequencing

Isolates were plated onto LB agar (DIFCO-BBL) and cultured aerobically at 37 °C overnight. Single colonies were selected, and genomic DNA was extracted usinq a DNeasy Blood and Tissue kit (Qiagen) or MagNA Pure LC DNA Isolation Kit (Roche). Libraries were generated with a Nextera XT DNA Library kit (Illumina) according to the manufacturers instructions and used to generate paired-end 150 bp sequencing data using Illumina MiSeq and NextSeq platforms (Illumina, San Diego, CA, USA).

2.3. Data availability

The sequencing reads and genome assemblies of the *E. coli* isolates used in this study have been uploaded in the NCBI Sequence Read Archive (Bioproject PRJNA398035). Individual GenBank accession numbers are provided in Table S1.

2.4. Genome sequences from public repositories

Draft or complete chromosomal sequences from 853 genomes of ST73/CC73 *E. coli* were downloaded from the NCBI (http://www.ncbi.nlm.nih.gov) and EnteroBase websites (http://enterobase.warwick.ac.uk) (databases accessed July 2020) and filtered according to descriptions for host, disease and geographical origin, resulting in a final dataset comprising of 558 genomes for downstream analyses and downloaded as FASTA files (Table S2). Sequences with incomplete information were still included if members of an underrepresented characteristic (e.g. environmental and animal isolates).

2.5. De novo genome assembly and determination of serotype, phylogroup, MLST, and virulence factors

This study's isolates were *de novo* assembled using Shovill version 1.0.4 (https://github.com/tseemann/shovill) with the SPAdes assembler. Further ST/CC73 assembled genomes were obtained from the NCBI Genomes and Enterobase databases. Assembled genomes were used for *in silico* typing utilizing settings with a minimum of 90 % coverage and 80 % identity. The phylogroup, virulence factors, serotype, plasmid replicons, and antimicrobial resistance, were identified using ABRicate v.0.9.0 (https://github.com/tseemann/abricate) with the VFDB, Ecoli_vf, EcOH, Plasmidfinder, Resfinder and NCBI's Bacterial Antimicrobial Resistance Reference Gene Databases (Feldgarden et al., 2019). MLST (multi-locus sequence typing) was done using MLST v.2.16 (https://github.com/tseemann/mLst). A custom database adapted from Reid et al. (2019) was used to detect the minimum profile of genes predicting the presence of the ColV-IncF plasmid. Genomes that contained one gene in each of at least four defined clusters (*iroBCDEN, cvaABC-cvi, iutA-iucABCD, sitABCD, hlyF-ompT, and etsABC)* were predicted to contain the plasmid. Chromosomal point-mutation resistance were detected with the ResFinder tool from the Center for Genomic Epidemiology (https://cge.cbs.dtu.dk/services/ResFinder/).

2.6. Core and whole genome phylogenetic analyses

To compare the core genomes of the 25 Brazilian isolates and the 558 other sequences available from online databases, a cgMLST (core genome multi-locus sequence typing) and single nucleotide polymorphisms (SNPs) phylogenetic analyses were carried out using the reference prototypic strain CFT073 (GenBank no. AE014075). The cgMLST employed a dataset based on a core of 2360 genes of the published *E. coli* scheme (INNUENDO https://doi.org/10.5281/zenodo.1323690) utilizing chewBBACA v.2.8.5 (Silva et al., 2018), creating a neighbor-joining tree based on the allele calls with GrapeTree (v.1.5) using the RapidNJ setting. The core-genome SNP analysis employed the NASP pipeline (v.1.0.0) (Sahl et al., 2016) inferring maximum-likelihood phylogenies for the alignments. SNPs were called with GATK (v.4.2.2) and recombinant regions removed with Gubbins (v.2.1). IQ-TREE (v.2.1.2) using ModelFinder and 100 bootstraps were applied to the resultant SNP matrix to construct the phylogenetic tree.

FigTree v.1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) and iTOL v.4 (https://itol.embl.de/) programs were used for visualization and adding the available metadata.

3. Results

3.1. Brazilian ST/CC73 isolates show consistent serotype, phylogroup, and MLST typing, overall similar virulence gene profiles and lack of extensive antibiotic resistance

Twenty-four of the Brazilian isolates used in this study were confirmed to be phylogroup B2, MLST sequence type (ST) 73 and predicted to have the O6:H1 serotype. In addition, one isolate was identified as ST1618, a member of the CC73 clonal complex, differing in only one allele to ST73 and predicted to be serotype O4:H1 (Table S1).

A number of adhesins are commonly linked to UPEC and SEPEC strains such as the P and S fimbriae (Mellata et al., 2013). All of the studied APEC and all but one of the Brazilian UPEC were positive for at least one gene of the *sfa* gene operon (96 %) (Table 1). Conversely, genes encoding the P fimbriae were detected in five of the APEC (45 %) and in nine UPEC (64 %). Several virulence genes frequently described in ExPEC were also detected among most of the isolates. These play an important role in virulence through mechanisms evading the immune system (K1 capsule, *omp*A, *pic*), as well as multiplication such as iron acquisition genes, besides the presence of diverse toxin genes (*cnf*1, *hly*A, *usp, vat*) (Table 1). Overall, the Brazilian APEC and UPEC shared remarkable similarities in the distribution of virulence genes. More marked differences were observed between the ST73 APEC and the sole CC73 APEC, regarding the genes *etsABC, hlyA, hlyF, cdt, and traT*. Six out of the 11 APEC isolates (54 %) were positive for the indicative presence of the ColV-IncF hybrid plasmid, one of the main markers characterizing APEC (Reid et al., 2019). The Brazilian UPEC had only three isolates positive for this plasmid prediction out of the total of 14 (Table S1).

The Brazilian APEC and UPEC did not appear to harbor significant numbers of transferrable antibiotic resistance genes according to the *in silico* genomic analysis. This was particularly true for the isolates from the 2006 collection, where most APEC and UPEC isolates were predicted to be fully susceptible or just exhibited resistance to one or two antibiotics. One UPEC isolate from 2006 presented MDR to beta-lactams ($bla_{\text{TEM-1}}$), chloramphenicol (*catA1*), sulfonamides (*sul2*), trimethoprim (*dfrA8*), fluoroquinolones (mutations in *gyrA*, *parC*), and aminoglycosides (*aph(6)-Id, aph(3'')-Ib*). Conversely, isolates from 2019/2020 displayed more antibiotic resistance, where all five human isolates showed resistance to at least two antibiotics or more, including to extended spectrum beta-lactams (ESBL) in isolate UPEC110 ($bla_{\text{SHV-48}}$; $bla_{\text{SHV-102}}$). The sole APEC isolate from 2019 showed resistance to macrolides (*mdfA*), chloramphenicol (*catA*), tetracyclines (*tetB*), and low-level resistance to fluoroquinolones (*qnrB*) (Table S1).

3.2. Comparison of Brazilian ST73 genomes with international ST73 isolates

3.2.1. O6:H1 is the dominant serotype among ST73 isolates and Brazilian genomes are phylogenetically similar to international ST/CC73 isolates

Comparison with 558 ST73 genomes obtained from diverse geographic regions, showed that O6:H1 is the most common serotype in conjunction with ST73 (N = 359, 64.5 %), regardless of geographical origin, host, or disease symptoms, followed by serotypes O2:H1 (N = 80, 14.4 %), O18:H1 (N = 40, 7.2 %) and O25:H1 (N = 38, 6.8 %), and less frequent serotypes (N = 65, 7.1 %) (Table S2).

Phylogenetic trees were generated for the 558 international ST/CC73 genomes and 25 Brazilian ST/CC73 genomes using core genome MLST and core genome SNPs. Both trees were similar in respect of clustering and comparable in the analyses but showed minor differences regarding the overall order of the isolates within particular clusters (Figs. 1 and 2).

Both trees revealed that the avian and human Brazilian isolates clustered into distinct parts of the trees, and grouped according to their region of sampling (south vs south-east of Brazil), and were found in clusters not strongly separated by disease symptoms or geographical origins (Figs. 1 and 2). There was also an intermingling of animal and human isolates, although there was an apparent underrepresentation of available ST/CC73 animal sequences for comparisons in the public databases. Overall, the Brazilian APEC and UPEC isolates clustered with isolates from Europe, North America, Australia, and another isolate (human UTI) from Brazil (Fig. 3).

Most avian isolates were located in two clusters, with Cluster I including two of the Brazilian UPEC sequences from the same region (Southeast) related to three APEC from this study (Fig. 3). Both groups nested within a larger cluster that included other closely related international ExPEC sequences spanning UPEC and SEPEC strains from humans and animals. Of interest, within Cluster I, the poultry isolates particularly clustered together in a subcluster that contained a Danish human sepsis isolate (isolate a342), and a recently described Brazilian human UTI sequence not belonging to our study (isolate a542) (Fig. 3).

The second cluster containing Brazilian APEC was located in a subcluster consisting almost exclusively of poultry isolates, but that also included close connections with two human isolates: a UTI isolate from the USA (a398) and a sepsis isolate from the UK (a054). Nearby clusters contained exclusively human isolates from Europe and Australia and from different clinical sources were also observed (Fig. 3).

A single CC73 APEC included (APEC69) was located in Cluster III, where it was found nested between CC73 isolates mainly from animal sources. A particular clustering could be observed with poultry sequences from the USA and UK. A single human UTI sequence from Denmark was found in a nearby cluster to this isolate (a545) (Fig. 3).

The Brazilian UPEC were overall very diverse, within the two periods of collection (2006 and 2020). These isolates were found dispersed in the phylogenies (Figs. 1 and 2), though tending to cluster with each other based on the region of collection (South vs Southeast). Two isolates were found in Cluster I (Fig. 3), and the remaining Brazilian UPEC were found in clusters mostly dominated by human international human isolates, although not showing specificity regarding disease (Figs. 1 and 2).

4. Discussion

ExPEC are globally important pathogens, causing significant mortality and morbidity in humans and animals. In this study we investigated Brazilian ExPEC from humans and chickens, and demonstrated that subgroups among ST/CC73 isolates are similar to isolates from other countries and from varied host sources worldwide. Four of the Brazilian strains were predicted to be multi-drug resistant, with resistance to three or more classes of antibiotics, which may contribute to dissemination of AMR. Our WGS analyses allowed for the first time to obtain more in-depth comparative results with ST/CC73 poultry strains. The results indicated that even for this uncommon clonal lineage among APEC, there are isolates that share many similarities with human ExPEC.

O6 is a rare serotype with regards to APEC but is frequently found in *E. coli* causing UTI and sepsis in humans and pets (Ewers et al., 2007; Johnson et al., 2008). It is reported that a clone of *E. coli* O6 isolated from a dog was also implicated as a possible source of zoonotic infection for humans inhabiting the same household (Manges and Johnson, 2012). As previously described by Bogema et al., 2020, O6:H1 is the predominant serotype among the ST73 lineage which was also observed in our avian and human Brazilian isolates (Table S1).

The genotypic comparisons among our APEC and UPEC sequences revealed many similarities and some differences in the combination of virulence determinants which are in alignment with the genome plasticity of *E. coli* and virulence factors for UPEC/SEPEC isolates (Sarowska et al., 2019). Differences in distribution of virulence factors have also been described by genotypic studies comparing select subsets of avian

Table 1
Virulence factors detected in the Brazilian APEC and UPEC isolates and grouped according to respective categories. Crosses indicate the presence of a gene.

Isolate	adhesins							invasine	iron uptake							protectins/serum resistance				toxins								Others	
ID	*fim*	*afa*	*dra*	*pap*	*sfa*	*foc*	*crl*	*ibe*	*iuc/ iut*	*irp*	*iroN*	*chu*	*ets*	*fyu*	*sit*	*kps, neuA*	*omp*	*iss*	*cvi/ cva*	*pic*	*sat*	*vat*	*hlyA*	*hlyF*	*cnf1*	*cdt*	*usp*	*traT*	*tsh*
APEC 69	+				+	+	+	+		+	+	+	+	+	+	+	+	+	+	+		+		+		+	+	+	
APEC T17	+				+	+		+		+	+	+		+	+	+	+	+	+	+		+	+		+		+		
APEC T1	+			+	+	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+				+		
APEC T20	+				+	+		+		+	+	+		+	+	+	+		+	+		+	+		+		+		
APEC T22	+				+	+		+		+	+	+		+		+	+		+	+		+	+		+		+		
APEC T3	+			+	+	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+		+		+		
APEC T30	+			+	+	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+				+		
APEC T38	+				+	+		+		+	+	+		+	+	+	+	+	+	+	+	+	+				+		
APEC T47	+				+	+		+		+	+	+		+	+	+	+	+	+	+		+	+				+		
APEC T49	+			+	+	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+				+		
APEC T7	+			+	+	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+				+		
UPEC U145	+			+	+	+		+		+	+	+		+	+	+	+	+	+	+		+	+		+	+	+		
UPEC U180	+				+	+		+		+	+	+		+	+	+	+	+	+	+		+	+		+		+		
UPEC U223	+			+	+	+		+		+		+		+	+	+	+	+		+		+	+		+	+	+		
UPEC U231	+			+	+	+		+		+		+		+	+	+	+	+		+		+	+		+	+	+		
UPEC U34	+				+	+		+		+	+	+		+	+	+	+	+	+	+		+	+		+		+		
UPEC U64	+	+		+				+	+	+		+		+	+	+	+	+		+		+	+				+	+	
UPEC UA196	+			+	+	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+				+		
UPEC 110	+			+	+	+		+	+	+	+	+		+	+	+	+	+		+	+	+	+		+		+	+	
UPEC 77	+			+	+	+		+		+	+	+		+	+	+	+	+	+	+		+	+		+		+		
UPEC 79	+				+	+		+		+	+	+		+	+	+	+	+	+	+		+	+		+		+		
UPEC 81	+				+	+		+		+	+	+		+	+	+	+	+	+	+	+	+	+		+	+	+		
UPEC 85	+				+	+		+		+	+	+		+	+	+	+	+	+	+	+	+	+		+	+	+		
UPEC V2-6	+			+	+	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+				+	+	
UPEC M164	+			+	+	+		+	+	+	+	+		+	+	+	+	+	+	+	+	+	+				+	+	

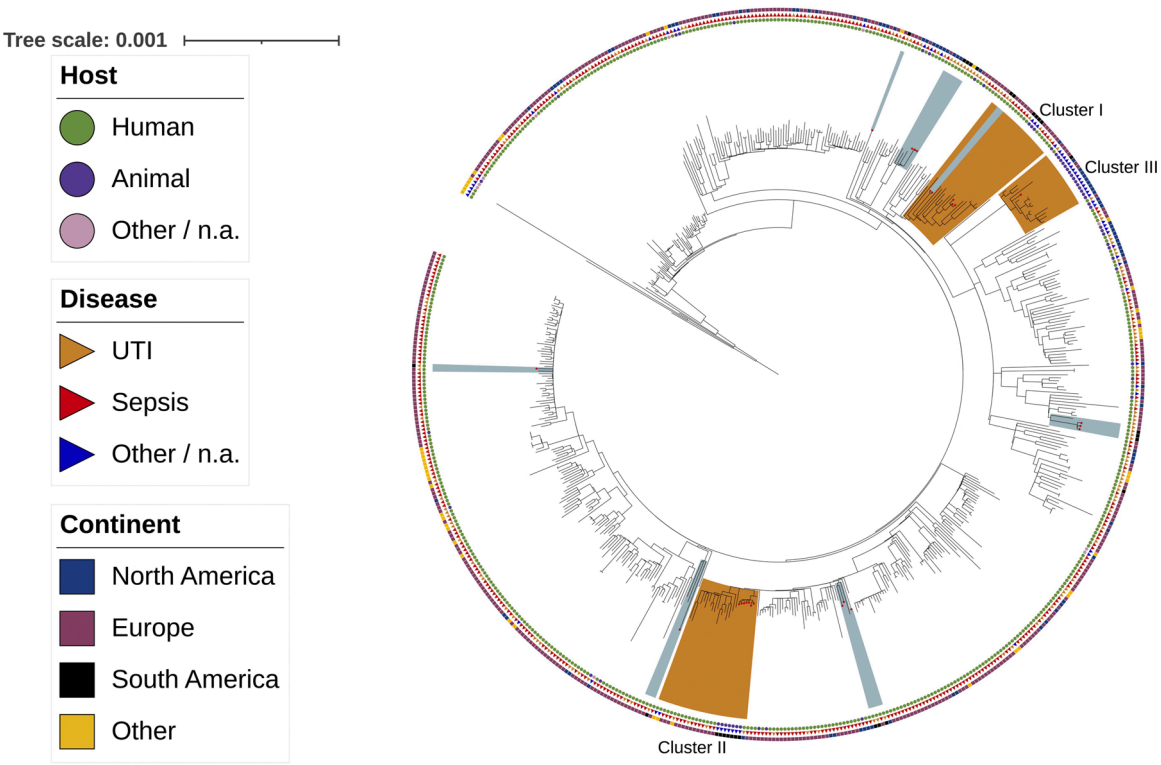

Fig. 1. Phylogeny of worldwide *E. coli* ST/CC73 isolates based on core genome single nucleotide polymorphisms. The tree is based on 558 genomes and rooted at mid-point using *E. coli* isolate CFT073 as reference. Classification according to host, disease/other or not available (n.a.), and continent are colored according to the figure's scheme. Related clusters containing the Brazilian APEC are highlighted in orange and the Brazilian UPEC are highlighted in blue. The individual sequences are marked with a red circle at the tip of the branch. The tree has been annotated and visualized using iTOL. Scalebar indicates substitutions per site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and human strains although mostly showing clear overlapping among them (Ewers et al., 2007; Mora et al., 2013). In this study, the Brazilian avian isolates and the Brazilian human isolates had for the most part similar genotypic profiles (Table 1). These findings could suggest that there is potential for transmission among avian and human hosts.

The presence of the ColV-IncF hybrid plasmid is considered one of the few markers for APEC strains (Jørgensen et al., 2019), but it is also found among subsets of human ExPEC, which may represent a recent spill over from avian reservoirs to humans (Liu et al., 2018). A total of six Brazilian APEC isolates (54 %) fulfilled the requirements for the suggestive presence of this plasmid, while 11 of the Brazilian UPEC (78 %) did not present the minimum set of genes predicting this plasmid (Table S1). These differences were also reflected in the phylogenies, as the Brazilian UPEC ColV-IncF positive were located quite apart from the Brazilian APEC and exclusively among other human isolates. No correlation between the presence of the plasmid in the Brazilian human isolates and a poultry origin can be currently hypothesized due to the lack of additional avian isolates for further comparisons.

The Brazilian ST73 isolates in general did not contain widespread antibiotic resistance genes, except for three human isolates and the poultry CC73 isolate, which were MDR (Table S1). These isolates clustered separately and were found dispersed in the phylogeny (Fig. 1). Acquired multidrug antimicrobial resistance in the ST73 lineage is of growing concern, as it can result in severe consequences for recurring infections and treatment failures (Alhashash et al., 2015). The avian isolate CC73 demonstrated that a recent isolate of the ST73 complex causing colibacillosis (collected in 2019) does have the capacity to exhibit MDR (resistance to macrolides, chloramphenicol, tetracyclines, and low-level quinolone resistance) (Table S1). However, analysis of a large panel of isolates is required to determine if this is true of all avian isolates more recently circulating in Brazil.

The phylogenetic analyses of this study's isolates demonstrated a degree of heterogeneity in terms of clustering, even among the avian and human isolates collected in the same geographical region (South vs South-East) and year (2006/2019/2020). This was mirrored by the varied distribution of pathotypes (UPEC/SEPEC) across the phylogenetic trees, with no definite overall clustering according to the geographical origins of the genomes, although the Brazilian isolates mostly clustered with European isolates (Fig. 3).

Studies comparing WGS ST73 isolates from UTI cases from dogs and cats in Australia previously demonstrated that, although most isolates tended to be found in host specific clusters, there were subgroups of animal and human isolates that intermingled in the SNP phylogenies suggesting bi-directional risks for infection (Kidsley et al., 2020a,b). Though the majority of this study's UPEC indeed tended to cluster among human isolates (Figs. 1 and 2), we observe a more indefinite pattern regarding the host specificity of some of the Brazilian APEC ST73 isolates given that they tended to intermingle both in the cgMLST and SNP trees among some of the Brazilian UPEC, and notably among the available international sequences originated both from human and other animal sources (Figs. 1 and 2).

One limitation of our study is that most ST73 isolates on databases for phylogenomic comparisons were biased towards human origins as these heavily outnumbered isolates from animal and environmental sources. There was also an over-representation of European and to a lesser-degree North American samples, and lack of other representative international genomes, particularly for South America. Recent studies have reported the well-established pandemic ST73 lineage as an important cause of UTI and sepsis even surpassing the most widespread ST131 clonal group (Riley, 2014); Manges et al., 2019). This is particularly reported in the UK and Denmark where studies aiming randomized sampling of clinical cases of ExPEC without a particular bias towards antimicrobial resistance, have shown its increasing predominance (Alhashash et al., 2015; Hertz et al., 2016; Rebelo et al., 2017). Of

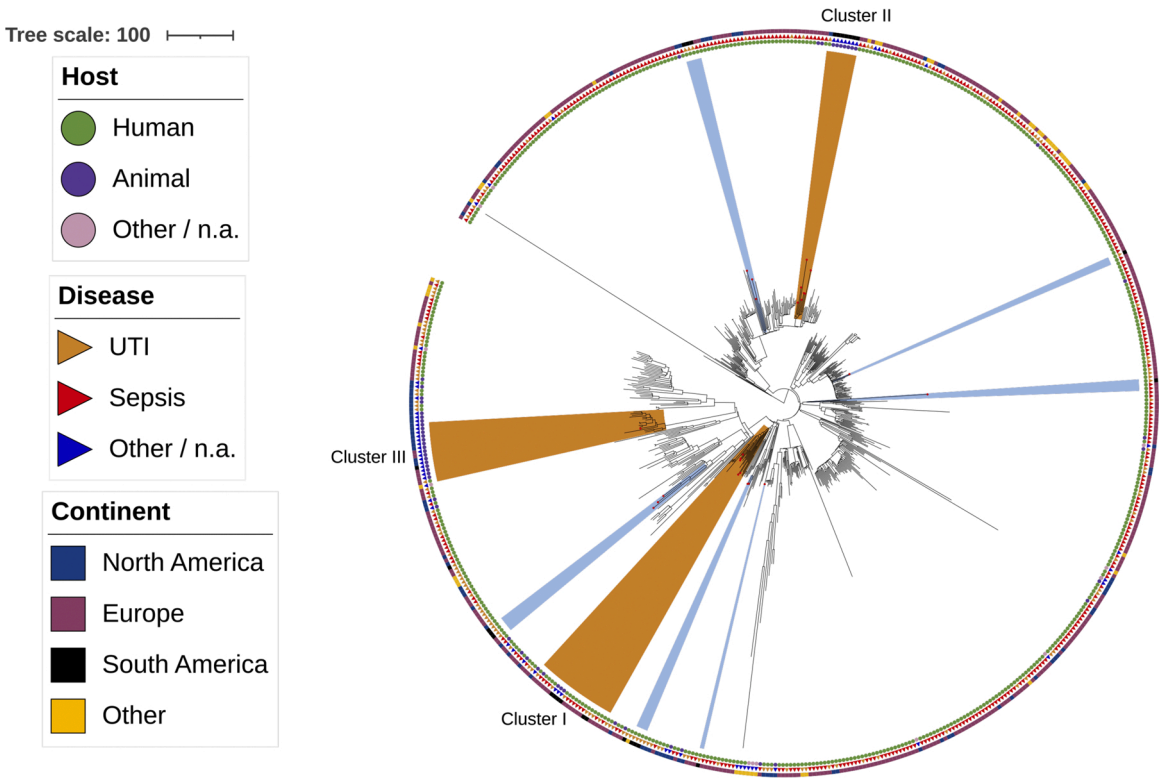

Fig. 2. Phylogeny of worldwide *E. coli* ST/CC73 isolates based on the core genome MLST (cgMLST). The tree is based on 558 genomes, rooted at mid-point using *E. coli* isolate CFT073 as reference, and allele calls were performed using a public scheme on a core of 2360 genes. Classification according to host, disease/other or not available (n.a.), are colored according to the figure's scheme. Related clusters containing the study's APEC are highlighted in orange and the UPEC are highlighted in blue. The individual sequences are marked with a red circle at the tip of the branch. The tree has been annotated and visualized using iTOL. Scalebar indicates substitutions per site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

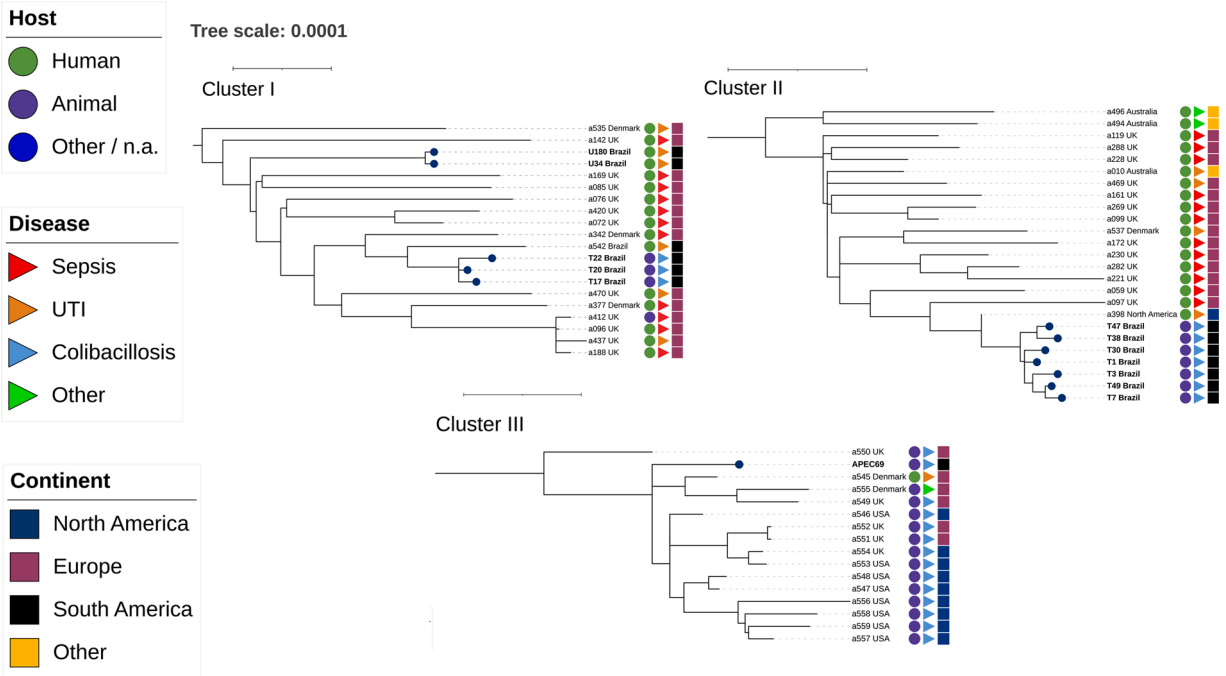

Fig. 3. Brazilian ST/CC73 *E. coli* APEC isolates are related to UTI, sepsis and other animal origin isolates but lack a clear host and phylogeographic signal. Core genome SNP phylogenetic trees showing the related clusters found in Clusters I, II and III. Classification according to host, disease, and continent are colored according to the figure's scheme. The Brazilian APEC are indicated with a blue circle at the tip of the branch and the ID in bold. The tree has been annotated and visualized using iTOL. Scalebar indicate substitutions per site. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

interest, the core-genome phylogeny illustrated that within Cluster I, three Brazilian poultry isolates clustered closely in vicinity of Danish and UK sequences from human clinical cases of UTI/sepsis. Conversely, Cluster II, which was almost exclusively composed of the remaining poultry isolates, also had a closely related UTI sequence from the USA and a sepsis case from the UK. Cluster III also showed this connection, with one isolate from a human UTI case from Denmark located in a related cluster to the sole CC73 APEC from our study (Fig. 3).

Although the zoonotic hypothesis for ExPEC has previously been explored, few genomic studies have directly compared APEC and other ExPEC sequences. The complete genome sequencing of an APEC O1 has shown high genotypic and phylogenetic similarities with human ExPEC strains (Johnson et al., 2007). ST117 isolates that caused severe outbreaks of colibacillosis in Nordic poultry farms were closely related to an isolate from human origin from the USA (Ronco et al., 2017). While studying the ST131 subtype *H*22, Liu et al. (2018) pointed out the transmission of ST131-*H*22 from retail poultry meat, and clinical cases of UTIs and sepsis in humans by performing WGS of contemporary isolates. SNP-based analysis demonstrated clusters with close phylogenetic similarities and subclusters of mixed human and poultry isolates which also carried the ColV-IncF plasmid. Similarly, Cummins et al. (2022) have recently analyzed of an extensive collection of ST95 isolates and showed the presence of particular clades where clonal human and avian isolates clustered and harbored the ColV plasmid belonging to multiple replicon F types. Therefore, these studies have been strongly indicating an avian origin for subgroups of ExPEC isolates and its potential as a foodborne pathogen causing UTIs and urosepsis in humans.

The fact that poultry and associated meat products frequently harbor ExPEC isolates similar to those found in humans reinforces this hypothesis (Nordstrom et al., 2013). Still, the different steps involved in transmission are not well established, possibly due to a time lag between a provisional establishment in the human intestinal tract and a possible subsequent urinary infection, and the huge diversity in lineages present in livestock (Manges, 2016). Our study could reflect the same conundrum, though the significant similarities between some of the Brazilian human and poultry isolates, as well as the close phylogenomic clustering of our APEC with human isolates from international UPEC/SEPEC cases, cannot be overlooked. Therefore, it is possible that Brazilian poultry meat may represent one common reservoir for national and international spread of pandemic STs, which may eventually include the ST73 clonal group among others already reported in exported poultry meat originating from the country (Müller et al., 2018). Besides the well-established pandemic clonal groups, emerging lineages are starting to be recognized and may play an important role that also need to be monitored such as the recent description of the ST457 lineage in poultry from Paraguay, which also shows non-host specificity, worldwide distribution, added of concerning MDR carriage (Nesporova et al., 2021).

WGS studies comparing human and avian ExPEC further indicate that some avian isolates and their mobile genomic contents could represent zoonotic threats which are particularly increased when acquiring/transmitting multidrug antimicrobial resistance (Manges, 2016; Cummins et al., 2022). As the number of available genomes of pandemic *E. coli* of poultry and human origins belonging to pandemic STs in common increase, so does the capacity to further compare and better define a meaningful public health importance for given clones. These studies will then be able to reinforce the need to establish preventive measures in a worldwide context regarding their spread and control.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetmic.2022.109372.

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