One Health 16 (2023) 100558

Contents lists available at ScienceDirect

One Health

journal homepage: www.elsevier.com/locate/onehlt

Genomics, biofilm formation and infection of bladder epithelial cells in potentially uropathogenic *Escherichia coli* (UPEC) from animal sources and human urinary tract infections (UTIs) further support food-borne transmission

Vanesa García [a,b], Luz Lestón [a], Ana Parga [c], Isidro García-Meniño [a,b,d], Javier Fernández [e,f,g,h], Ana Otero [c], John E. Olsen [i], Ana Herrero-Fresno [i,1], Azucena Mora [a,b,*]

[a] Laboratorio de Referencia de Escherichia coli (LREC), Dpto. de Microbioloxía e Parasitoloxía, Universidade de Santiago de Compostela (USC), Lugo 27002, Spain
[b] Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), Santiago 15706, Spain
[c] Departamento de Microbioloxía e Parasitoloxía, Facultade de Bioloxía, Edificio CIBUS, Universidade de Santiago de Compostela, Santiago de Compostela 15782, Spain
[d] Department for Biological Safety, German Federal Institute for Risk Assessment, Berlin 10589, Germany
[e] Servicio de Microbiología, Hospital Universitario Central de Asturias (HUCA), Oviedo 33011, Spain
[f] Grupo de Microbiología Traslacional, Instituto de Investigación Sanitaria del Principado de Asturias (ISPA), Oviedo 33011, Spain
[g] Research & Innovation, Artificial Intelligence and Statistical Department, Pragmatech AI Solutions, Oviedo 33011, Spain
[h] CIBER de Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Madrid, Spain
[i] Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg 1870, Denmark

ARTICLE INFO

Keywords:
E. coli
UPEC
UTI
Cell infection
Biofilm
ST1193
Food-borne

ABSTRACT

Escherichia coli is the main cause of urinary tract infections (UTI). While genomic comparison of specific clones recovered from animals, and human extraintestinal infections show high identity, studies demonstrating the uropathogenicity are lacking. In this study, comparative genomics combined with bladder-cell and biofilm formation assays, were performed for 31 *E. coli* of different origins: 7 from meat (poultry, beef, and pork); 2 from avian-farm environment; 12 from human uncomplicated UTI, uUTI; and 10 from human complicated UTI, cUTI. These isolates were selected based on their genetic uropathogenic (UPEC) status and phylogenetic background. *In silico* analysis revealed similar virulence-gene profiles, with flagella, type 1 and curli fimbriae, outer-membrane proteins (*agn43*, *ompT*, *iha*), and iron-uptake (*iutA*, *entA*, and *fyuA*) associated-traits as the most prevalent (>65%). In bladder-cell assays, moderate to strong values of association (83%, 60%, 77.8%) and invasion (0%, 70%, 55.5%) were exhibited by uUTI, cUTI, and animal-derived isolates, respectively. Of interest, uUTI isolates exhibited a significantly lower invasive capacity than cUTI isolates ($p < 0.05$). All isolates but one produced measurable biofilm. Notably, 1 turkey meat isolate O11:H6-F-ST457, and 2 cUTI isolates of the pandemic lineages O83:H42-F-ST1485-CC648 and O25b:H4-B2-ST131, showed strong association, invasion and biofilm formation. These isolates showed common carriage of type 1 fimbriae and *csg* operons, toxins (*hlyF*, *tsh*), iron uptake systems (*iutA*, *entA*, *iroN*), colicins, protectins (*cvaC*, *iss*, *kpsM*, *traT*), *ompT*, and *malX*. In summary, the similar *in vitro* behaviour found here for certain *E. coli* clones of animal origin would further reinforce the role of food-producing animals as a potential source of UPEC. Bladder-cell infection assays, combined with genomics, might be an alternative to *in vivo* virulence models to assess uropathogenicity.

1. Introduction

Urinary tract infections (UTIs) are one of the most recurrent and widespread infectious diseases in humans, accounting for around 150 million cases annually, representing an important public health problem with high economic impact on the health-care system [1,2]. Clinical

* Corresponding author at: Laboratorio de Referencia de *Escherichia coli* (LREC), Dpto. de Microbioloxía e Parasitoloxía, Universidade de Santiago de Compostela (USC), Lugo 27002, Spain.
E-mail address: azucena.mora@usc.es (A. Mora).
[1] Present address: National Food Institute, Technical University of Denmark, 2800 Kongens Lyngby, Denmark.

https://doi.org/10.1016/j.onehlt.2023.100558
Received 8 October 2022; Received in revised form 29 April 2023; Accepted 1 May 2023
Available online 3 May 2023

manifestations of UTI vary in severity from benign to life-threatening infections, thus, UTIs have been typically classified as uncomplicated UTI (uUTI, lower uncomplicated cystitis and/or upper uncomplicated pyelonephritis) and complicated UTI (cUTI, UTIs in patients with risk factors or with anatomical or functional abnormalities of the urinary tract, and/or with other associated immunocompromising diseases) [3,4].

A wide range of pathogens are implicated in UTI, including Gram-positive and Gram-negative bacteria as well as fungi, however, the most common agent is *Escherichia coli* [1]. *E. coli* strains able to cause UTI are known as uropathogenic *E. coli* (UPEC), as a subgroup included within the category of extraintestinal pathogenic *E. coli* (ExPEC) [5,6]. The human gut is the main reservoir of UPEC, where they rarely cause any complications. However, the expression of multiple virulence factors, such as adhesins, toxins and iron acquisition systems, together with the capability to adapt to harsh environment and evade the immune system, allow UPEC to disseminate and colonize the urinary tract [1,2]. Unlike the intestinal pathotypes of *E. coli*, which are clearly distinguished by specific virulence genes, extraintestinal pathogenic isolates are considered UPEC based on their site of infection and isolation (urinary tract) rather than their virulence content [7]. Despite of this fact, Spurbeck et al., [8] found that the carriage of specific virulence factor-encoding genes (*vat*, *fyuA*, *chuA*, and *yfcV*) was statistically associated with a higher capability of effectively colonizing the urinary tract. Therefore, the presence of ≥3 of these genes is considered as indicative of UPEC status [8,9].

Previous studies have claimed that food-producing animals act as a source of human ExPEC isolates, potentially transmitted to humans *via* food [10]. The hypothesis of poultry as carrier of ExPEC was evidenced by close genetic similarity, presence of common virulence genes, and shared pathogenic potential between *E. coli* recovered from human extraintestinal infections and avian *E. coli* [10–13]. Besides, several studies proved that meat, especially chicken and turkey, is a source of high-risk clonal groups of *E. coli* associated with human extraintestinal and/or uropathogenic pathologies [14–16]. In particular, clonal lineages such as ST69, ST95, ST117, ST648, or ST131, commonly associated with UTIs worldwide [7,9,17], have been also identified in poultry-meat *E. coli* isolates [14–16].

To gain knowledge on the uropathogenicity of food/animal isolates, we aimed here the comparison of a representative *E. coli* collection of human (cUTI, uUTI) and farm animal-derived isolates based on genomics, the capability to infect bladder epithelial cells (association and invasion), and the capability to form biofilm in order to assess a screening strategy that could eventually prevent the spread of these high-risk clones to humans through the food chain.

2. Material and methods

2.1. E. coli collection

Thirty-one *E. coli* isolates previously recovered from meat (poultry, beef, pork) (7 isolates), poultry farm (2), human uUTI (12), and human complicated cUTI (10) were analysed in the study (Table 1). These isolates were selected based on their genetic features and phylogenetic background. Briefly, meat and human *E. coli* isolated in 2020 came from a wide sampling performed during the same period in different supermarkets, in the Hospital Universitario Central de Asturias (HUCA) (cUTI), and at health-care centres (uUTI) [9] of the same city (Oviedo, Spain). The remaining 5 isolates belonging to the clonal complex (CC) 648, including 3 poultry-derived isolates (chicken meat, avian-farm environment [18]), displayed high identity (>85%) in the PFGE *XbaI*-macrorestriction comparison (data not shown). All farm animal-derived *E. coli* selected for the present study, conformed to the genetic features for the uropathogenic (UPEC) status. In the phenotypic assays, the non-pathogenic *E. coli* strain K12 [19], and the UPEC prototype strains UTI89 [20] and CFT073 [21], were used as controls and for comparative purposes.

Table 1
E. coli isolates characterized in this study.

Isolate	Sample origin	Year of isolation	Phylogroup	ST/CC	UPEC status
LREC-201	Chicken meat	2017	F	1485/648	*chuA, vat, yfcV*
LREC-231	cUTI	2017	F	1485/648	*chuA, yfcV*
LREC-232	Avian-farm environment	2010	F	648/648	*chuA, fyuA, yfcV*
LREC-234	Avian-farm environment	2012	F	648/648	*chuA, fyuA, yfcV*
LREC-235	cUTI	2005	F	648/648	*chuA, fyuA, yfcV*
LREC-243	Beef meat	2020	B2	1257	*chuA, fyuA, vat, yfcV*
LREC-248	Turkey meat	2020	F	457	*chuA, fyuA, yfcV*
LREC-251	Chicken meat	2020	G	117	*chuA, fyuA, vat*
LREC-252	Turkey meat	2020	B2	428	*chuA, fyuA, vat, yfcV*
LREC-253	Turkey meat	2020	B2	1236	*chuA, fyuA, vat, yfcV*
LREC-259	Pork meat	2020	G	117	*chuA, fyuA, vat*
LREC-264	uUTI	2020	G	117	*chuA, fyuA, vat*
LREC-265	uUTI	2020	B2	1193/14	*chuA, fyuA, vat, yfcV*
LREC-266	uUTI	2020	B2	404/14	*chuA, fyuA, vat, yfcV*
LREC-267	uUTI	2020	B2	131/131	*chuA, fyuA, yfcV*
LREC-268	uUTI	2020	F	59/59	*chuA, fyuA, yfcV*
LREC-269	uUTI	2020	B2	1193/14	*chuA, fyuA, vat, yfcV*
LREC-270	uUTI	2020	B2	1193/14	*chuA, fyuA, vat, yfcV*
LREC-271	uUTI	2020	F	59/59	*chuA, fyuA, yfcV*
LREC-272	uUTI	2020	F	59/59	*chuA, fyuA, yfcV*
LREC-273	uUTI	2020	B2	1193/14	*chuA, fyuA, vat, yfcV*
LREC-274	uUTI	2020	F	59/59	*chuA, fyuA, yfcV*
LREC-275	uUTI	2020	B2	1193/14	*chuA, fyuA, vat, yfcV*
LREC-278	cUTI	2020	B2	131/131	*chuA, fyuA, yfcV*
LREC-279	cUTI	2020	B2	9126/131	*chuA, fyuA, yfcV*
LREC-280	cUTI	2020	B2	131/131	*chuA, fyuA, yfcV*
LREC-281	cUTI	2020	B2	131/131	*chuA, fyuA, yfcV*
LREC-285	cUTI	2020	B2	9126/131	*chuA, fyuA, yfcV*
LREC-286	cUTI	2020	B2	131/131	*chuA, fyuA, yfcV*
LREC-287	cUTI	2020	B2	131/131	*chuA, fyuA, yfcV*
LREC-289	cUTI	2020	B2	131/131	*chuA, fyuA, yfcV*

2.2. Conventional typing

The UPEC status based on specific genes (*chuA*, *fyuA*, *vat* and *yfcV*) [8] was first screened by PCR. Then, the phylogenetic group and sequence type (ST) were assigned following the Clermont et al. [22] and Achtman typing [23] schemes, respectively.

2.3. Whole genome sequencing (WGS) and in silico analysis

WGS and genome assembly was performed as previously described [9]. Briefly, DNA was extracted with the DNeasey Blood & Tissue Kit (Qiagen, Hilen, Germany) according to the manufacturer's instructions. The genomic DNA libraries for sequencing were prepared using the Nextera XT Library Prep kit (Illumina, CA, USA) according to the manufacturer's recommendation. Then, libraries were pooled in equimolar amounts according to the quantification data provided by the Qubit dsDNA HS Assay (Thermo Fisher Scientific). Lastly, the libraries were sequenced in an Illumina NovaSeq PE150 platform, obtaining 100–150 bp paired end reads which were trimmed (Trim Galore 0.6.0) and filtered according to quality criteria (FastQC 0.11.9). The quality-filtered reads were assembled *de novo* using Unicycler (v0.4.8) [24] which uses an adapted SPAdes (v3.14.0) assembling algorithm [25]. The assembled contigs were analysed using the Center for Genomic Epidemiology (CGE) databases, and applying the thresholds suggested by default when required (minimum identity of 90% and coverage of 60%): SeroTypeFinder 2.0 [26], CHTyper 1.0 [27], MLST 2.0 [23,28], cgMLSTFinder1.1 [29], and ResFinder 4.1 [30–32]. Virulence genes were identified using ABRIcate v1.0.1 run against the virulence factor database (VFDB) [33] where results were filtered only for *E. coli* entries, as well as using the web-based tool VirulenceFinder 2.0 of CGE [34,35].

2.4. Infection of human epithelial bladder cells

The ability of the isolates to infect human epithelial J82 cells, derived from a bladder carcinoma (ATCC HTB-1), was tested following the previously described protocol [36]. Briefly, J82 cells were infected with the bacterial isolates at a multiplicity of infection (MOI) of approx. 100:1. Colony forming unit (CFU) counts of the bacterial inoculum were verified by plating onto Luria-Bertani (LB, Oxoid, Denmark) agar plates. After 1 h of infection, cells were washed twice with Dulbecco's phosphate-buffered saline (DPBS, Gibco, Denmark) to eliminate not-associated bacteria. Then, J82 cells were lysed by addition of 1 mL 0.1% Triton X-100 (v/v), and serial dilutions of the lysates were plated onto the LB agar for CFU/mL quantification to estimate associated bacteria at 1 h post-infection. For invasion assessment, a gentamicin protection assay was performed as follows: after 1 h post-infection and DPBS wash, the infected bladder cells were incubated in Dulbecco's modified eagle medium (DMEM, Gibco, Denmark) with 10% fetal bovine serum (FBS, Gibco, Denmark) and 100 μg/mL gentamicin (Gibco, Denmark) for 1 h followed by washing, lysing and bacterial plating as described above. Association and invasion values were calculated using ratios of bacterial counts: (i) CFU/mL at 1 h post-infection over the initial inoculum (association); (ii) CFU/mL of intracellular bacteria at 2 h (after 1-h gentamicin exposure) over the initial inoculum (invasion). Then, the association and invasion rates were classified in low, moderate and strong, taking as reference the *E. coli* K-12 values, namely: low (0–0.6% association; 0–0.06% invasion), moderate (0.6–3% association; 0.06–0.3% invasion) and strong (>3% association; >0.3% invasion).

2.5. Biofilm formation systems

Before the screening of the complete collection, *E. coli* isolates LREC-201, 231, 232, 234, 235, and strains K12, UTI89 and CFT073 were first assayed with three different biofilm formation methods, namely, the standard 96-well microtiter plate method, the Amsterdam Active Attachment (AAA) biofilm cultivation method [37], and the xCELLigence equipment (Acea Biosciences, USA) for real-time biofilm monitoring [38]. The three methods were inoculated with the same suspensions of *E. coli*, cultured for 12 h at 37 °C with shaking (100 rpm) in 10 mL of LB medium (10 g/L Bacto™ Tryptone, Becton Dickinson (BD), Spain; 10 g/L NaCl, Scharlau, Spain; 5 g/L Bacto™ Yeast Extract, BD, Spain), and the minimal medium M9 (BD, Spain). Cultures were centrifuged (5 min, 5000 rpm), and pelleted cells were resuspended in fresh LB medium and adjusted to an OD_{600} of 0.5.

For the 96-well microtiter plates method, 20 μL of the cell suspension were inoculated into 180 μL of LB in a 96-well microtitre plate (BRAND®, Merck, Spain) and incubated at 37 °C for 24 h. Next, the medium was removed, wells were washed with 200 μL of LB to remove non-attached bacteria, and biofilms were allowed to dry. Then, 200 μL of 0.04% crystal violet solution (Panreac, Spain) was added to all wells and plates were incubated for 20 min at room temperature. The excess dye was removed by washing twice with distilled water, bound crystal violet was released by adding 200 μL of 33% acetic acid (Scharlab, Spain), and the sample absorbance was measured at OD_{590} (MULTISKAN SkyHigh, ThermoScientific, Spain). Basal absorbance was corrected using wells containing only LB medium, stained, and quantified as stated above. This method was used to screen the complete collection of *E. coli* isolates. The results from the comparison of the selected standard 96-well microtiter plate method with the other biofilm methods (AAA, xCELLigence), are fully described in the Supplementary material.

Following the Stepanovic et al. [39] criterion, the isolates were classified as non-biofilm formers ($OD \leq 0.124$), weak biofilm formers (≥ 0.125 $OD \leq 0.248$), moderate biofilm formers (≥ 0.249 $OD \leq 0.496$), or strong biofilm formers ($OD \geq 0.497$).

2.6. Statistical analysis

Statistical analyses were performed using Prism 8 (GraphPad, San Diego, CA, USA), and significant differences were determined at $p < 0.05$. Specifically for cell infection assays, one-way ANOVA followed by Dunett's (for comparison of isolates with *E. coli* K12) and Mann-Whitney post-tests (for comparison between different categories) were applied. Regarding biofilm formation, statistical differences between isolates were determined by means of two-tailed Kruskal-Wallis tests for non-normally distributed samples. Correlation analysis between biofilm formation, and cell invasion and association abilities were calculated using Spearman's r for non-normally distributed samples. Pairwise comparisons performed by a two-tailed Fisher's exact probability test were considered statistically significant when p values <0.05.

3. Results and discussion

UPEC is part of the extraintestinal pathogenic *E. coli* (ExPEC) group, which includes any *E. coli* that causes disease outside the gut [5,6]. In previous studies, we proved that ExPEC, including potential UPEC, can be present in poultry meat [13,15]. High genetic identity has been reported from the comparison of ExPEC isolates recovered from human UTI and food-producing animals or meat (poultry origin mainly). This provides support for the hypothesis of food-borne transmission for UTI-causing *E. coli* [40–43]. However, there is lack of studies that perform parallel assessment of the uropathogenic potential of both human community and hospital UTI, and food-producing animal isolates. A recent study [44] acknowledged the difficulty of a molecular diagnosis due to the incredible genetic heterogeneity of the UPEC group, and claimed the utility of phenotypic screening to better predict the severity of UTI that *E. coli* strains may cause.

The selection criteria of the *E. coli* analysed in the study, included i) to carry specific virulence factor-encoding genes statistically associated with a higher capability of effectively colonizing the urinary tract [8] (for the farm animal-derived *E. coli*), ii) to be the etiological agent of UTI (for those of human origin), iii) to be recovered in the same period and city (27 of the 31 human UTI and animal-derived sources) (Table 1).

3.1. In silico characterization

Table 2 summarizes the main traits of the *in silico* characterization of the 31 *E. coli*, which revealed 12 clonal groups of 3 phylogroups (B2, F, G), including the clinically relevant F-ST59 (clonotype, CH32–41), G-ST117 (CH45–97), B2-ST131 (CH40–30), F-ST648 (CH4–58) or B2-ST1193 (CH14–64) [9,17,45].

The increasing emergence of UPEC extended-spectrum-betalactamase (ESBL)-producing isolates currently challenges the therapeutic management of UTI worldwide [1]. In our collection, ResFinder revealed the presence of resistance genes to 10 different antimicrobial categories. Most isolates (83%) harboured macrolide-encoding genes, and > 30% carried peroxide, beta-lactam, aminoglycosides, sulphonamides, tetracycline, and trimethoprim encoding genes. Besides, point mutations for quinolone-resistance were detected in 19 isolates of CC648 (2 avian-farm environment isolates, 1 chicken meat, and 2 cUTI), ST1193 (5 uUTI) and CC131 (1 uUTI, 8 cUTI). Seventeen isolates were categorized as multidrug-resistant (MDR) (≥ 3 antimicrobial categories) [46], including 12 of 13 ESBL-producers (Table 2).

Table 2 and Table S1 show different genes encoding flagellar, fimbriae, toxins, biofilm formation, protectins, and iron uptake retrieved by means of VirulenceFinder and VFDB databases.

In detail, flagellar genes of operons *flgBCDEFGHIJ*, *flhBAE*, *fliAZY*, *flgKL* and *motAB*, associated with biofilm formation were predicted in all genomes (Table S1, Table S2). According to Niba et al. [47] assays, mutation of these genes caused defective biofilm formation in *E. coli* K12 strain BW25113. Besides that, previous literature demonstrated the critical role of flagellum-mediated motility in ascension of the urinary tract [48] although their expression was not studied here.

Bacterial adherence constitutes the key step to develop a UTI. UPEC express several fimbrial adhesins that promote the attachment to bladder epithelial cell layer, of which type 1 and P fimbriae are the best characterized and most relevant virulence factors for infection [1,2]. However, while type 1 fimbriae are critical for establishing cystitis, they are dispensable for colonization of other sites in the urinary tract [44]. Type 1 fimbriae-encoding genes were found in the 31 *E. coli*, although the *fimA-I* operon was complete in 18 genomes and incomplete in all cUTI, 1 turkey and 1 pork meat isolates. P-fimbriae genes were predicted in 28 isolates, with the entire *pap* operon in 7 isolates of both human and animal origins. F1C and S fimbriae genes were not identified in the collection (Fig. 1).

Other highly prevalent virulence genes (≥ 20 isolates) found here, were those encoding: siderophores (*iutA*, *entA* and *fyuA*), which seems to be crucial for UPEC survival since the urinary tract is iron-limited [49]; protectins (*iss*, *kpsM* and *traT*); the outer membrane proteins (*ompT, agn43*); *malX*; and the uropathogen-specific protein (*usp*). On the contrary, the toxin-encoding genes (*astA*, *hlyA*, *tsh*, *pic, cnf-1* and *ibeA*) were found in few isolates (1 to 4), and only *sat*, *hlyF* and *vat* genes were predicted in ≥11 isolates. In addition, the colicin-encoding genes were detected in 14 isolates (Fig. 1, Table 2). The *iss* (serum resistance) and *kpsM* (capsule production) genes, which are considered virulence factors commonly detected in UPEC isolates [49], were determined in 22 and 26 isolates, respectively. The high occurrence of *iss*, *kpsM* and colicin-encoding genes in the poultry-derived isolates (71%, 100% and 71%, respectively) (Table 2) might confer competitive advantage with potential pathogenicity in humans [12,13].

UPEC isolates can form biofilm-like intracellular bacterial communities, a relevant pathogenetic mechanism that provides protection against neutrophils, antibiotics, and stresses [1,49]. There are multiple surface structures involved in biofilm production, a subset of which are also involved in bladder colonization [49]. Among them, type 1 fimbriae, essential for bacterial adherence, and in the initial phases of biofilm development. Besides the flagella, there are curli fibers (*csgBA* and *csgDEFG* operons), found in 30 of 31 isolates, which mediate the attachment to abiotic surface [50]. Other genes described in biofilm formation identified here were: *agn43* (promotes cell-to-cell adhesion) [50], and *iha* (adherence protein) [51], found in 71% of the isolates; *cah* (calcium-binding antigen 43 homolog) [52], found in 13%; and *upaH* (autotransporter protein implicated in biofilm formation) [53], found in 6.5% (Fig. 1, Table S1).

3.2. Infection of human epithelial bladder cells

Cell culture assays have been widely used to determine the mechanisms and effects of UPEC interactions with host cells [54]. Here, we infected J82 bladder epithelial cells to assess and compare the pathogenicity of our collection. All isolates were able to associate to cells, with association values ranging from 0.12% (LREC-267) to 12.7% (LREC-231). Four isolates: 2 cUTI (LREC-231 and LREC-286), 1 uUTI (LREC-266) and 1 turkey meat (LREC-248) significantly exceeded *E. coli* K-12 (0.2%) (Fig. 2). Similarly, the isolates showed very different invasion capability, ranging from 0.009% (LREC-232) to 1.006% (LREC-231). Three cUTI (LREC-231, LREC-278 and LREC-286) and 1 turkey meat (LREC-248) isolates exhibited significantly higher invasion rates than *E. coli* K-12 (0.024%) (Fig. 2).

Regarding origin, the poultry *E. coli* isolates exhibited moderate (80%) or strong (20%) association, and moderate (40%) or strong (20%) invasion; the single beef isolate showed moderate association and invasion, while the pork isolate displayed weak result for both association and invasion (Figs. 1 and 2). Few studies have assessed pathogenicity in bladder cells of animal-derived isolates, with heterogenous response. Thus, similar results were observed among poultry-origin *E. coli* strains carrying the traits associated with urinary tract infection in India [43]. On the contrary, extended-spectrum cephalosporin resistant *E. coli* from retail chicken meat in Norway exhibited high capability of association but low invasion; while ExPEC poultry isolates in a study conducted in USA showed weak association, and most of them were non-invasive [42,55]. These differences can be easily attributed, among others, to the high genetic heterogeneity of the *E. coli* tested in each study. Most isolates assayed here belong to specific high-risk clonal groups of phylogroups B2, F and G and STs commonly implicated in UTIs [7,9,17]. The phylogenetic background of the isolates assayed in other studies are not usually reported, which can make comparisons difficult. In any case, our findings on infection in bladder epithelial cells suggest that certain poultry-origin *E. coli* showed the pathogenic potential to initiate a UTI.

Within the human UTI isolates, a relevant finding was the difference found regarding invasion pattern and type of infection. Thus, 100% of the uUTI isolates showed low invasive capability, while 75% of the cUTI *E. coli* exhibited moderate or strong invasion ($p = 0.0126$) (Fig. 2). Most uUTI and cUTI *E. coli* displayed moderate or strong association to J82 cells (83% and 62.5%, respectively). This would correlate with lower capability of uUTI isolates to cause a severe infection *in vivo*, while being capable of causing a more benign syndrome (cystitis) in the host. In agreement with a recent study on the effectiveness of phenotypic characterization of isolates to predict the infectivity [56], cellular infection assays would be of utility to assess the infection severity of human isolates. We suggest here that it could be used also to predict the potential pathogenicity of animal-derived sources, in combination with genomic characterization for the screening of preeminent virulence factors for urinary tract adhesion, invasion and colonization.

3.3. Biofilm formation

Biofilm formation is an important pathogenic determinant in UPEC [1,49], which facilitates bacterial persistence leading to chronic or recurrent UTI. It is also frequently implicated in catheter-associated UTI [4].

Under the selected condition, all isolates but one (LREC-287, cUTI) formed measurable biofilm. The LREC-287 isolate was classified as non-biofilm producer despite the carriage of *csg* operons, *agn43* and *iha*, which might indicate that they were not functional, or expressed in the assayed conditions. On the other hand, 2 isolates stood out as biofilm

Table 2
In silico characterization of *E. coli* isolates.

ID code[1]	Origin	O:H antigens[2]	ST#1/ ST#2[3]	cgST[4]	CHType[5]	Acquired resistances and point mutations (in bold)[6]	Virulence genes[7]
LREC-201	Chicken meat	O83:H42	1485/ND	58794	231–58	*bla*$_{TEM-1B}$, *aac(3)-IIa*, *aadA1*, *aph(3″)-Ib*, *aph(6)-Id*, *sul1*, *sul2*, *sul3*, *tet(A)*, *dfrA1*, *qacE*, *sitABCD*, **gyrA S83L, parC S80I**	*air*, *chuA*, *cvaC*, *eilA*, *etsC*, *gad*, *hlyF*, *hra*, *iroN*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsMII_K5*, *lpfA*, *mchF*, *mcmA*, *ompT*, *sitA*, *terC*, *traT*, *tsh*, *yfcV*
LREC-231	cUTI	O83:H42	1485/ND	58794	231–58	*bla*$_{SHV-12}$, *bla*$_{TEM-1B}$, *aadA1*, *aadA2b*, *aadA13*, *aph(3′)-Ia*, *aph(3″)-Ib*, *aph(6)-Id*, *cmlA1*, *catA1*, *sul1*, *sul2*, *sul3*, *tet(A)*, *dfrA14*, *qacE*, *sitABCD*, **gyrA S83L, gyrA D87H, parC S80I**	*air*, *chuA*, *cib*, *cvaC*, *eilA*, *etsC*, *gad*, *hlyF*, *hra*, *iroN*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsMII_K5*, *lpfA*, *mchF*, *mcmA*, *ompT*, *papC*, *sitA*, *terC*, *traT*, *tsh*, *yfcV*
LREC-232	Avian-farm environment	O25:H4	648/ND	24888	4–58	*bla*$_{CTX-M-32}$, *sul2*, *tet(B)*, *sitABCD*, **gyrA S83L**	*air*, *chuA*, *eilA*, *etsC*, *fyuA*, *gad*, *hlyF*, *hra*, *ireA*, *irp2*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsMII_K52*, *lpfA*, *ompT*, *papA_F20*, *papC*, *sitA*, *terC*, *traT*, *yfcV*
LREC-234	Avian-farm environment	O25:H4	648/ND	24888	4–58	*bla*$_{CTX-M-32}$, *sul2*, *tet(B)*, *sitABCD* **gyrA S83L, parC S80R**	*air*, *astA*, *chuA*, *cma*, *eilA*, *etsC*, *fyuA*, *gad*, *hlyF*, *hra*, *iha*, *ireA*, *irp2*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsMII_K52*, *lpfA*, *ompT*, *papA_F20*, *papC*, *sitA*, *terC*, *traT*, *yfcV*
LREC-235	cUTI	O25:H4	648/ND	24888	4–58	*bla*$_{CTX-M-32}$, *aph(6)-Id*, *aph(3″)-Ib*,*sul2*, *sitABCD* **gyrA S83L**	*air*, *chuA*, *eilA*, *etsC*, *fyuA*, *gad*, *hlyF*, *hra*, *irp2*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsMII_K52*, *lpfA*, *ompT*, *sitA*, *terC*, *traT*, *yfcV*
LREC-243	Beef meat	O8: H10	1257/122	10228	39–9	*bla*$_{SHV-12}$, *mdf(A)*, *tet(B)*, *sitABCD*	*chuA*, *etsC*, *fyuA*, *gad*, *hlyF*, *ibeA*, *iroN*, *irp2*, *iss*, *mchF*, *ompT*, *papC*, *pic*, *sitA*, *terC*, *traT*, *vat*, *yfcV*
LREC-248	Turkey meat	O11:H6	457/829	125009	88–145	*mdf(A)*, *sitABCD*	*air*, *cba*, *chuA*, *cia*, *cma*, *cvaC*, *eilA*, *etsC*, *fyuA*, *gad*, *hlyF*, *iroN*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsMII*, *lpfA*, *mchF*, *ompT*, *papC*, *sitA*, *terC*, *traT*, *tsh*, *yfcV*
LREC-251	Chicken meat	O161:H4	117/48	84901	45–97	*mdf(A)*, *tet(A)*, *sitABCD*	*cba*, *chuA*, *cia*, *cma*, *etsC*, *fyuA*, *hlyF*, *iha*, *ireA*, *iroN*, *irp2*, *iss*, *iucC*, *iutA*, *lpfA*, *mchB*, *mchC*, *mchF*, *ompT*, *papA_F11*, *papC*, *pic*, *sitA*, *terC*, *traT*, *vat*
LREC-252	Turkey meat	O117:H4	428/73	75536	40–22	*mdf(A)*	*cea*, *chuA*, *cia*, *cvaC*, *etsC*, *fyuA*, *hlyF*, *ibeA*, *ireA*, *iroN*, *irp2*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsMII_K1*, *mchF*, *neuC*, *ompT*, *sitA*, *terC*, *traT*, *tsh*, *usp*, *vat*, *yfcV*
LREC-253	Turkey meat	O68:H5	1236/ unknown	75761	195–253	*mdf(A)*, *sul2*	*cba*, *chuA*, *cia*, *cma*, *cvaC*, *etsC*, *fyuA*, *gad*, *ibeA*, *ireA*, *iroN*, *irp2*, *iss*, *iucC*, *iutA*, *kpsE*, *mchF*, *ompT*, *sitA*, *terC*, *traT*, *usp*, *vat*, *yfcV*
LREC-259	Pork meat	O119:H4	117/641	46662	45–97	*mdf(A)*, *tet(A)*, *sitABCD*	*chuA*, *cia*, *cma*, *cnf1*, *cvaC*, *etsC*, *fyuA*, *hlyF*, *hra*, *iha*, *ireA*, *iroN*, *irp2*, *iss*, *iucC*, *iutA*, *lpfA*, *mchB*, *mchC*, *mchF*, *ompT*, *papA_F14*, *papC*, *pic*, *sitA*, *terC*, *traT*, *vat*
LREC-264	uUTI	O119:H4	117/48	99386	45–97	*bla*$_{TEM-1B}$, *aph(3″)-Ib*, *aph(6)-Id*, *mdf (A)*, *sul2*, *dfrA5*, *sitABCD*	*chuA*, *cia*, *cvaC*, *etsC*, *fyuA*, *hlyF*, *hra*, *ireA*, *iroN*, *irp2*, *iss*, *iucC*, *iutA*, *lpfA*, *mchB*, *mchC*, *mchF*, *mcmA*, *ompT*, *papA_F11*, *papC*, *pic*, *sitA*, *terC*, *traT*, *vat*
LREC-265	uUTI	O75:H5	1193/53	4085	14–64	*bla*$_{TEM-1B}$, *aph(3″)-Ib*, *aph(6)-Id*, *mdf (A)*, *sul2*, *sitABCD* **gyrA S83L, gyrA D87N, parC S80I, parE L416F**	*chuA*, *fyuA*, *gad*, *iha*, *irp2*, *iucC*, *iutA*, *kpsE*, *kpsMII_K1*, *neuC*, *ompT*, *papA_F43*, *sat*, *senB*, *sitA*, *terC*, *usp*, *vat*, *yfcV*
LREC-266	uUTI	O75:H5	404/6	91334	14–27	*mdf(A)*, *sitABCD*	*afaA*, *afaB*, *afaC*, *afaD*, *afaE*, *chuA*, *clbB*, *fyuA*, *gad*, *iha*, *irp2*, *iucC*, *iutA*, *kpsE*, *kpsMII_K5*, *ompT*, *papA_F43*, *sat*, *senB*, *sitA*, *terC*, *traT*, *usp*, *vat*, *yfcV*
LREC-267	uUTI	O25:HNT	131/43	10774	40–1196	*mdf(A)*, *sitABCD* **gyrA S83L, gyrA D87N, parC S80I, parC E84V, parE I529L**	*chuA*, *fyuA*, *gad*, *hra*, *iha*, *irp2*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsMII_K5*, *ompT*, *papA_F43*, *sat*, *senB*, *sitA*, *terC*, *usp*, *yfcV*
LREC-268	uUTI	O1:H7	59/34	61756	32–41	*mdf(A)*, *sitABCD*	*air*, *capU*, *chuA*, *eilA*, *fyuA*, *iha*, *ireA*, *irp2*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsMII_K1*, *lpfA*, *ompT*, *papA_feiA_F8*, *papC*, *sat*, *senB*, *sitA*, *terC*, *traT*, *usp*, *yfcV*
LREC-269	uUTI	O75:H5	1193/53	4085	14–64	*bla*$_{TEM-1B}$, *aph(3″)-Ib*, *aph(6)-Id*, *mdf (A)*, *mph(A)*, *sul2*, *dfrA17*, *sitABCD* **gyrA S83L, gyrA D87N, parC S80I, parE L416F**	*chuA*, *fyuA*, *gad*, *iha*, *irp2*, *iucC*, *iutA*, *kpsE*, *kpsMII_K1*, *neuC*, *ompT*, *papA_F43*, *sat*, *senB*, *sitA*, *terC*, *usp*, *vat*, *yfcV*
LREC-270	uUTI	ONT:H5	1193/53	72142	14–64	*mdf(A)*, *sitABCD* **gyrA S83L, gyrA D87N, parC S80I, parE L416F**	*cia*, *fyuA*, *gad*, *iha*, *irp2*, *iucC*, *iutA*, *kpsE*, *kpsMII_K1*, *senB*, *terC*, *usp*
LREC-271	uUTI	O1:H7	59/34	6704	32–41	*mdf(A)*, *sitABCD*	*air*, *capU*, *chuA*, *eilA*, *fyuA*, *iha*, *ireA*, *irp2*, *iucC*, *iutA*, *kpsE*, *kpsMII_K1*, *lpfA*, *neuC*, *ompT*, *papA_F9*, *papA_feiA_F8*, *papC*, *sat*, *sitA*, *terC*, *usp*, *yfcV*
LREC-272	uUTI	O1:H7	59/34	6704	32–41	*mdf(A)*, *sitABCD*	*air*, *capU*, *chuA*, *eilA*, *fyuA*, *iha*, *ireA*, *irp2*, *iucC*, *iutA*, *kpsE*, *kpsMII_K1*, *lpfA*, *neuC*,

(*continued on next page*)

Table 2 (*continued*)

ID code[1]	Origin	O:H antigens[2]	ST#1/ ST#2[3]	cgST[4]	CHType[5]	Acquired resistances and point mutations (in bold)[6]	Virulence genes[7]
							ompT, *papA_F9*, *papA_feiA_F8*, *papC*, *sat*, *sitA*, *terC*, *usp*, *yfcV*
LREC-273	uUTI	O75:H5	1193/53	4085	14–64	*mdf(A)*, *sitABCD* **gyrA S83L, gyrA D87N, parC S80I, parE L416F**	*chuA*, *fyuA*, *gad*, *iha*, *irp2*, *iucC*, *iutA*, *kpsE*, *kpsMII_K1*, *neuC*, *ompT*, *papA_F43*, *sat*, *senB*, *sitA*, *terC*, *usp*, *vat*, *yfcV*
LREC-274	uUTI	O1:H7	59/34	124035	32–41	*mdf(A)*, *sitABCD*	*air*, *capU*, *chuA*, *eilA*, *fyuA*, *iha*, *irp2*, *iucC*, *iutA*, *kpsE*, *kpsMII_K1*, *lpfA*, *neuC*, *ompT*, *papA_feiA_F8*, *sat*, *sitA*, *terC*, *usp*, *yfcV*
LREC-275	uUTI	O75:H5	1193/53	4085	14–64	bla_{TEM-1B}, *aph(3″)-Ib*, *aph(6)-Id*, *mdf (A)*, *sul2*, *dfrA14*, *sitABCD* **gyrA S83L, gyrA D87N, parC S80I, parE L416F**	*chuA*, *fyuA*, *gad*, *iha*, *irp2*, *iucC*, *iutA*, *kpsE*, *kpsMII_K1*, *neuC*, *ompT*, *papA_F43*, *sat*, *senB*, *sitA*, *terC*, *usp*, *vat*, *yfcV*
LREC-278	cUTI	O25:H4	131/43	12614	40–30	$bla_{CTX-M-15}$, *aadA5*, *mdf(A)*, *mph(A)*, *sul1*, *tet(A)*, *dfrA17*, *qacE*, *sitABCD* **gyrA S83L, gyrA D87N, parC S80I, parC E84V, parE I529L**	*chuA*, *fyuA*, *gad*, *hra*, *iha*, *irp2*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsMII_K5*, *ompT*, *papA_F43*, *papC*, *sat*, *senB*, *sitA*, *terC*, *traT*, *usp*, *yfcV*
LREC-279	cUTI	O25:H4	9126/43	7829	1267–30	$bla_{CTX-M-15}$, *mdf(A)*, *sitABCD* **gyrA S83L, gyrA D87N, parC S80I, parC E84V, parE I529L**	*celb*, *chuA*, *fyuA*, *gad*, *iha*, *irp2*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsMII_K5*, *ompT*, *papA_F43*, *sat*, *sitA*, *terC*, *usp*, *yfcV*
LREC-280	cUTI	O25:H4	131/43	142625	40–30	$bla_{CTX-M-15}$, bla_{TEM-1A}, *aadA2*, *mdf(A)*, *mph(A)*, *sul1*, *dfrA12*, *qacE*, *sitABCD*, **gyrA S83L, gyrA D87N, parC S80I, parC E84V, parE I529L**	*chuA*, *cnf1*, *fyuA*, *gad*, *hra*, *iha*, *irp2*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsMII_K5*, *ompT*, *papA_F43*, *papC*, *sat*, *sitA*, *terC*, *traT*, *usp*, *yfcV*
LREC-281	cUTI	O25:H4	131/43	116708	40–30	$bla_{CTX-M-15}$, bla_{OXA-1}, *aac(3)-IIa*, *catB3*, *aac(6′)-Ib-cr*, *qnrS1*, *mdf(A)*, *dfrA14*, *sitABCD* **gyrA S83L, gyrA D87N, parC S80I, parC E84V, parE I529L**	*chuA*, *cnf1*, *fyuA*, *gad*, *hra*, *iha*, *irp2*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsMII_K5*, *ompT*, *papA_F43*, *papC*, *sat*, *senB*, *sitA*, *terC*, *traT*, *usp*, *yfcV*
LREC-285	cUTI	O25:H4	9126/43	7829	1267–30	$bla_{CTX-M-15}$, bla_{OXA-1}, bla_{TEM-1B}, *catB3*, *aac(6′)-Ib-cr*, *mdf(A)*, *sitABCD* **gyrA S83L, gyrA D87N, parC S80I, parC E84V, parE I529L**	*celb*, *chuA*, *fyuA*, *gad*, *iha*, *irp2*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsMII_K5*, *ompT*, *papA_F43*, *sat*, *sitA*, *terC*, *traT*, *usp*, *yfcV*
LREC-286	cUTI	O25:H4	131/43	139233	40–30	$bla_{CTX-M-15}$, bla_{OXA-1}, *aadA5*, *catB3*, *aac (6′)-Ib-cr*, *mdf(A)*, *mph(A)*, *sul1*, *dfrA17*, *qacE*, *sitABCD* **gyrA S83L, gyrA D87N, parC S80I, parC E84V, parE I529L**	*afaA*, *afaC*, *afaD*, *chuA*, *fyuA*, *gad*, *iha*, *irp2*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsMII_K5*, *nfaE*, *ompT*, *papA_F43*, *sat*, *sitA*, *terC*, *usp*, *yfcV*
LREC-287	cUTI	O25:H4	131/43	122338	40–30	$bla_{CTX-M-15}$, bla_{OXA-1}, bla_{TEM-1B}, *aadA5*, *aph(3″)-Ib*, *aph(6)-Id*, *aac(6′)-Ib-cr*, *mdf(A)*, *mph(A)*, *sul1*, *sul2*, *tet(A)*, *dfrA17*, *qacE*, *sitABCD* **gyrA S83L, gyrA D87N, parC S80I, parC E84V, parE I529L**	*chuA*, *fyuA*, *gad*, *iha*, *irp2*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsMII_K5*, *ompT*, *papA_F43*, *sat*, *senB*, *sitA*, *terC*, *traT*, *usp*, *yfcV*
LREC-289	cUTI	O25:H4	131/43	71301	40–30	$bla_{CTX-M-15}$, bla_{OXA-1}, *aac(3)-IIa*, *catB3*, *aac(6′)-Ib-cr*, *mdf(A)*, *tet(A)*, *sitABCD* **gyrA S83L, gyrA D87N, parC S80I, parC E84V, parE I529L**	*chuA*, *cnf1*, *fyuA*, *gad*, *hra*, *iha*, *irp2*, *iss*, *iucC*, *iutA*, *kpsE*, *kpsMII_K5*, *ompT*, *papA_F43*, *papC*, *sat*, *senB*, *sitA*, *terC*, *traT*, *usp*, *yfcV*

[1] Isolate and genome (LREC) identification.

[2] O and H antigen prediction with SerotypeFinder 2.0.

[3] Sequence types (ST#1 and ST#2) based on two different MLST schemes were applied: *E. coli* #1 (23) and *E. coli* #2 (28), respectively, and retrieved with MLST 2.0.4; ND: not determined.

[4] Core genome ST obtained with cgMLSTFinder1.1. software run against the Enterobase database.

[5] Clonotypes, [6]acquired antimicrobial resistance genes and/or chromosomal mutations and [7]virulence genes were also predicted using: CHtyper 1.0, ResFinder 4.1, and VirulenceFinder 2.0 online tools at the Center of Genomic Epidemiology (http://www.genomicepidemiology.org/services/), respectively.

[6] Resistome: Acquired resistance genes: beta-lactam: bla_{OXA-1}, bla_{TEM-1A}, bla_{TEM-1B}, bla_{SHV-12}, $bla_{CTX-M-15}$, $bla_{CTX-M-32}$, aminoglycosides: *aac(3)-IIa, aadA1, aadA2, aadA2b, aadA5, aadA13, aph(3′)-Ia, aph(3″)-Ib, aph(6)-Id;* phenicols*: cmlA1, catA1,catB3;* fluoroquinolones*: aac(6′)-Ib-cr, qnrS1,* macrolides: *mdf(A), mph(A)*; sulphonamides: *sul1*, *sul2, sul3*; tetracycline: *tet(A), tet(B);* trimethoprim: *dfrA1*, *dfrA5*, *dfrA14, dfrA12, dfrA17*; quaternary ammonium compounds: *qacE,* peroxide: *sitABCD* (mediates transport of iron and manganese and resistance to hydrogen peroxide). Point mutations: quinolones and fluoroquinolones: *gyrA* S83L: TCG-TTG, *gyrA* D87N: GAC-AAC, *parC* S80I: AGC-ATC, *parC* S80R:AGC-AGA, *parC* E84V:GAA-GTA, *parE* L416F: CTT-TTT, *parE* I529L:ATT-CTT.

[7] Virulence determinants: *afaA*: transcriptional regulator; *afaB*: periplasmic chaperone; *afaC*: Outer membrane usher protein; *afaD*: afimbrial adhesin; *afaE*: adhesin protein; *air*: enteroaggregative immunoglobulin repeat protein; *cba*: colicin B; *capU*: hexosyltransferase homolog; *cea*: colicin E1; *chuA*: outer membrane hemin receptor; *cia*: colicin ia; *cib*: Colicin ib.; *clb*: hybrid non-ribosomal peptide / polyketide megasynthase; *cma*: colicin M; *cnf1*: cytotoxic necrotizing factor; *cvaC*: microcin C; *eilA*: *Salmonella* HilA homolog; *etsC*: putative type I secretion outer membrane protein; *fyuA*: siderophore receptor; *gad*: glutamate decarboxylase; *hlyF*: hemolysin F; *hra*: heat-resistant agglutinin; *ibeA*: invasin of brain endothelial cells; *iha*: adherence protein; *iroN*: enterobactin siderophore receptor protein; *ireA*: siderophore receptor; *irp2*: high molecular weight protein 2 non-ribosomal peptide synthetase; *iss*: increased serum survival; *iucC*: aerobactin synthetase; *iutA*: ferric aerobactin receptor; *kpsE*: capsule polysaccharide export inner-membrane protein; *kpsMII_K1*: polysialic acid transport protein group 2 capsule; *kpsMII_K5*: polysialic acid transport protein, Group 2 capsule; *kpsMII_K52*: polysialic acid transport protein, Group 2 capsule; *lpfA*: long polar fimbriae; *mchB*: microcin H47 part of colicin H; *mchC*: MchC protein; *mchF*: ABC transporter protein MchF; *mcmA*: Microcin M part of colicin H; *neuC*: polysialic acid capsule biosynthesis protein; *ompT*: outer membrane protease (protein protease 7); *papA_F9*: Major pilin subunit F9; *papA_feiA_F18*: major pilin subunit F8; *papA_F11*: major pilin subunit F11; *papA_F14*: major pilin subunit F14; *papA_F20*: major pilin subunit F20; *papA_F43*: major pilin subunit F43; *papC*: outer membrane usher P fimbriae; *pic*: serine protease autotransporters of Enterobacteriaceae (SPATE); *sat*: secreted autotransporter toxin; *senB*: plasmid-encoded enterotoxin; *sitA*: iron transport protein; *terC*: tellurium ion resistance protein; *traT*: Outer membrane protein complement resistance; *tsh*: temperature-sensitive hemagglutinin; *usp*: uropathogenic specific protein; *vat*: vacuolating autotransporter toxin; *yfcV*: fimbrial protein.

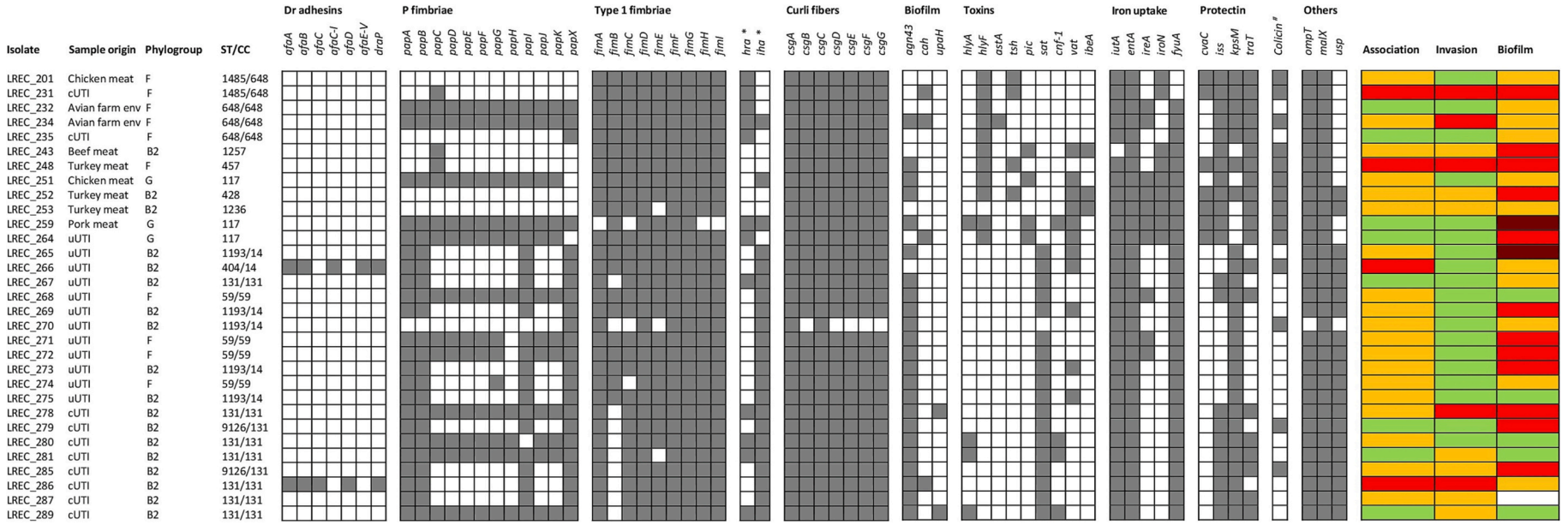

Fig. 1. Genomic traits associated with UPEC found within the studied *E. coli* collection and correlation with bladder-cell infection and biofilm assays. The most relevant virulence genes associated with extraintestinal pathogenic *E. coli* [40] and detected by WGS using VirulenceFinder and VFDB databases are indicated in grey (presence) and white (absence). *Genes categorized as adhesins. #In grey when at least one colicin-encoding gene was detected. Different categories of association, invasion and biofilm formation are depicted in different shades: low (green), moderate (orange), strong (light red), no biofilm-producer (white) and hyper biofilm producer (dark red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

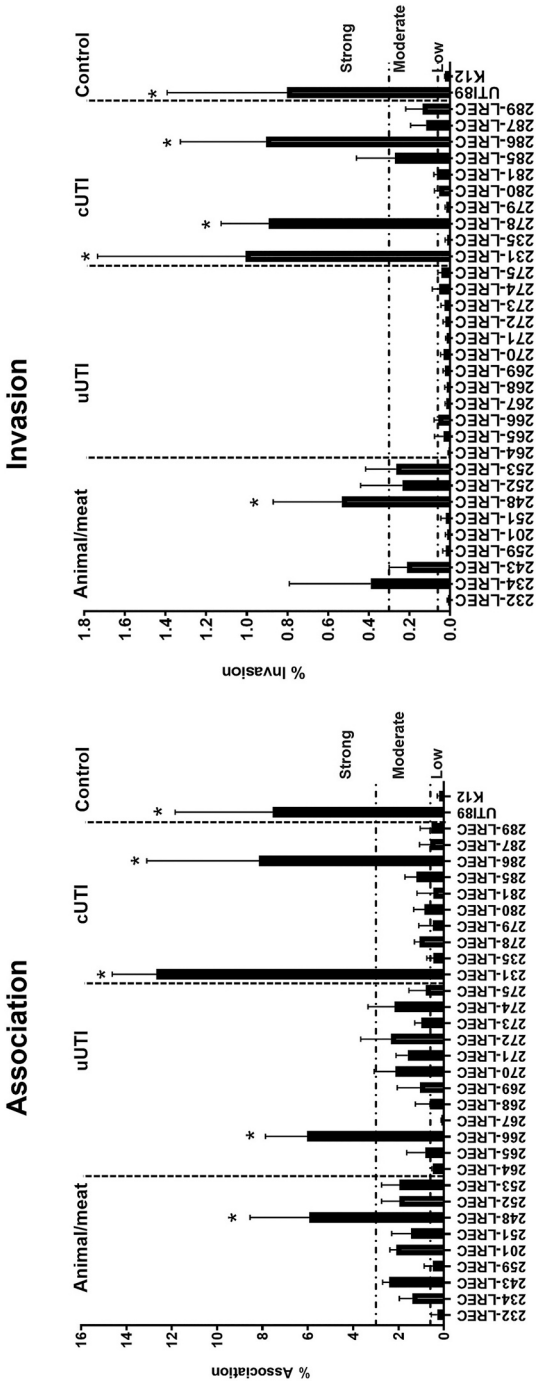

Fig. 2. Percentage and standard deviation (SD) for *E. coli* association and invasion of J82 epithelial bladder cells. Vertical dashed lines separate the isolates according to their origin. Horizontal broken lines indicate the low, moderate, and strong association or invasion established for classification of the isolates. The data represent means and SD of at least 3 replicates. *Statistical significance ($P < 0.05$) using one-way ANOVA followed by Dunnett's in comparison with K12.

hyperproducers in comparison with *E. coli* K12 (Fig. 3), namely, 1 pork meat O119:H4-G-ST117 (LREC-259) and 1 uUTI O75:H5-B2-ST1193 (LREC-265) isolates. Although we did not observe a unique genotypic profile associated with these strains regarding biofilm production, they showed common carriage of flagella, curli fibers (*csgBA* and *csgDEFG*) operons, *agn43* and *iha* (adherence protein), besides P-fimbriae (in LREC-259) and type-1 fimbriae operon (in LREC-265). There could be other biofilm-formation genes implicated, but not detected here, such as those of lipopolysaccharide biosynthesis or required for the integrity of outer membrane [47]. The remaining collection was classified as weak (5 isolates, 16%), moderate (11, 35%) or strong (12, 38%) biofilm producers.

Comparing results across different studies may be difficult, as the biofilm production greatly depends on the culture medium and assayed conditions [40,57]. While some authors reported biofilm-formation [41], and strong ability to form biofilm [43] in ExPEC poultry isolates, the results differed in other studies [40,55].

3.4. Population structure, uropathogenicity and multidrug-resistance

No correlation was observed here between cell infection capacity and origin or ST. Moreover, our results suggest similar *in vitro* behaviour for isolates of both origins, human and animal. Thus, we observed moderate or strong values of adhesion for 73% isolates of animal origin, 83% uUTI, 62.5% cUTI; of invasion for 54.5%, 0%, 75% (animal, uUTI, cUTI, respectively); of biofilm formation for 100%, 83%, 50% (animal, uUTI, cUTI, respectively).

Comparisons between the ability to associate and/or invade bladder cells and the biofilm production revealed only a positive correlation between cell association and biofilm formation for isolates belonging to phylogroup F (Spearman $r = 0.75$, $p < 0.05$) (Supplementary material, Fig. 3). Notably, 1 turkey meat isolate O11:H6-F-ST457, and 2 cUTI isolates belonging to pandemic lineages [17] O83:H42-F-ST1485-CC648 and O25b:H4-B2-ST131, showed strong association, invasion and biofilm formation (Figs. 1 and 2). These isolates showed common carriage of complete type 1 fimbriae and *csg* operons, toxins (*hlyF*, *tsh*), iron uptake systems (*iutA*, *entA*, *iroN*), colicins, protectins (*cvaC*, *iss*, *kpsM*, *traT*), *ompT*, and *malX*. A recent study analysed in-depth chicken-source isolates of phylogroup F (including ST59, ST457 and ST648). The authors performed, among others, biofilm-formation assays, and pathogenicity in animal models to demonstrate not only their ability to cause UTI but also sepsis and meningitis [58]. In agreement with this study, and despite the variability observed, our 10 isolates of phylogroup F showed biofilm-formation and bladder-cell infection ability.

Most of the human isolates analysed here belong to the pandemic ExPEC lineages CC131 and ST1193 [17], which share common features such as phylogroup B2 and fluoroquinolone resistance. While CC131 is recognized as the predominant clonal group causing UTI and bloodstream infections and its pathogenicity has been widely investigated [59–62], the new emerging ST1193 clone needs to be analysed in-depth. Our 5 O75/ONT:H5-B2-ST1193 isolates showed moderate association and low invasion capability, and all formed measurable biofilm (80% moderate or strong, including 1 isolate standing out as hyperproducer). Huang et al., [62] analysed 15 clinical ST1193 isolates, which exhibited association and invasion to bladder cancer T24 cells, and most (93%) formed biofilm. However, only 27% were reported as moderate or strong producers. As mentioned above, discrepancies might be derived by different assay conditions, namely, LB in our study and Todd-Hewitt broth in Huang et al., [62]. Besides, the authors reported similar pathogenicity among ST1193 and ST131 isolates [62], in contrast to the variability detected here for CC131 in our infection assays, which could be consistent with the genetic heterogenicity of this clonal group [60]. Indeed, our CC131 isolates displayed 5 different virotypes (A, C2, D3, E and F), based on the presence/absence of specific virulence genes [59,60], which in previous studies [60] correlated with different *in vivo* virulence patters using the murine model of sepsis [60].

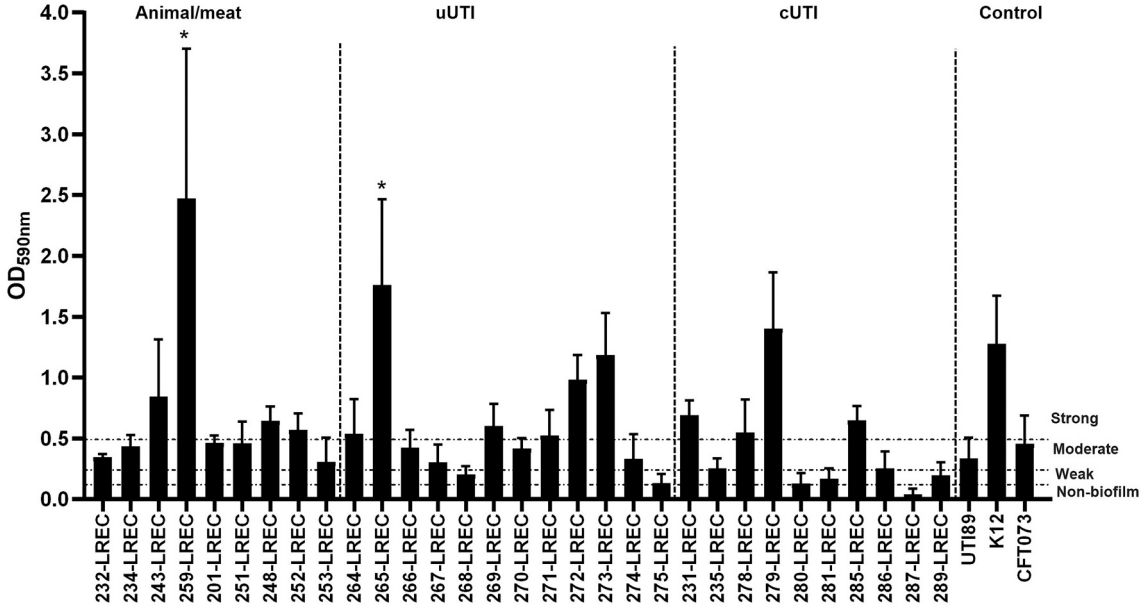

Fig. 3. Biofilm formation of *E. coli* isolates. Biofilm formation was assessed during growth at 37 °C in LB. Vertical dashed lines separate the isolates according to their origin. Horizontal broken lines indicate non-biofilm, weak, moderate and strong biofilm production established for classification of the isolates. Values represents the mean OD_{590} and standard desviation of 6 replicates. *Statistical significance ($p < 0.05$) using two-tailed Kruskal-Wallis in comparison with K12 showing hyper-producer isolates.

The MDR determined in 17 isolates (55%), including 14 fluoroquinolone-resistant (FQR) and 12 ESBL-producers, did not correlated with a higher biofilm production. Thus, 71% and 93% of MDR and non-MDR isolates, respectively, exhibited strong or moderate biofilm formation ($p > 0.05$).

Poultry meat as UPEC reservoir has been extensively described, however, pork and beef meat have not been analysed in-depth so far. Here, 1 pork (O119:H4-G-ST117) and 1 beef meat (O8:H10-B2-ST1257) isolates showed low and moderate ability to infect bladder cells, together with hyper and strong biofilm-production, respectively. This finding would indicate that other food-producing animals different from poultry can carry potentially UPEC high-risk clones, which deserves further surveillance.

4. Conclusions

Our results suggest similar *in vitro* behaviour for certain *E. coli* clones of animal origin positive for the UPEC status, compared to human UTI isolates, which reinforces the role of food-producing animals as a potential source of UPEC for consumers.

Bladder-cell infection assays, together with genomics, might be of utility to predict the infection severity of human *E. coli* isolates and the potential pathogenicity of animal-derived sources. Future studies assessing a larger set of *E. coli* isolates are required to validate the hypothesis that the combination of phylogenetic group, the set of UPEC status-associated genes, type 1 fimbriae and *csg* operons, iron uptake systems, colicins, protectins (*iss*, *kpsM*) may be preeminent traits for uropathogenicity prediction, as observed here.

Nucleotide sequence accession numbers

The nucleotide sequences of the isolates were deposited in the European Nucleotide Archive (ENA) and are part of BioProject IDs PRJNA558228, PRJEB49681, PRJEB55215 and PRJEB55220.

Funding

This study was supported by the projects and funds PID2019-104439RB-C21/AEI/10.13039/501100011033 from the Agencia Estatal de Investigación (AEI, Spain), co-funded by the European Regional Development Fund of the European Union: a Way to Make Europe (ERDF); ED431C 2021/11 and ED431B 2020/13 from the Consellería de Cultura, Educación e Ordenación Universitaria (Xunta de Galicia) and ERDF. GRUPIN IDI/2022/000033 by the Regional Ministry of Science of Asturias (IDI/2022/000033). Co-funded by Independent Research Found Denmark (grant number 4184–00050) to the project Combining non-essential metabolic targets into suitable targets for control of uropathogenic *Escherichia coli*.

Ethical approval

Not required.

CRediT authorship contribution statement

Vanesa García: Investigation, Data curation, Formal analysis, Validation, Visualization, Writing – original draft, Writing – review & editing. **Luz Lestón:** Investigation, Data curation, Formal analysis. **Ana Parga:** Investigation, Data curation, Formal analysis. **Isidro García-Meniño:** Investigation, Writing – review & editing. **Javier Fernández:** Funding acquisition, Resources, Writing – review & editing. **Ana Otero:** Methodology, Formal analysis, Supervision, Funding acquisition, Resources. **John E. Olsen:** Funding acquisition, Resources, Supervision, Writing – review & editing. **Ana Herrero-Fresno:** Methodology, Formal analysis, Validation, Supervision, Writing – review & editing. **Azucena Mora:** Conceptualization, Methodology, Funding acquisition, Resources, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

All authors declare no conflicts of interest.

Data availability

Data will be made available on request.

Acknowledgements

VG and IG-M acknowledge the Consellería de Cultura, Educación e Ordenación Universitaria, Xunta de Galicia for their post-doctoral grants (Grant Number ED481D-2022-012 and ED481B-2021-006, respectively). LL acknowledges the Ministry of Education of Spain for her pre-doctoral Grant FPU19/01127. AP acknowledges the Regional Government of Galicia for her pre-doctoral grant (ED481A-2019/194).

The authors would like to thank AllGenetics & Biology SL (www.allgenetics.eu) for supporting us with the genomic analysis service.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2023.100558.

References

[1] A.L. Flores-Mireles, J.N. Walker, M. Caparon, S.J. Hultgren, Urinary tract infections: epidemiology, mechanisms of infection and treatment options, Nat. Rev. Microbiol. 13 (5) (2015) 269–284, https://doi.org/10.1038/nrmicro3432.

[2] R. Mann, D.G. Mediati, I.G. Duggin, E.J. Harry, A.L. Bottomley, Metabolic adaptations of Uropathogenic *E. coli* in the urinary tract, Front. Cell. Infect. Microbiol. 7 (2017) 241, https://doi.org/10.3389/fcimb.2017.00241.

[3] F.M.E. Wagenlehner, T.E. Bjerklund Johansen, T. Cai, B. Koves, J. Kranz, A. Pilatz, et al., Epidemiology, definition and treatment of complicated urinary tract infections, Nat. Rev. Urol. 17 (10) (2020) 586–600, https://doi.org/10.1038/s41585-020-0362-4.

[4] S.E. Geerlings, Clinical presentations and epidemiology of urinary tract infections, Microbiol. Spectr. 4 (5) (2016), https://doi.org/10.1128/microbiolspec.UTI-0002-2012.

[5] T.A. Russo, J.R. Johnson, Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC, J. Infect. Dis. 181 (5) (2000) 1753–1754, https://doi.org/10.1086/315418.

[6] J.B. Kaper, J.P. Nataro, H.L. Mobley, Pathogenic *Escherichia coli*, Nat. Rev. Microbiol. 2 (2) (2004) 123–140, https://doi.org/10.1038/nrmicro818.

[7] M. Biggel, B.B. Xavier, J.R. Johnson, K.L. Nielsen, N. Frimodt-Møller, V. Matheeussen, et al., Horizontally acquired papGII-containing pathogenicity islands underlie the emergence of invasive uropathogenic *Escherichia coli* lineages, Nat. Commun. 11 (1) (2020) 5968, https://doi.org/10.1038/s41467-020-19714-9.

[8] R.R. Spurbeck, P.C. Dinh Jr., S.T. Walk, A.E. Stapleton, T.M. Hooton, L.K. Nolan, et al., *Escherichia coli* isolates that carry *vat, fyuA, chuA*, and *yfcV* efficiently colonize the urinary tract, Infect. Immun. 80 (12) (2012) 4115–4122, https://doi.org/10.1128/IAI.00752-12.

[9] I. García-Meniño, P. Lumbreras, L. Lestón, M. Álvarez-Álvarez, V. García, J. A. Hammerl, et al., Occurrence and genomic characterization of clone ST1193 Clonotype 14-64 in uncomplicated urinary tract infections caused by *Escherichia coli* in Spain, Microbiol. Spectr. 10 (3) (2022), e0004122, https://doi.org/10.1128/spectrum.00041-22.

[10] A.R. Manges, *Escherichia coli* and urinary tract infections: the role of poultry-meat, Clin. Microbiol. Infect. 22 (2) (2016) 122–129, https://doi.org/10.1016/j.cmi.2015.11.010.

[11] L. Jakobsen, P. Garneau, G. Bruant, J. Harel, S.S. Olsen, L.J. Porsbo, et al., Is *Escherichia coli* urinary tract infection a zoonosis? Proof of direct link with production animals and meat, Eur. J. Clin. Microbiol. Infect. Dis. 31 (6) (2012) 1121–1129, https://doi.org/10.1007/s10096-011-1417-5.

[12] A. Mora, C. López, G. Dabhi, M. Blanco, J.E. Blanco, M.P. Alonso, et al., Extraintestinal pathogenic *Escherichia coli* O1:K1:H7/NM from human and avian origin: detection of clonal groups B2 ST95 and D ST59 with different host distribution, BMC Microbiol. 9 (2009) 132, https://doi.org/10.1186/1471-2180-9-132.

[13] A. Mora, S. Viso, C. López, M.P. Alonso, F. García-Garrote, G. Dabhi, et al., Poultry as reservoir for extraintestinal pathogenic *Escherichia coli* O45:K1:H7-B2-ST95 in humans, Vet. Microbiol. 167 (3–4) (2013) 506–512, https://doi.org/10.1016/j.vetmic.2013.08.007.

[14] R. Yamaji, C.R. Friedman, J. Rubin, J. Suh, E. Thys, P. McDermott, et al., A population-based surveillance study of shared genotypes of *Escherichia coli* isolates from retail meat and suspected cases of urinary tract infections, mSphere. 3 (4) (2018), https://doi.org/10.1128/mSphere.00179-18.

[15] D. Díaz-Jiménez, I. García-Meniño, J. Fernández, V. García, A. Mora, Chicken and Turkey meat: consumer exposure to multidrug-resistant Enterobacteriaceae including *mcr*-carriers, uropathogenic *E. coli* and high-risk lineages such as ST131, Int. J. Food Microbiol. 331 (2020), 108750, https://doi.org/10.1016/j.ijfoodmicro.2020.108750.

[16] D. Díaz-Jiménez, I. García-Meniño, A. Herrera, L. Lestón, A. Mora, Microbiological risk assessment of Turkey and chicken meat for consumer: significant differences regarding multidrug resistance, *mcr* or presence of hybrid aEPEC/ExPEC pathotypes of *E. coli*, Food Control (2021) 123, https://doi.org/10.1016/j.foodcont.2020.107713.

[17] A.R. Manges, H.M. Geum, A. Guo, T.J. Edens, C.D. Fibke, J.D.D. Pitout, Global Extraintestinal pathogenic *Escherichia coli* (ExPEC) lineages, Clin. Microbiol. Rev. 32 (3) (2019), https://doi.org/10.1128/CMR.00135-18.

[18] V. Gómez Gómez, Caracterización de cepas de origen aviar de *Escherichia coli* productoras de betalactamasas de espectro extendido y del grupo clonal de alto riesgo ST131, University of Santiago de Compostela, 2017. https://minerva.usc.es/xmlui/handle/10347/15479.

[19] F.R. Blattner, G. Plunkett, C.A. Bloch, N.T. Perna, V. Burland, M. Riley, et al., The complete genome sequence of *Escherichia coli* K-12, Science. 277 (5331) (1997) 1453–1462, https://doi.org/10.1126/science.277.5331.1453.

[20] S.L. Chen, C.S. Hung, J. Xu, C.S. Reigstad, V. Magrini, A. Sabo, et al., Identification of genes subject to positive selection in uropathogenic strains of *Escherichia coli*: a comparative genomics approach, Proc. Natl. Acad. Sci. U. S. A. 103 (15) (2006) 5977–5982, https://doi.org/10.1073/pnas.0600938103.

[21] H.L. Mobley, D.M. Green, A.L. Trifillis, D.E. Johnson, G.R. Chippendale, C. V. Lockatell, et al., Pyelonephritogenic *Escherichia coli* and killing of cultured human renal proximal tubular epithelial cells: role of hemolysin in some strains, Infect. Immun. 58 (5) (1990) 1281–1289, https://doi.org/10.1128/iai.58.5.1281-1289.1990.

[22] O. Clermont, J.K. Christenson, E. Denamur, D.M. Gordon, The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups, Environ. Microbiol. Rep. 5 (1) (2013) 58–65, https://doi.org/10.1111/1758-2229.12019.

[23] T. Wirth, D. Falush, R. Lan, F. Colles, P. Mensa, L.H. Wieler, et al., Sex and virulence in *Escherichia coli*: an evolutionary perspective, Mol. Microbiol. 60 (5) (2006) 1136–1151, https://doi.org/10.1111/j.1365-2958.2006.05172.x.

[24] R.R. Wick, L.M. Judd, C.L. Gorrie, K.E. Holt, Unicycler: resolving bacterial genome assemblies from short and long sequencing reads, PLoS Comput. Biol. 13 (6) (2017), e1005595, https://doi.org/10.1371/journal.pcbi.1005595.

[25] A. Bankevich, S. Nurk, D. Antipov, A.A. Gurevich, M. Dvorkin, A.S. Kulikov, et al., SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing, J. Comput. Biol. 19 (5) (2012) 455–477.

[26] K.G. Joensen, A.M. Tetzschner, A. Iguchi, F.M. Aarestrup, F. Scheutz, Rapid and easy in silico serotyping of *Escherichia coli* isolates by use of whole-genome sequencing data, J. Clin. Microbiol. 53 (8) (2015) 2410–2426, https://doi.org/10.1128/JCM.00008-15.

[27] L. Roer, T.B. Johannesen, F. Hansen, M. Stegger, V. Tchesnokova, E. Sokurenko, et al., CHTyper, a web tool for subtyping of Extraintestinal pathogenic *Escherichia coli* based on the *fumC* and *fimH* alleles, J. Clin. Microbiol. 56 (4) (2018), https://doi.org/10.1128/JCM.00063-18.

[28] F. Jaureguy, L. Landraud, V. Passet, L. Diancourt, E. Frapy, G. Guigon, et al., Phylogenetic and genomic diversity of human bacteremic *Escherichia coli* strains, BMC Genomics 9 (2008) 560, https://doi.org/10.1186/1471-2164-9-560.

[29] P.T.L.C. Clausen, F.M. Aarestrup, O. Lund, Rapid and precise alignment of raw reads against redundant databases with KMA, BMC Bioinformat. 19 (1) (2018) 307, https://doi.org/10.1186/s12859-018-2336-6.

[30] C. Camacho, G. Coulouris, V. Avagyan, N. Ma, J. Papadopoulos, K. Bealer, et al., BLAST+: architecture and applications, BMC Bioinformat. 10 (2009) 421, https://doi.org/10.1186/1471-2105-10-421.

[31] E. Zankari, R. Allesøe, K.G. Joensen, L.M. Cavaco, O. Lund, F.M. Aarestrup, PointFinder: a novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens, J. Antimicrob. Chemother. 72 (10) (2017) 2764–2768, https://doi.org/10.1093/jac/dkx217.

[32] V. Bortolaia, R.S. Kaas, E. Ruppe, M.C. Roberts, S. Schwarz, V. Cattoir, et al., ResFinder 4.0 for predictions of phenotypes from genotypes, J. Antimicrob. Chemother. 75 (12) (2020) 3491–3500, https://doi.org/10.1093/jac/dkaa345.

[33] B. Liu, D. Zheng, Q. Jin, L. Chen, J. Yang, VFDB 2019: a comparative pathogenomic platform with an interactive web interface, Nucleic Acids Res. 47 (D1) (2019) D687–D692, https://doi.org/10.1093/nar/gky1080.

[34] K.G. Joensen, F. Scheutz, O. Lund, H. Hasman, R.S. Kaas, E.M. Nielsen, et al., Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*, J. Clin. Microbiol. 52 (5) (2014) 1501–1510, https://doi.org/10.1128/JCM.03617-13.

[35] A.M. Malberg Tetzschner, J.R. Johnson, B.D. Johnston, O. Lund, F. Scheutz, Genotyping of *Escherichia coli* isolates for Extraintestinal virulence genes by use of whole-genome sequencing data, J. Clin. Microbiol. 58 (10) (2020), https://doi.org/10.1128/JCM.01269-20.

[36] A.I.S. Andersen-Civil, S. Ahmed, P.R. Guerra, T.E. Andersen, Y.M.G. Hounmanou, J.E. Olsen, et al., The impact of inactivation of the purine biosynthesis genes, *purN* and *purT*, on growth and virulence in uropathogenic *E. coli*, Mol. Biol. Rep. 45 (6) (2018) 2707–2716, https://doi.org/10.1007/s11033-018-4441-z.

[37] A. Muras, C. Mayer, P. Otero-Casal, R.A.M. Exterkate, B.W. Brandt, W. Crielaard, et al., Short-chain N-Acylhomoserine lactone quorum-sensing molecules promote periodontal pathogens in in vitro Oral biofilms, Appl. Environ. Microbiol. 86 (3) (2020), https://doi.org/10.1128/AEM.01941-19.

[38] A. Muras, C. Mayer, M. Romero, T. Camino, M.D. Ferrer, A. Mira, et al., Inhibition of *Streptococcus mutants* biofilm formation by extracts of Tenacibaculum sp. 20J, a bacterium with wide-spectrum quorum quenching activity, J. Oral Microbiol. 10 (1) (2018) 1429788, https://doi.org/10.1080/20002297.2018.1429788.

[39] S. Stepanović, D. Vuković, V. Hola, G. Di Bonaventura, S. Djukić, I. Cirković, et al., Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci, APMIS. 115 (8) (2007) 891–899, https://doi.org/10.1111/j.1600-0463.2007.apm_630.x.

[40] M. Mellata, J.R. Johnson, R. Curtiss 3rd., *Escherichia coli* isolates from commercial chicken meat and eggs cause sepsis, meningitis and urinary tract infection in rodent models of human infections, Zoonoses Public Health 65 (1) (2017) 103–113, https://doi.org/10.1111/zph.12376.

[41] Z.R. Stromberg, J.R. Johnson, J.M. Fairbrother, J. Kilbourne, A. Van Goor, R. R. Curtiss, et al., Evaluation of *Escherichia coli* isolates from healthy chickens to determine their potential risk to poultry and human health, PLoS One 12 (7) (2017), e0180599, https://doi.org/10.1371/journal.pone.0180599.

[42] X. Xia, J. Meng, P.F. McDermott, S. Zhao, *Escherichia coli* from retail meats carry genes associated with uropathogenic *Escherichia coli*, but are weakly invasive in human bladder cell culture, J. Appl. Microbiol. 110 (5) (2011) 1166–1176, https://doi.org/10.1111/j.1365-2672.2011.04978.x.

[43] P.R. Meena, P. Yadav, H. Hemlata, K.K. Tejavath, A.P. Singh, Poultry-origin extraintestinal *Escherichia coli* strains carrying the traits associated with urinary tract infection, sepsis, meningitis and avian colibacillosis in India, J. Appl. Microbiol. 130 (6) (2021) 2087–2101, https://doi.org/10.1111/jam.14905.

[44] A.E. Shea, A.E. Frick-Cheng, S.N. Smith, H.L.T. Mobley, Phenotypic assessment of clinical *Escherichia coli* isolates as an Indicator for Uropathogenic potential, mSystems. 7 (6) (2022), e0082722, https://doi.org/10.1128/msystems.00827-22.

[45] A. Mora, M. Blanco, C. López, R. Mamani, J.E. Blanco, M.P. Alonso, et al., Emergence of clonal groups O1:HNM-D-ST59, O15:H1-D-ST393, O20:H34/HNM-D-ST354, O25b:H4-B2-ST131 and ONT:H21,42-B1-ST101 among CTX-M-14-producing *Escherichia coli* clinical isolates in Galicia, Northwest Spain, Int. J. Antimicrob. Agents 37 (1) (2011) 16–21, https://doi.org/10.1016/j.ijantimicag.2010.09.012.

[46] A.P. Magiorakos, A. Srinivasan, R.B. Carey, Y. Carmeli, M.E. Falagas, C.G. Giske, et al., Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance, Clin. Microbiol. Infect. 18 (3) (2012) 268–281, https://doi.org/10.1111/j.1469-0691.2011.03570.x.

[47] E.T. Niba, Y. Naka, M. Nagase, H. Mori, M. Kitakawa, A genome-wide approach to identify the genes involved in biofilm formation in *E. coli*, DNA Res. 14 (6) (2007) 237–246, https://doi.org/10.1093/dnares/dsm024.

[48] M.C. Lane, C.J. Alteri, S.N. Smith, H.L. Mobley, Expression of flagella is coincident with uropathogenic *Escherichia coli* Ascension to the upper urinary tract, Proc. Natl. Acad. Sci. U. S. A. 104 (42) (2007) 16669–16674, https://doi.org/10.1073/pnas.0607898104.

[49] S. Subashchandrabose, H.L.T. Mobley, Virulence and fitness determinants of Uropathogenic *Escherichia coli*, Microbiol. Spectr. 3 (4) (2015), https://doi.org/10.1128/microbiolspec.UTI-0015-2012.

[50] G. Sharma, S. Sharma, P. Sharma, D. Chandola, S. Dang, S. Gupta, et al., *Escherichia coli* biofilm: development and therapeutic strategies, J. Appl. Microbiol. 121 (2) (2016) 309–319, https://doi.org/10.1111/jam.13078.

[51] P.I. Tarr, S.S. Bilge, J.C. Vary, S. Jelacic, R.L. Habeeb, T.R. Ward, et al., Iha: a novel *Escherichia coli* O157:H7 adherence-conferring molecule encoded on a recently acquired chromosomal island of conserved structure, Infect. Immun. 68 (3) (2000) 1400–1407, https://doi.org/10.1128/iai.68.3.1400-1407.2000.

[52] A.G. Torres, N.T. Perna, V. Burland, A. Ruknudin, F.R. Blattner, J.B. Kaper, Characterization of Cah, a calcium-binding and heat-extractable autotransporter protein of enterohaemorrhagic *Escherichia coli*, Mol. Microbiol. 45 (4) (2002) 951–966, https://doi.org/10.1046/j.1365-2958.2002.03094.x.

[53] L.P. Allsopp, M. Totsika, J.J. Tree, G.C. Ulett, A.N. Mabbett, T.J. Wells, et al., UpaH is a newly identified autotransporter protein that contributes to biofilm formation and bladder colonization by uropathogenic *Escherichia coli* CFT073, Infect. Immun. 78 (4) (2010) 1659–1669, https://doi.org/10.1128/IAI.01010-09.

[54] A.E. Barber, J.P. Norton, T.J. Wiles, M.A. Mulvey, Strengths and limitations of model Systems for the Study of urinary tract infections and related pathologies, Microbiol. Mol. Biol. Rev. 80 (2) (2016) 351–367, https://doi.org/10.1128/MMBR.00067-15.

[55] M.L. Buberg, S.S. Mo, C. Sekse, M. Sunde, Y. Wasteson, I.L. Witsø, Population structure and uropathogenic potential of extended-spectrum cephalosporin-resistant *Escherichia coli* from retail chicken meat, BMC Microbiol. 21 (1) (2021) 94, https://doi.org/10.1186/s12866-021-02160-y.

[56] A. Shea, A. Frick-Cheng, S. Smith, H. Mobley, Phenotypic assessment of clinical *Escherichia coli* isolates predicts Uropathogenic potential, mSystems 7 (6) (2022), e0082722, https://doi.org/10.1128/msystems.00827-22.

[57] P. Naves, G. del Prado, L. Huelves, M. Gracia, V. Ruiz, J. Blanco, et al., Measurement of biofilm formation by clinical isolates of *Escherichia coli* is method-dependent, J. Appl. Microbiol. 105 (2) (2008) 585–590, https://doi.org/10.1111/j.1365-2672.2008.03791.x.

[58] X. Zhuge, Z. Zhou, M. Jiang, Z. Wang, Y. Sun, F. Tang, et al., Chicken-source *Escherichia coli* within phylogroup F shares virulence genotypes and is closely related to extraintestinal pathogenic *E. coli* causing human infections, Transbound. Emerg. Dis. 68 (2) (2021) 880–895, https://doi.org/10.1111/tbed.13755.

[59] G. Dahbi, A. Mora, R. Mamani, C. López, M.P. Alonso, J. Marzoa, et al., Molecular epidemiology and virulence of *Escherichia coli* O16:H5-ST131: comparison with H30 and H30-Rx subclones of O25b:H4-ST131, Int. J. Med. Microbiol. 304 (8) (2014) 1247–1257, https://doi.org/10.1016/j.ijmm.2014.10.002.

[60] A. Mora, G. Dahbi, C. López, R. Mamani, J. Marzoa, S. Dion, et al., Virulence patterns in a murine sepsis model of ST131 *Escherichia coli* clinical isolates belonging to serotypes O25b:H4 and O16:H5 are associated to specific virotypes, PLoS One 9 (1) (2014), e87025, https://doi.org/10.1371/journal.pone.0087025.

[61] S.C. Flament-Simon, M.H. Nicolas-Chanoine, V. García, M. Duprilot, N. Mayer, M. P. Alonso, et al., Clonal structure, virulence factor-encoding genes and antibiotic resistance of *Escherichia coli*, causing urinary tract infections and other Extraintestinal infections in humans in Spain and France during 2016, Antibiotics (Basel). 9 (4) (2020), https://doi.org/10.3390/antibiotics9040161.

[62] J. Huang, S. Zhang, Z. Zhao, Y. Cao, M. Chen, B. Li, A comparative study of fluoroquinolone-resistant *Escherichia coli* lineages portrays indistinguishable pathogenicity- and survivability-associated phenotypic characteristics between ST1193 and ST131, Infect. Drug Resist. 13 (2020) 4167–4175, https://doi.org/10.2147/IDR.S277681.