Genotypic characteristics of Uropathogenic *Escherichia coli* isolated from complicated urinary tract infection (cUTI) and asymptomatic bacteriuria—a relational analysis

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ABSTRACT

Background. Uropathogenic *Escherichia coli* (UPEC) is the predominant agent causing various categories of complicated urinary tract infections (cUTI). Although existing data reveals that UPEC harboured numerous virulence determinants to aid its survival in the urinary tract, the reason behind the occurrence of differences in the clinical severity of uninary tract infections (UTI) demonstrated by the UPEC infection is poorly understood. Therefore, the present study aims to determine the distribution of virulence determinants and antimicrobial resistance among different phylogroups of UPEC isolated from various clinical categories of cUTI and asymptomatic bacteriuria (ASB) *E. coli* isolates. The study will also attempt a relational analysis of the genotypic characteristics of cUTI UPEC and ASB *E. coli* isolates.

Methods. A total of 141 UPEC isolates from cUTI and 160 ASB *E. coli* isolates were obtained from Universiti Malaya Medical Centre (UMMC). Phylogrouping and the occurrence of virulence genes were investigated using polymerase chain reaction (PCR). Antimicrobial susceptibility of the isolates to different classes of antibiotics was determined using the Kirby Bauer Disc Diffusion method.

Results. The cUTI isolates were distributed differentially among both Extraintestinal Pathogenic *E. coli* (ExPEC) and non-ExPEC phylogroups. Phylogroup B2 isolates were observed to possess the highest average aggregative virulence score (7.17), a probable representation of the capability to cause severe disease. Approximately 50% of the cUTI isolates tested in this study were multidrug resistant against common antibiotics used to treat UTI. Analysis of the occurrence of virulence genes among different cUTI categories demonstrated that UPEC isolates of pyelonephritis and urosepsis were highly virulent and had the highest average aggregative virulence scores of 7.80 and 6.89 respectively, compared to other clinical categories. Relational analysis of the occurrence of phylogroups and virulence determinants of UPEC and ASB *E. coli* isolates showed

that 46.1% of UPEC and 34.3% of ASB *E. coli* from both categories were distributed in phylogroup B2 and had the highest average aggregative virulence score of 7.17 and 5.37, respectively. The data suggest that UPEC isolates which carry virulence genes from all four virulence genes groups studied (adhesions, iron uptake systems, toxins and capsule synthesis) and isolates from phylogroup B2 specifically could predispose to severe UTI involving the upper urinary tract. Therefore, specific analysis of the genotypic characteristics of UPEC could be further explored by incorporating the combination of virulence genes as a prognostic marker for predicting disease severity, in an attempt to propose a more evidence driven treatment decision-making for all UTI patients. This will go a long way in enhancing favourable therapeutic outcomes and reducing the antimicrobial resistance burden among UTI patients.

INTRODUCTION

Urinary tract infections (UTIs) are among the most common bacterial infections, affecting around 150 million people annually worldwide (*Chegini et al., 2021*; *Maniam et al., 2022*). Despite current advances in healthcare management, the incidence of both community and healthcare-associated UTIs remains higher and causes a significant burden on the management and healthcare expenditure every year (*Mann et al., 2017*). Complicated urinary tract infections (cUTI) are the UTI category associated with heterogeneous complicated comorbidities or conditions such as anatomical and functional abnormalities of the urinary tract, coexistence with immunosuppressing diseases, indwelling urinary catheter, pregnancy, being male in gender and recurrent infection (*Lodise et al., 2022*; *Sabih & Leslie, 2021*). In comparison with uncomplicated UTIs which are easy to be resolved with antibiotics, the treatment of cUTI often poses significant failure rates of up to 27% (*Eliakim-Raz et al., 2019*). In many circumstances, cUTI often results in recurrent infections, extended hospitalization and urosepsis which may lead to fatality (*Sabih & Leslie, 2021*).

According to the European Association of Urology (EAU) urological infection guidelines, cUTI can be grouped into a few categories such as cystitis, pyelonephritis, urosepsis, recurrent UTI, and catheter-associated UTI (*Bonkat et al., 2017*). In this study, the cUTI cases which could not be clinically classified into these five clinical categories were grouped into cUTI with co-morbidities. This category of cUTI with co-morbidities represents general cUTI isolates that could probably cause any of the defined cUTI clinical manifestations. Treating UTIs with antibiotics is common in practice. However, the emergence of antimicrobial resistance (AMR) among the causative agents will hamper the desired treatment outcome (*Dadgostar, 2019*). The prescription of broad-spectrum
antibiotics further enhances the emergence of multidrug resistance (MDR) organisms in the community (*Kot, 2019*).

Although various Gram-negative and Gram-positive bacterial species are known to cause all types of UTIs, the strains of *Escherichia coli* (*E. coli*) are the predominant agent responsible for more than 85% of all UTI categories (*Barber et al., 2016*; *Davari Abad, Khameneh & Vahedi, 2019*). *E. coli*, based on the outcome of its association with the host, can be categorized into three groups such as commensals, intestinal pathogenic *E. coli* (InPEC), and extraintestinal pathogenic *E. coli* (ExPEC) which causes UTI. A subgroup of this ExPEC associated with UTIs is known as uropathogenic *E. coli* (UPEC), which is very diverse in its pathogenic capability. UPEC harbours various virulence determinants such as adhesin (*fimH, papC, sfa, afa*), siderophore (*iroN, fyuA, iutA, chuA*), toxins (*cnf, hly, sat, vat, usp*) and capsule synthesis (*kpsMTII, ompT, traT*); encoded in different combinations in its pathogenicity islands (PAI), mobile genetic elements and plasmids (*Dadi et al., 2020*; *Rezatofighi, Mirzarazi & Salehi, 2021*; *Tanabe et al., 2022*). The acquisition of these virulence genes was observed to play an important role in the pathogenesis of UTI (*Terlizzi, Gribaudo & Maffei, 2017*). Typing the bacterial pathogen is the utmost important aspect in understanding the epidemiology of a disease and enabling diagnosis and prognosis of infectious diseases and their management (*Kotłowski et al., 2020*). Clermont's *E. coli* phylogrouping scheme is widely reported for isolates from various niches and hosts across literature, and was able to easily classify the *E. coli* strains in different groups which were shown to be congruent with results from multilocus sequence typing (MLST) (*Beghain et al., 2018*; *Clermont et al., 2013*). Phylogenetically, *E. coli* can be categorized into seven phylogroups; namely A, B1, B2, C, D, E, F and cryptic clade 1 (CC1) *Clermont et al., 2013*). *E. coli* isolated from UTI mostly belong to phylogroups B2 and D and to a lesser extent to phylogroups A and B1 (*Tanabe et al., 2022*). Analysis of the phylogroups combined with analysis of the occurrence of virulence genes in these phylogroups are expected to provide insights to pathogenicity of the UPEC, leading to better treatment decisions and outcomes.

Asymptomatic bacteriuria (ASB) represents bacterial colonization in the urinary tract without causing any clinical symptoms to the host (*Maniam et al., 2022*; *Roos et al., 2006*). Patients with ASB may carry the bacteria in their urinary tract, mimicking the communalistic existence with the host. The occurrence of ASB strains in the urinary tract was known to benefit the host by preventing the colonization of virulent strains (*Stork et al., 2018*). ASB state is not treated in healthy individuals except for pregnant women and patients with urological procedures. Data from the literature have revealed that ASB isolates also belonged to ExPEC phylogroups and possessed various UPEC-associated virulence genes such as *fimH*, *papC*, *iroN*, *chuA*, *kpsMTII*, *ompT*, and *usp*. The presence of the UPEC-associated virulence genes among ASB *E. coli* isolates indicate a greater risk of developing symptomatic infections in persons with ASB (*Maniam et al., 2022*).

The incidence of UTI's severity followed by developments of its associated complications were often associated with the anatomical site of infection, the integrity of the host defense mechanism and also pathogen-related factors in terms of virulence nature and MDR potential of the organism involved (*Tarchouna et al., 2013*). Early population-based studies on molecular characterization of UPEC reveal that isolates of UPEC are highly

heterogeneous in their virulence potential and the possession of these genes was often shown to increase its fitness in the urinary tract (*Lara et al., 2017*; *Rezatofighi, Mirzarazi & Salehi, 2021*; *Schifano et al., 2019*). Relational analysis of the occurrence of virulence genes in relation to phylogroups and cUTI clinical categories are not reported in the literature.

This study intended to determine (i) the distribution of virulence determinants and AMR among different phylogroups of UPEC isolated from clinically manifesting UTI cases; and (ii) relational analysis of the occurrence of phylogroups, virulence determinants and MDR UPEC isolates from different cUTI categories and ASB *E. coli* isolates. The findings may reveal the differential virulence potential of the UPEC isolated from various UTI manifestations and ASB *E. coli*. An explicit understanding of the virulence potential of UPEC isolates from different cUTI categories may aid in the development of new diagnostic algorithms, leading to better management and prognosis.

MATERIALS & METHODS

Ethics approval

Ethical approval for this study was obtained from the Universiti Malaya Medical Centre (UMMC) Medical research ethics committee (MRECID 201765-5313). All the cUTI *E. coli* isolates and ASB *E. coli* isolates used in this study were obtained between July 2017 and October 2019 from the Medical Microbiology Diagnostic Laboratory, Urology ward and Obstetrics and Gynecology Department of UMMC. Written informed consent was obtained before sample collection from patients. Ethical approval also included the collection of diagnosed and reported isolates of *E. coli* from Medical Microbiology Diagnostic Laboratory. UPEC isolates were grouped into cUTI clinical categories such as cystitis, pyelonephritis, urosepsis, recurrent UTI, and catheter associated-UTI (CAUTI) based on reported clinical evaluation. Isolates with undetermined cUTI category were grouped into a category 'cUTI with co-morbidities'. This category 'cUTI with co-morbidities' represents possible isolates that could cause any of the five defined clinical category manifestations. This was done to ensure the inclusion of all the clinical isolates in the study.

Identification of cUTI UPEC isolates

All the bacterial isolates were further confirmed using standard biochemical tests (*Odonkor & Ampofo, 2013*). *E. coli* isolates were stored frozen in Luria-Bertani (LB) (HiMedia, Maharashtra, India) broth containing 30% glycerol at −70 °C until further investigation. The identification was confirmed further by detecting the presence of 16s rRNA in *E. coli* isolates using conventional polymerase chain reaction (PCR) (*Huijsdens et al., 2002*).

Phylogenetic analysis

Bacterial DNA was extracted using the boiling method (*Tewawong et al., 2020*). Isolates were assigned to one of the eight phylogenetic groups of *E. coli* (A, B1, B2, C, D, E, F, CC1) by the quadruplex PCR assay as described by *Clermont et al. (2013)*.

Identification of virulence genes

A conventional PCR technique was used to detect 12 virulence genes that are associated with UPEC type 1 fimbriae (*fimH),* P fimbriae (*papC*), F1 C fimbriae (*sfa*), salmochelin receptor

(*iroN*), heme receptor (*chuA*), yersiniabactin receptor (*fyuA*), cytotoxic necrotizing factor (*cnf*), secreted autotransporter protein (*sat*), hemolysin (*hlyC*), outer membrane protein T (*ompT*), kps M group II capsule (*kpsMTII*) and uropathogen specific protein (*usp*), using specific primers (Table S1) (*Ejrnæs, 2011*; *Idress et al., 2010*; *Lara et al., 2017*; *Momtaz et al., 2013*; *Shreya Basu, Hazra & Mukherjee, 2013*; *Tabasi et al., 2016*; *Yun et al., 2014*). PCR was performed in a 25 µL reaction volume containing 5 µL of PCR master mix 5X (Promega, Madison, WI, USA), 18 µL of RNA-free water, 0.5 µL of forward and reverse primer (10 µmol) (Integrated DNA Technologies, Singapore), and 1 µL of template DNA. The PCR amplification was performed using the following conditions; initial denaturation at 95 °C for 5 min, 25 cycles of denaturation at 94 °C for 30 s, annealing at respective gene's temperature (Table S1) for 30 s, extension at 68 °C for 3 min and final extension at 72 °C for 10 min (CFX, 96, BioRad, Hercules, CA, USA). PCR products stained with SYBR safe dye (Invitrogen, Waltham, MA, USA) were resolved on 1.5% agarose gels by electrophoresis at 120 V for 60 min. On completion of the electrophoresis, the gel was visualized under UV-induced fluorescence.

Antibiotic sensitivity test

Kirby-Bauer disc diffusion method was used to determine the antimicrobial susceptibility profile of UPEC isolates used in this study. The methods and conditions were exactly the same as previously described in the work by *Maniam et al. (2022)*. Results obtained were interpreted according to Clinical Laboratory Standards International (CLSI) guidelines (*CLSI, 2018*). The antibiotics used for testing were: ampicillin (AMP) 10 µg, amikacin (AK) 30 µg, amoxicillin/clavulanate (AMC) 20/10 µg, ampicillin/sulbactam (SAM) 10/10 µg, aztreonam (ATM) 30 µg, cefuroxime (CXM) 30 µg, cefoxitin (FOX) 30 µg, cefotaxime (CTX) 30 µg, cefepime (FEP) 30 µg, ceftriaxone (CRO) 30 µg, imipenem (IPM) 10 µg, gentamicin (CN) 30 µg, norfloxacin (NOR) 30 µg, and piperacillin/tazobactam (TP) 100/10 µg. *E. coli* ATCC 25922 was used as the quality control strain in antimicrobial susceptibility determination (*Shahbazi et al., 2018*). A strain showing resistance to at least three antibiotic classes tested was considered an MDR strain (*Magiorakos et al., 2012*).

Phylogrouping, virulence gene detection and antibiotic susceptibility test of ASB *E. coli* isolates

The same methodology adopted for UPEC isolates was also used for phylogrouping, virulence gene detection and antibiotic susceptibility testing of ASB *E. coli* isolates, which was reported in another preliminary report published by the research group (*Maniam et al., 2022*).

Statistical analysis

Statistical analyses were performed using IBM Statistical Package for Social Sciences (SPSS) software (version 25.0). The Kruskal–Wallis test was used for the analysis of the occurrence of virulence determinants and antibiotic resistance among phylogroups of UPEC. Analysis of the occurrence of virulence determinants among different clinical categories was also performed through Kruskal–Wallis test. The Pearson Chi-square test was used to perform a comparison for the occurrence of virulent determinants between ExPEC with non-ExPEC

phylogroups of UPEC and for overall comparison between UPEC and ASB *E. coli* isolates. Bonferroni adjusted *p* value for multiple comparisons was used to identify the significant differences in occurrences.

The aggregative virulence score for the occurrence of virulence genes in this study was calculated by averaging the number of virulence genes identified in each strain among the phylogroups, virulence functional groups and cUTI categories (*Rezatofighi, Mirzarazi & Salehi, 2021*). The co-occurrence analysis of the virulence genes was performed using pair-wise correlation using the Phi coefficient (*Johnson et al., 2001a*; *Tabasi et al., 2016*).

RESULTS

Phylogenetic analysis of cUTI UPEC isolates

The study included a total of 141 UPEC isolates obtained. The phylogenetic analysis revealed that the majority of the cUTI UPEC isolates were from phylogroup B2 (46.1%), followed by group A (14.9%), group D (12.0%), group B1 (11.3%), group E (5.7%), group C (4.3%), group F (2.2%) and CC1 (0.7%). There were only four isolates (2.9%) which were non-typeable (NT) as per the classification proposed by *Clermont et al. (2013)*. The comparison of the distribution of phylogroups between cUTI UPEC and ASB *E. coli* is depicted in Fig. 1.

Distribution of virulence genes among phylogroups

Considering the cUTI UPEC isolates as a whole, the occurrence of the *fimH* (87.9%) gene was observed highest followed by *usp* (58.9%), *kpsMTII* (50.4%), *fyuA* (44.7%), *hlyA* (41.8%), *papC* (37.4%), *iroN* (36.2%), *ompT* (31.9%), *cnf* (29.8%), *sfa* (25.5%) and *sat* (12.8%) in descending order (Table 1). Analysis of the distribution of virulence genes among phylogroups revealed that UPEC from phylogroups B2 and E harboured all 12 virulence genes tested in this study in different permutations and combinations. It was observed that, there were significant differences (*p* value <0.004) for the occurrence of virulence genes among UPEC of ExPEC (B2, D, E and F) and non-ExPEC (A, B1 and C) for *fyuA*, *chuA*, *kpsMTII* and *usp* (Table 2). Analysis of the distribution of total virulence genes screened revealed that 43 (30.5%) isolates harboured at least three of the virulence genes tested, followed by 53 (37.6%) with four to six virulence genes and 29 (20.6%) with seven to nine virulence genes. There were 16 isolates of UPEC identified with 10 to 12 genes tested and the majority of these isolates (14 isolates) were from phylogroup B2. With regard to each of the virulence genes tested, there were significant differences identified in the presence of virulence genes among phylogroups for genes *sfa*, *papC*, *fyuA*, *chuA*, *ompT*, *kpsMTII* and *usp*. The occurrence of these genes was observed significantly higher among UPEC from phylogroup B2 (Table 1).

Antibiotic susceptibility test results

UPEC isolated from different UTI manifestations were tested against 14 antibiotics representing nine antimicrobial classes. Resistance observed was highest against AMP (85.8%), followed by CTX (66.7%), AMC (63.1%), AK (60.3%), GN (53.9), TP (51.1), FEP (49.6%), CXM (43.3%), SAM (41.1%), IPM (39.7), NOR (32.6%), CRO (31.9%) and
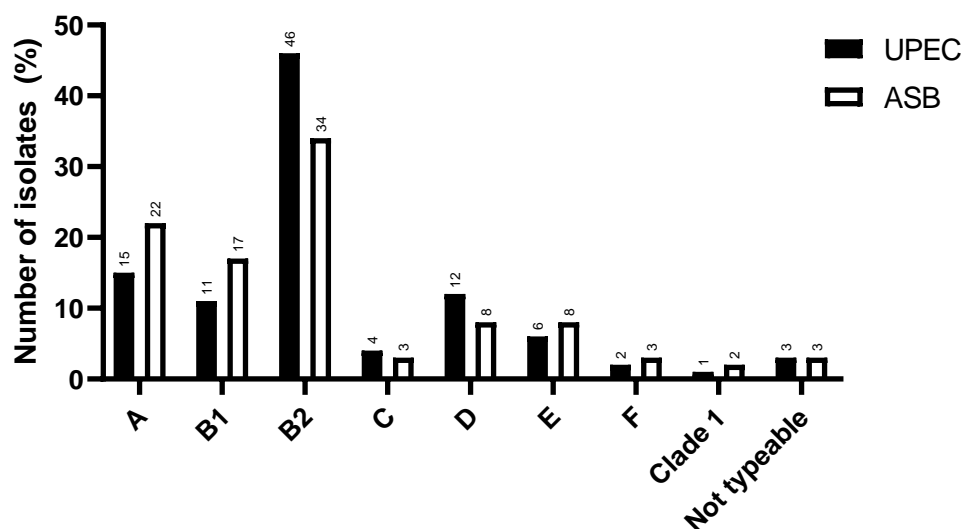
Figure 1 **Distribution of phylogroups among cUTI UPEC and ASB *E. coli* isolates.** Data for ASB *E. coli* isolates adapted and representation obtained from *Maniam et al. (2022)*.

ATM (29.1%) (Table 3). Among the antibiotics tested in this study, resistance to FOX was observed to be the lowest (12.1%). There was no statistical difference observed for the occurrence of antibiotic resistance among phylogroups. Of the 141 UPEC, 123 (87.2%) isolates exhibited resistance to at least one antimicrobial tested, while 52.5% of UPEC were identified as MDR.

Distribution of phylogroups, virulence genes and multidrug resistance among different cUTI categories

The data on the distribution of phylogroups, virulence genes, and MDR of UPEC isolated from different cUTI manifestations are depicted in Table 4. Analysis of the distribution of phylogroups among UPEC of various cUTI categories demonstrated a common trend of higher distribution of isolates from phylogroup B2. Among the cUTI categories, the occurrence of phylogroup B2 was observed highest among isolates of pyelonephritis, followed by urosepsis and cystitis. Isolates of CAUTI, and recurrent UTI exhibited higher diversity in the distribution of phylogroups, especially the occurrence of isolates from phylogroups C, E, F, and CC1. The assembled cUTI with co-morbidities category also showed the same trend with high diversity with the presence of isolates from all the phylogroups except for CC1.

Data from the analysis on the distribution of virulence genes among UPEC from different cUTI categories revealed that isolates from cystitis, urosepsis, recurrent UTI and CAUTI (also cUTI with co-morbidities category) harbour all virulence genes tested in this study. Isolates from pyelonephritis cases notably lacked *sat* gene. Of the different cUTI categories included, isolates of pyelonephritis and urosepsis showed higher occurrence for all the virulence genes tested in this study. UPEC isolates from pyelonephritis showed the highest occurrence for *fimH* (100%), *sfa* (100%), *papC* (90.0%), *chuA* (90.0%), *usp* (90.0%) and

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Table 1 **Distribution of uropathogenic *Escherichia coli* (UPEC) virulence genes among different phylogroups, *n* (%).**

Virulence genes	A	B1	B2	C	D	E	F	CC1	NT	Total	*p*-value
	21	16	65	6	17	8	3	1	4	141	
Adhesion											
fimH	15 (71.4)	15 (93.8)	60 (92.3)	6 (100)	14 (82.4)	7 (87.5)	2 (66.7)	1 (100)	4 (100)	124 (87.9)	0.245
sfa	3 (14.3)[ab]	3 (18.8)[ab]	27 (41.5)[a]	0[c]	0[c]	1 (12.5)[ab]	1 (33.3)[ab]	0[c]	1 (25.0)[ab]	36 (25.5)	0.013[*]
papC	5 (23.8)[ab]	5 (31.0)[ab]	38 (57.9)[a]	0[c]	5 (29.4)[ab]	2 (25.0)[ab]	0[c]	0[c]	1 (25.0)[ab]	56 (37.4)	0.029[*]
Siderophore											
iroN	7 (33.3)	2 (12.5)	30 (46.2)	3 (50.0)	4 (23.5)	1 (12.5)	2 (66.7)	0	2 (50.0)	51 (36.2)	0.134
fyuA	4 (19.0)[b]	2 (12.5)[b]	42 (64.6)[a]	2 (33.3)[ab]	9 (52.9)[ab]	2 (25.0)[ab]	0[b]	0[b]	2 (50.0)[ab]	63 (44.7)	<0.001[*]
chuA	0[b]	0[b]	65 (100)[a]	0[b]	17 (100)[a]	8 (100)[a]	8 (100)[a]	0[b]	0[b]	98 (69.4)	<0.001[*]
Toxin											
cnf	1 (4.8)[b]	2 (13.3)[b]	32 (49.3)[a]	2 (33.3)[ab]	2 (11.8)[b]	1 (12.5)[ab]	0[b]	0[b]	2 (50.0)[a]	42 (29.8)	<0.001[*]
hlyA	7 (33.3)	6 (37.5)	33 (50.8)	3 (50.0)	4 (23.5)	1 (12.5)	2 (66.7)	0	3 (75.0)	59 (41.8)	0.179
sat	1 (4.8)	0	10 (15.4)	1 (16.7)	3 (17.6)	2 (25.0)	0	0	1 (25.0)	18 (12.8)	0.582
usp	6 (28.6)[b]	6 (37.5)[b]	58 (89.2)[a]	2 (33.3)[b]	5 (29.40)[b]	5 (62.5)[ab]	0[b]	0[b]	1 (25.0)[b]	83 (58.9)	<0.001[*]
Capsule synthesis											
ompT	6 (28.6)	3 (18.8)	25 (38.5)	2 (33.3)	2 (11.8)	3 (37.5)	3 (100)	0	1 (25.0)	45 (31.9)	0.118
kpsMTII	4 (19.0)[b]	0[b]	47 (72.3)[a]	1 (16.7)[b]	12 (70.6)[a]	4 (50.0)[ab]	1 (33.3)[ab]	0[b]	2 (50.0)[ab]	71 (50.4)	<0.001[*]

Notes.

Group with different lowercase alphabets superscripted are statistically different based on pairwise comparison. Kruskal–Wallis test was used for statistical comparison and *p*-value <0.05[*] was considered as significant. Significance values have been adjusted by the Bonferroni correction for multiple comparisons.

Table 2 Comparison of virulence gene occurrence among the cUTI UPEC isolates and ASB *E. coli* isolates, (*n*) (%).

Virulence genes	cUTI UPEC ExPEC phylogroups n (%)	cUTI UPEC non- ExPEC phylogroups n (%)	p value	UPEC n (%)	ASB n (%)	p value
fimH	84 (67.7)	40 (32.3)	0.464	124 (87.9)	121 (75.6)	0.006
sfa	29 (80.6)	7 (19.4)	0.041	36 (25.5)	27 (16.9)	0.066
papC	45 (80.4)	11 (19.6)	0.005	56 (37.4)	33 (20.6)	<0.001[*]
iroN	37 (72.5)	14 (27.5)	0.265	51 (36.2)	39 (24.4)	0.026
fyuA	53 (84.1)	10 (15.9)	<0.001[*]	63 (44.7)	51 (31.9)	0.022
chuA	94 (95.9)	4 (5.1)	<0.001[*]	98 (69.4)	88 (55.0)	0.010
cnf	35 (83.3)	7 (16.7)	0.006	42 (29.8)	36 (22.5)	0.150
hlyA	40 (67.8)	19 (32.2)	0.809	59 (41.8)	44 (27.5)	0.009
sat	16 (88.9)	2 (11.1)	0.032	18 (12.8)	12 (7.5)	0.128
usp	68 (81.9)	15 (18.1)	<0.001[*]	83 (58.9)	55 (34.4)	0.001[*]
ompT	33 (73.3)	12 (26.7)	0.250	45 (31.9)	24 (15.0)	0.001[*]
kpsMTII	65 (91.5)	6 (8.5)	<0.001[*]	71 (50.4)	61 (38.1)	0.033

Notes.

Chi-square test was used for statistical comparison and *p*-value has been adjusted by the Bonferroni correction for multiple comparisons (*p* < 0.004[*]).

fyuA (50.0%). While, UPEC isolated from urosepsis exhibited the highest occurrence of *iroN* (62.5%), *cnf* (75.0%), *hlyA* (75.0%), *ompT* (62.5%) and *kpsMTII* (70.0%). The distribution of these virulence genes among the *other* cUTI categories such as cystitis, recurrent UTI and CAUTI (also cUTI with co-morbidities category) were moderate to low in comparison with isolates of pyelonephritis and urosepsis. Overall, statistical differences in the distribution of virulence genes were observed for *papC* (*p* < 0.006) and *cnf* (*p* < 0.007) genes among UPEC of different cUTI categories.

Analysis of the occurrence of MDR UPEC among the different clinical categories revealed that 90% of pyelonephritis isolates were MDR, followed by 72.0% of cystitis isolates, 62.5% of urosepsis isolates, 50% of recurrent UTI isolates, and 42.5% of CAUTI isolates in the descending order. The assembled cUTI with co-morbidities category also had 45.1% MDR isolates.

Relational analysis of the occurrence of phylogroups and virulence determinants among UPEC isolates from different cUTI categories and ASB *E. coli* isolates

Table 5 shows an analysis of aggregative virulence score for UPEC and ASB *E. coli* isolates according to phylogroups. Results obtained from this study demonstrate that 38.3% of UPEC isolates possessed a minimum of four to a maximum of six virulence genes, followed by 29.8% of strains with a minimum of one to a maximum of three virulence genes, 20.6% of strains with a minimum of seven to a maximum of nine virulence genes and 11.3% of strains with a minimum of 10 to a maximum of 12 virulence genes in different permutations and combinations. Analysis of average aggregative virulence scores
Table 3 Antimicrobial resistance pattern observed among different phylogroups of cUTI UPEC, *n* (%).

Antibiotics	Phylogroups										*p*-value
	A 21	B1 16	B2 65	C 6	D 17	E 8	F 3	CC 1 1	NT 4	Total 141	
AMP	18 (85.7)	13 (81.3)	55 (84.6)	5 (83.3)	16 (94.1)	7 (87.5)	3 (100)	0	4 (100)	121 (85.8)	0.384
AMC	15 (71.4)	10 (62.5)	36 (55.4)	2 (33.3)	13 (76.5)	7 (87.5)	3 (100)	0	3 (75.0)	89 (63.1)	0.155
SAM	9 (42.9)	8 (50.0)	24 (36.9)	1 (16.7)	8 (47.1)	2 (25.0)	3 (100)	0	3 (75.0)	58 (41.1)	0.221
TP	9 (42.9)	8 (50.0)	33 (50.8)	3 (50.0)	12 (70.6)	2 (25.0)	3 (100)	0	2 (50.0)	72 (51.1)	0.325
CXM	8 (38.1)	7 (43.8)	30 (46.2)	2 (33.3)	7 (41.2)	2 (25.0)	3 (100)	0	2 (50.0)	61 (43.3)	0.622
FOX	0	3 (18.8)	7 (10.8)	1 (16.7)	3 (17.6)	2 (25.0)	0	0	1 (25.0)	17 (12.1)	0.528
CTX	14 (66.7)	10 (62.5)	45 (69.2)	4 (66.7)	11 (64.7)	4 (50.0)	3 (100)	0	3 (75.0)	94 (66.7)	0.803
FEP	8 (38.1)	9 (56.3)	34 (52.3)	4 (66.7)	6 (35.3)	3 (37.5)	3 (100)	0	3 (75.0)	70 (49.6)	0.335
CRO	6 (28.6)	5 (31.3)	20 (30.8)	3 (50.0)	5 (29.4)	1 (12.5)	3 (100)	0	2 (50.0)	45 (31.9)	0.266
ATM	5 (23.8)	6 (37.5)	17 (26.2)	2 (33.3)	5 (29.4)	1 (12.5)	3 (100)	0	2 (50.0)	41 (29.1)	0.203
IPM	5 (23.8)	5 (31.3)	32 (49.2)	2 (33.3)	5 (29.4)	2 (25.0)	2 (66.7)	0	3 (75.0)	56 (39.7)	0.231
AK	16 (76.2)	11 (68.8)	35 (53.8)	4 (66.7)	9 (52.9)	4 (50.0)	3 (100)	0	3 (75.0)	85 (60.3)	0.391
GN	9 (42.9)	9 (56.3)	37 (56.9)	3 (50.0)	8 (47.1)	4 (50.0)	3 (100)	0	3 (75.0)	76 (53.9)	0.676
NOR	9 (42.9)	6 (37.5)	18 (27.7)	2 (33.3)	5 (29.4)	2 (25.0)	2 (66.7)	0	2 (50.0)	46 (32.6)	0.823
MDR	11 (52.4)	8 (50.0)	37 (50.0)	3 (50.0)	7 (41.2)	2 (25.0)	3 (100)	0	3 (75.0)	74 (52.5)	–
Non-MDR	10 (47.6)	8 (50.0)	37 (50.0)	3 (50.0)	10 (58.8)	6 (75.0)	0	1 (100)	1 (25.0)	67 (47.5)	–

Notes.

MDR, Multidrug resistance; Non-MDR, Non multidrug resistance.

ampicillin (AMP,10μg), amoxicillin/clavulanate (AMC, 20/10μg), ampicillin/sulbactam (SAM, 10/10 μg), piperacillin /tazobactam (TP, 100/10μg), cefuroxime (CXM, 30μg), cefoxitin (FOX, 30μg), cefotaxime (CTX, 30μg), cefepime (FEP, 30μg), ceftriaxone (CRO, 30μg), aztreonam (ATM, 30μg), imipenem (IMP, 10μg), amikacin (AK, 30μg), gentamicin (CN, 30μg) and norfloxacin (NOR, 30μg).

Kruskal–Wallis test was used for statistical comparison and *p*-value <0.05 was considered as significant. Significance values have been adjusted by the Bonferroni correction for multiple comparisons.

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Table 4 Distribution of phylogroups, virulence genes, multidrug resistance of UPEC isolates according to different cUTI categories; *n* (%).

	Complicated UTI categories							
	Cystitis	Pyelonephritis	Urosepsis	Recurrent UTI	Catheter-associated UTI	cUTI with co-morbidities	Total	*p*-value
	18 (12.8)	10 (7.1)	8 (5.7)	14 (9.9)	40 (28.4)	51 (36.2)	141 (100)	
A	1 (5.6)	1 (10.0)	0	3 (21.4)	7 (17.5)	9 (17.6)	–	–
B1	2 (11.1)	0	1 (12.5)	1 (7.1)	5 (12.5)	7 (13.7)	–	–
B2	11 (61.1)	9 (90.0)	5 (62.5)	4 (28.6)	17 (42.5)	19 (37.3)	–	–
C	1 (5.6)	0	0	0	3 (7.5)	2 (3.9)	–	–
D	2 (11.1)	0	1 (12.5)	3 (21.4)	4 (10.0)	7 (13.9)	–	–
E	1 (5.6)	0	1 (12.5)	2 (14.3)	1 (2.5)	3 (5.9)	–	–
F	0	0	0	0	1 (2.5)	2 (3.9)	–	–
CC1	0	0	0	0	1 (1.6)	1 (1.9)	–	–
Not typeable				1 (7.1)	1(1.6)	1 (1.9)	–	–
fimH	16 (88.9)	10 (100)	7 (87.5)	11 (78.6)	35 (87.5)	45 (88.2)	124 (87.9)	0.770
sfa	4 (22.2)	4 (40.0)	2 (25.0)	2 (14.3)	9 (22.5)	15 (29.4)	36 (25.5)	0.774
papC	7 (38.9)[bc]	9 (90.0)[a]	6 (75.0)[ab]	7 (50.0)[ab]	16 (40.0)[bc]	11 (21.6)[c]	56 (39.7)	<0.001[*]
iroN	3 (16.9)	6 (60.0)	5 (62.5)	5 (35.7)	13 (32.5)	19 (37.3)	51 (36.2)	0.155
fyuA	7 (38.9)	5 (50.0)	4 (50.0)	6 (42.9)	17 (42.5)	24 (47.1)	63 (44.7)	0.985
chuA	14 (77.8)	9 (90.0)	7 (87.5)	10 (71.4)	25 (62.5)	33 (64.7)	98 (69.5)	0.386
cnf	4 (22.8)[b]	7 (70.0)[a]	6 (75.0)[a]	3 (21.4)[b]	11 (27.5)[b]	11 (21.6)[b]	42 (29.8)	0.003[*]
hlyA	6 (33.3)	6 (60.0)	6 (75.0)	6 (42.9)	13 (32.5)	22 (43.1)	59 (41.8)	0.226
sat	2 (11.1)	0	1 (12.5)	2 (14.3)	4 (10.0)	9 (17.6)	18 (12.8)	0.718
usp	14 (77.8)	9 (90.0)	3 (37.5)	8 (57.1)	9 (22.5)	24 (27.1)	45 (31.9)	0.058
ompT	5 (27.8)	3 (30.0)	5 (62.5)	7 (50.0)	21 (52.5)	17 (33.3)	71 (50.4)	0.305
kpsMTII	8 (44.4)	7 (70.0)	6 (75.0)	6 (42.9)	26 (65.0)	23 (45.1)	83 (58.9)	0.718
MDR	13 (72.2)	9 (90.0)	5 (62.5)	7 (50.0)	17 (42.5)	23 (45.1)	74 (52.5)	–
Non-MDR	5 (27.8)	1 (10.0)	3 (37.5)	7 (50.0)	23 (57.5)	28 (54.9)	67 (47.5)	–

Notes.

Group with different lowercase alphabets superscripted are statistically different based on pairwise comparison. Kruskal–Wallis test was used for statistical comparison and *p*-value <0.05[*] was considered as significant. Significance values have been adjusted by the Bonferroni correction for multiple comparisons.

of UPEC from different phylogroups revealed that isolates from phylogroup B2 had the highest aggregative virulence score (7.17), and there were 14 isolates of these phylogroups possessing a minimum of 10 virulence genes. Lower average aggregative virulence score was observed for UPEC isolates from phylogroup A (2.90) and B1 (2.81) and most of the isolates from these phylogroups only possessed a maximum of three virulence genes.

In comparison with cUTI isolates, ASB *E. coli* isolates were identified to possess a reduced number of virulence genes (Table 5). Statistical analysis of the occurrence of virulence genes among cUTI and ASB *E. coli* isolates using the Chi-square test revealed that, there were statistical differences in the occurrence of virulence genes among these isolates for the occurrence of three virulence genes tested (*papC*: $p < 0.001$, *ompT*: $p < 0.001$, and *usp*; $p < 0.001$) (Table 2). It was observed that 56.3% of ASB *E. coli* isolates possessed zero to a maximum of three virulence genes, followed by 26.9% with a minimum of four to a maximum of six virulence genes, 14.4% strains with a minimum of seven to a maximum of nine virulence genes and only four strains were harbouring a minimum of 10 to a maximum of 12 virulence genes in different permutations and combinations. Similar to UPEC isolates, ASB *E. coli* isolates from phylogroup B2 were also observed with the highest average aggregative virulence score (5.37), which was lower than the average aggregative virulence scores of phylogroup B2 cUTI UPEC isolates.

The analysis of comparison of average aggregative virulence score among UPEC from different cUTI categories revealed that UPEC isolated from pyelonephritis had the highest score (7.80), followed by urosepsis (6.87), cystitis (5.00), recurrent UTI (5.00), and CAUTI (4.70). The assembled cUTI with the comorbidities category had an average aggregative virulence score of 4.80 (Table 6). It was observed that 57.1% of UPEC isolated from recurrent UTI, 45.0% UPEC from CAUTI and 41.2% of UPEC from cUTI with co-morbidities harboured a minimum of one to a maximum of three virulence genes tested in this study in different combinations. In comparison with UPEC from the above mentioned cUTI categories, 83.9% cystitis isolates were observed to harbour four to six virulence genes tested, followed by 60.0% of pyelonephritis isolates and 37.5% of urosepsis isolates with seven to nine virulence genes, respectively.

The analysis of the average aggregative virulence score for the individual virulence functional groups (adhesin, iron uptake, toxin and capsule synthesis) is depicted in Table 7. Data demonstrated that UPEC isolates of pyelonephritis and urosepsis have higher average aggregative virulence score for all the virulence functional categories tested in this study. Isolates of pyelonephritis and urosepsis possessed a minimum of two to three virulence genes from each virulence category tested in this study. In comparison with UPEC strains, ASB *E. coli* isolates were identified to have lower average aggregative virulence scores for each of the virulence functional categories included in this study. However, it was also observed that there were few UPEC isolates of other UTI categories (cystitis, recurrent UTI, CAUTI, and UTI with comorbidities), which did not possess any of the tested virulence genes encoding for a particular virulence functional group. The number of ASB isolates with no virulence genes in some of the functional groups was higher than that of UPEC isolates.

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Table 5 Average aggregative virulence scores of cUTI UPEC and ASB *E. coli* isolates among the phylogroups, *n* (%).

Total number of virulence genes	A		B1		B2		C		D		E		F		CC1		NT		Total	
	UPEC *n*=16	ASB *n*=35	UPEC *n*=16	ASB *n*=27	UPEC *n*=65	ASB *n*=55	UPEC *n*=6	ASB *n*=5	UPEC *n*=17	ASB *n*=13	UPEC *n*=8	ASB *n*=13	UPEC *n*=3	ASB *n*=5	UPEC *n*=1	ASB *n*=3	UPEC *n*=4	ASB *n*=4	UPEC *n*=141	ASB *n*=160
0 to 3	15	18	11	15	5	35	3	3	3	9	4	5	0	2	0	1	1	3	42 (29.8)	90 (56.3)
4 to 6	4	9	5	7	20	13	3	2	13	2	3	5	3	2	1	2	2	1	54 (38.3)	43 (26.9)
7 to 9	2	9	0	5	25	5	0	0	1	2	0	2	0	0	0	0	0	0	29 (20.6)	23 (14.4)
10 to 12	0	0	0	0	14	2	0	0	0	0	1	1	0	1	0	0	1	0	16 (11.3)	4 (2.4)
Average Aggregative virulence score	2.90	2.13	2.81	2.11	7.17	5.37	3.83	3.60	4.53	3.64	4.63	3.36	3.61	4.40	2.00	2.67	5.75	1.75	–	–

Notes.

Average aggregative virulence scores were calculated by averaging the total number of virulence genes identified in each strain among the phylogroups.

Table 6   Average aggregative virulence scores of cUTI UPEC isolates based on clinical categories, n (%).

Total virulence genes	cUTI categories					
	Cystitis	Pyelonephritis	Urosepsis	Recurrent UTI	Catheter-associated UTI	cUTI with co-morbidities
	18 (12.8)	10 (7.1)	8 (5.7)	14 (9.9)	40 (28.4)	51 (36.2)
0 to 3	2 (11.1)	0	2 (25.0)	8 (57.1)	18 (45.0)	21 (41.2)
4 to 6	15 (83.9)	1 (10.0)	1 (17.5)	2 (14.3)	12 (30.0)	17 (33.3)
7 to 9	1 (5.5)	6 (60.0)	3 (37.5)	2 (14.3)	5 (12.5)	8 (15.7)
10 to 12	0	3 (30.0)	2 (25.0)	2 (14.3)	5 (12.5)	5 (9.8)
Average aggregative virulence score	5.00	7.80	6.89	5.00	4.71	4.80

Notes.

Average aggregative virulence scores were calculated by averaging the total number of virulence genes identified in each strain of the cUTI categories.

Co-occurrence analysis for the occurrence of virulence genes among cUTI UPEC and ASB *E. coli* isolates

The co-occurrence analysis of the occurrence of virulence genes among cUTI UPEC isolates, revealed a strong positive correlation ($r > 0.5$) between *sfa* and *cnf* ($r = 0.508$), *iroN* with *hlyA* ($r = 0.858$), and *chuA* with *kpsMTII* ($r = 0.513$) (Table 8). A moderate positive correlation ($0.4 < r < 0.5$) was noticed for *fyuA* and *kpsMTII* ($r = 0.493$).

The co-occurrence analysis performed for ASB *E. coli* isolates revealed similar strong positive correlations observed for *sfa* with *cnf* ($r = 0.556$) *iroN* with *hlyA* ($r = 0.563$), and *iroN* with *cnf* ($r = 0.531$) (Table 8). Moderate correlations were observed between *sfa* with *iroN* ($r = 0.444$), *sfa* with *hlyA* ($r = 0.433$), *papC* with *hlyA* ($r = 0.447$), *fyuA* with *kpsMTII* ($r = 0.430$), *chuA* with *kpsMTII* ($r = 0.425$), and *chuA* with *usp* ($r = 0.433$).

DISCUSSION

To the best of our knowledge, the present work is the very first study describing the occurrence of phylogroups, virulence genes and MDR potential of UPEC isolated from more than three cUTI categories (cystitis, pyelonephritis, urosepsis, recurrent UTI, and CAUTI). Complicated UTI with co-morbidities is a group of heterogeneous isolates, put together by the authors, as the patients they were isolated from were not clinically assigned to any specified cUTI category.

Analysis of the occurrence of phylogroups

UPEC isolates in this study were differentially distributed among eight phylogroups. The phylogenetic distribution pattern of cUTI UPEC isolates was similar to that reported in other studies, with a greater occurrence of isolates from phylogroup B2 (*Dadi et al., 2020*; *Halaji et al., 2022*; *Tewawong et al., 2020*). A meta-analysis of 28 studies reporting UPEC virulence genes also identified a similar pattern (*Halaji et al., 2022*). Overall, 68.1% of UPEC isolates were from ExPEC-associated phylogroups, while 31.9% were from non-ExPEC. The present study showed that the UPEC isolates in traditional phylogroups (A, B1, B2 and D) were redistributed to Clermonts' extended new phylogroups (C, E and F). These findings

Table 7 Comparison of virulence gene occurrence and average aggregative virulence score in cUTI UPEC and ASB *E. coli* isolates based on functional virulence category, *n* (%).

Functional Virulence category	Cystitis (18)	Pyelonephritis (10)	Urosepsis (8)	Recurrent UTI (14)	Catheter associated UTI (40)	UTI with co-morbidities (51)	Overall UPEC isolates (141)	ASB *E. coli* (160)
Adhesion								
0 gene	1 (5.56)	0	0	3 (21.43)	5 (12.50)	3 (5.88)	12 (8.51)	29 (18.13)
1 gene	7 (38.89)	1 (10.00)	2 (25.00)	4 (28.57)	15 (37.50)	30 (58.82)	59 (41.84)	91 (56.88)
2 genes	10 (55.56)	5 (50.00)	5 (62.50)	5 (35.71)	15 (37.50)	13 (25.49)	53 (37.59)	30 (18.75)
3 genes	0	4 (40.00)	1 (12.50)	2 (14.29)	5 (12.50)	5 (9.80)	17 (12.06)	10 (6.25)
AVS	1.50	2.30	1.88	1.43	1.50	1.39	1.53	1.13
Iron uptake system								
0 gene	4 (22.22)	0	0	4 (28.57)	7 (17.50)	12 (23.53)	29 (20.57)	49 (30.63)
1 gene	6 (33.33)	2 (20.00)	2 (25.00)	4 (28.57)	16 (40.00)	11 (21.57)	39 (27.66)	54 (33.75)
2 genes	6 (33.33)	3 (30.00)	3 (37.50)	1 (7.14)	12 (30.00)	19 (37.25)	46 (32.62)	47 (29.38)
3 genes	2 (11.11)	5 (50.00)	3 (37.50)	5 (35.71)	5 (12.50)	9 (17.65)	27 (19.15)	10 (6.25)
AVS	1.33	2.20	2.00	1.50	1.38	1.49	1.50	1.11
Toxin								
0 gene	0	1 (10.00)	0	5 (35.71)	7 (17.50)	13 (25.49)	26 (18.44)	68 (42.50)
1 gene	10 (55.56)	1 (10.00)	1 (12.50)	5 (35.71)	19 (47.50)	20 (39.22)	56 (39.72)	55 (34.38)
2 genes	8 (44.44)	3 (30.00)	3 (37.50)	1 (7.14)	8 (20.00)	10 (19.61)	33 (23.40)	19 (11.88)
3 genes	0	5 (50.00)	4 (50.00)	2 (14.29)	5 (12.50)	7 (13.71)	23 (16.31	18 (11.25)
4 genes	0	0	0	1 (7.14)	1 (2.50)	1 (1.96)	3 (2.13)	0
AVS	1.44	2.20	2.38	1.21	1.35	1.27	1.44	0.92
Capsule synthesis								
0 gene	8 (44.44)	3 (30.00)	2 (25.00)	3 (21.43)	18 (45.00)	18 (35.29)	52 (36.88)	83 (51.88)
1 gene	7 (38.89)	4 (40.00)	4 (50.00)	7 (50.00)	14 (35.00)	26 (50.98)	62 (43.97)	69 (43.13)
2 genes	3 (16.67)	3 (30.00)	2 (25.00)	4 (28.57)	8 (20.00)	7 (13.73)	27 (19.15)	8 (5.00)
AVS	0.72	1.00	1.00	1.07	0.75	0.78	0.82	0.53

Notes.

0 gene: absence of any tested virulence gene in the specific functional group, 1 gene: one gene is present among the tested three genes in the specific functional group, 2 genes: two genes are present among the tested three genes in the specific functional group etc., AVS- average aggregative virulence score.

were also consistent with the meta-analysis report by *Halaji et al. (2022)*. Phylogenetic analysis indicated that the occurrence of UPEC from non-EXPEC phylogroups was lower than reported in other studies (*Gatya Al-Mayahie et al., 2022*; *Tanabe et al., 2022*). It was observed that the distribution of UPEC into the NT group was also lower than the reported 27% from Iran (*Iranpour et al., 2015*).

Similar to the trends of phylogroups distribution among cUTI isolates, ASB *E. coli* isolates were also reported to be highly distributed among ExPEC representing phylogroups (53.78%) than non-ExPEC phylogroups (41.82%) (*Maniam et al., 2022*). Such similar higher occurrences of ASB *E. coli* from ExPEC representing phylogroup B2 were also reported by *Amarsy et al. (2019)* and *Lavigne et al. (2011)*. The higher distribution of ASB *E. coli* in ExPEC phylogroups suggests that ASB strains have the potential to cause symptomatic UTI. Any treatment decisions regarding the ASB should involve a detailed

Table 8 Co-occurrence of virulence genes among cUTI UPEC and ASB *E. coli* isolates.

		fimH	*sfa*	*papC*	*iroN*	*fyuA*	*chuA*	*cnf*	*hlyA*	*sat*	*ompT*	*kpsMTII*	*usp*
fimH	UPEC	1	.117	.167*	.052	.245**	.086	.241**	.093	.076	−.027	.068	.025
	ASB	1	.061	.074	−.118	.388**	−.104	−.043	−.042	.162*	.157*	.086	−.079
sfa	UPEC		1	.090	.372**	.194*	.176*	.508**	.295**	−.078	.192*	.191*	.227**
	ASB		1	.224**	.444**	.157*	.173*	.556**	.433**	−.128	−.049	.299**	.377**
papC	UPEC			1	.324**	.174*	.223**	.327**	.369**	−.050	.097	.139	.189*
	ASB			1	.178*	.314**	.088	.354**	.447**	.148	.089	.300**	.249**
iroN	UPEC				1	.303**	.114	.349**	.858**	−.155	.213*	.068	.032
	ASB				1	.049	.192*	.531**	.563**	−.162*	−.157*	.244**	.294**
fyuA	UPEC					1	.347**	.350**	.221**	.255**	.242**	.493**	.177*
	ASB					1	.187*	.145	.089	.314**	.051	.430**	.239**
chuA	UPEC						1	.263**	.062	.161	.090	.513**	.366**
	ASB						1	.187*	.135	−.029	.099	.425**	.443**
cnf	UPEC							1	.328**	−.063	.120	.275**	.252**
	ASB							1	.406**	−.153	.025	.317**	.398**
hlyA	UPEC								1	−.152	.159	−.020	.031
	ASB								1	−.122	−.141	.179*	.291**
sat	UPEC									1	.240**	.167*	−.009
	ASB									1	.013	.314**	−.206**
ompT	UPEC										1	.132	.026
	ASB										1	−.041	.028
kpsMTII	UPEC											1	.360**
	ASB											1	.543**
usp	UPEC												1
	ASB												1

Notes.

Significance levels:

**$p \leq 0.01$.

*$0.01 < p < 0.05$.

+positive correlation.

−negative correlation.

evaluation of the virulence characteristics of these isolates in the best interest of favourable treatment outcome and prevention of future complications.

Distribution of virulence genes among phylogroups

In parallel with other studies (*Dadi et al., 2020*; *Er et al., 2015*; *Rezatofighi, Mirzarazi & Salehi, 2021*; *Tanabe et al., 2022*), analysis of the distribution of virulence genes among phylogroups in this study revealed that cUTI UPEC isolates from phylogroup B2 had a higher presence of all the virulence genes tested. In comparison with isolates from non-ExPEC phylogroups, UPEC from ExPEC phylogroups B2 and D showed higher percentage distribution of all the virulence genes studied, particularly the occurrence of *sfa, papC, fyuA, cnf, usp* and *kpsMTII* genes. These findings were in agreement with the report by *Belmont-Monroy et al. (2022)*, in which phylogroup B2 was shown to possess six to ten virulence genes, in comparison to non-ExPEC phylogroups possessing one to five virulence genes. Isolates from phylogroup E, which is a sub-group from D, was identified
to harbour all the virulence genes tested. UPEC isolates from non-ExPEC phylogroups A, B1 and C were generally observed to possess a reduced number of virulence genes than the ExPEC phylogroups. Considering ExPEC and non-ExPEC phylogroups together, there were statistically significant differences in the presence of virulence genes *fyuA*, *chuA*, *usp* and *kpsMTII* ($p < 0.001$) (Table 2).

The analysis of the aggregative virulence score among phylogroups demonstrates that there is a potential association between the phylogroups and the virulence of the isolates. In both cUTI UPEC and ASB *E. coli* isolates, strains from phylogroup B2 demonstrated the highest average aggregative virulence scores of 7.17 and 5.37, respectively, than strains from other phylogroups. Similar higher average aggregative virulence scores of 9.00 and 5.95 for UPEC from phylogroup B2 was reported by *Kudinha et al. (2013)* and *Rezatofighi, Mirzarazi & Salehi (2021)*, respectively. Data obtained from the analysis of average aggregative virulence scores among the strains of cUTI UPEC and ASB *E. coli* isolates revealed that *E. coli* isolates of ExPEC, particularly isolates of B2 were highly virulent, possessing a greater number of virulence genes than strains from non-ExPEC phylogroup.

Data across the literature has demonstrated that ASB *E. coli* isolates of various phylogroups were also possessing the virulence-associated genes and the presence of these genes were hypothesized to facilitate the survival of the bacterium in the urinary tract of the host (*Eghbalpour et al., 2022*; *Maniam et al., 2022*). Data from the previous study by *Maniam et al. (2022)* on genotypic characteristics of ASB *E. coli* isolates revealed that these isolates also carried all the virulence genes tested in this study in different permutations and combinations among its isolates. It was observed that the occurrence of these virulence genes were lower in frequency among all the phylogroups for ASB *E. coli* isolates. There were statistically significant differences observed in the overall occurence of virulence genes *fyuA*, *chuA*, *usp* and *kpsMTII* ($p < 0.001$) between UPEC and ASB *E. coli* isolates (Table 2).

Relational analysis of the occurrence of virulence genes

In this study, the occurrence of *fimH* gene was also observed as the highest (number) among tested virulence genes for each cUTI category. Adhesin virulence factors of UPEC are crucial to facilitate the colonisation and initiation of the infection process, of which the occurrence of type 1 fimbriae (*fimH*) gene was identified as highly abundant and conserved among the isolates of *E. coli* of various phylogroups and pathotypes (*Firoozeh et al., 2022*; *Norinder et al., 2012*). The presence of *fimH* gene was known to promote the binding of this bacterium to mannosylated glycoproteins of uroplakin 1a which are abundant along the urothelium of the urinary bladder (*Johnson et al., 2005b*). Adhesin between UPEC and superficial facet cells in host urothelium was known to aid in the invasion of this bacterium into the cytoplasm, and thus promotes the production of intracellular bacterial communities (IBC) (*Schwartz et al., 2013*). This subsequently protects the bacteria from the sheer force of urination and the host's innate immune system and promotes prolonged colonisation in the urinary tract. The highest occurrence of these genes among both cUTI and ASB *E. coli* indicate the importance of this gene as a vital element of survival of most *E. coli* isolates of various pathotypes. Although the occurrence of *fimH* was observed to

be common among UPEC and commensal *E. coli*, the expression of this gene is partially regulated by phase variation of promoter gene (*fimS*), which eventually results in phase ON (expression) or OFF (non-expression) orientations (*Firoozeh et al., 2022*; *Foroogh et al., 2021*; *Subashchandrabose & Mobley, 2015*). However, the expression analysis of *fimH* and occurrence of *fimS* were not investigated in this study.

The presence of the S-fimbriae (*sfa*) gene is associated with UPEC's ability to bind to the terminal of sialyl-galactoside residues of urothelium present on various parts of the kidney including tubules of glomeruli (*Desvaux et al., 2020*). Therefore, the occurrence of *sfa* gene is often associated with UPEC survival strategy in the upper urinary tract, in turn associating the gene with severe UTI categories of pyelonephritis and urosepsis. In this study, the distribution of *sfa* gene was observed among 40% of the isolates of pyelonephritis and 25% among isolates of urosepsis. The isolates of other cUTI categories were also observed to possess *sfa* gene with minimal frequency.

The occurrence of the p-fimbriae (*pap*) gene, was significantly associated with pyelonephritis. UPEC possessing *pap* gene will be able to bind to the Gal α (1–4) - Gal moieties (glycosphingolipids) located in the urothelial cells, especially in the kidney. Although the occurrence of *pap* gene was lower than other *fimH* genes, the highest occurrence of this gene was observed among pyelonephritis isolates. Higher distribution of *pap* gene (75%) was also observed among isolates of urosepsis, indicating the association of this gene with increased disease severity. The occurrence of *papC* genes among other cUTI isolates indicates the probability of the development of more severe diseases like pyelonephritis or urosepsis in these patients.

Iron is crucial for the survival of UPEC, required for various cellular and biological processes of nucleotide biosynthesis, electron transport and peroxidase reduction (*Robinson, Heffernan & Henderson, 2018*). However, iron is limiting in the hostile environment of the urinary tract. As part of its survival strategies, UPEC is able to synthesize various small and high-affinity molecules called siderophores, which enable them to scavenge ferric iron from host cells. Results from this study reveal that UPEC isolated from all UTI categories, were identified to possess varying distribution of salmochelin (*iroN*), yersiniabactin (*fyuA*) and heme receptor (*chuA*) genes. In addition to facilitating the iron acquisition, *Gao et al. (2012)* demonstrated that the acquisition of both *iroN* and *fyuA* genes could predispose UPEC to invade the urothelial, thus causing infection severity.

In addition to siderophore molecules, the isolates of UPEC were also able to scavenge the heme-bound iron. *Chu* is an important heme transport gene possessed mostly by UPEC, also known to facilitate the acquisition of iron to aid in bacterial survival. Similarly, the occurrence of *chuA* gene is reported to be associated with increased resistance of invading uropathogens against antimicrobial peptides and thus predisposes to biofilm formation, which could further enhance the fitness of UPEC in the urinary tract (*Hancock & Klemm, 2007*).

Toxins are known to be associated with UTI severity. UPEC possessing toxins are able to invade the host cells, allowing the bacterium to survive in a more quiescent state, promoting long-term bacterial persistence within the urinary tract (*Dhakal & Mulvey, 2012*). Hemolysin A (*hlyA*) gene encodes for pore-forming toxins, which increases the

permeability of the cell membrane, causing cell death at high concentration (*Wiles & Mulvey, 2013*). As per the literature, the occurrence of *hlyA* is often associated with urosepsis and pyelonephritis, with an increased likelihood of renal complications later (*Jahandeh et al., 2015*).

Cytotoxic necrotising factor (*cnf*) gene in UPEC activates small Rho-family GTPases which promotes various events in the host cells, especially multinucleation and cell spreading, apoptosis of urothelium, decreasing polymorphonuclear leucocytes and also activation of nuclear factor, which could predispose to the disease severity. Acquisition of *cnf* and *hlyA* increases the chances of invasion of UPEC into urothelial cells of the kidney, which increases the risk of urosepsis. Based on the results from this study, the isolates from all cUTI categories possess these two virulence genes in varying distribution. However, a higher distribution of these genes was observed among isolates of urosepsis and pyelonephritis corresponding with its known disease severity. In contrast to the report by *Dadi et al. (2020)*, which reported nearly 29% of the occurrence of *cnf* gene among pyelonephritis isolates, the occurrence of this gene in this study was remarkably high (70%). Although, a reduced occurrence of these genes was observed among the isolates of other cUTI categories such as cystitis, recurrent UTI, CAUTI and cUTI with comorbidities; the isolates with these genes may predispose patients to a higher risk of severe UTI (*Vejborg et al., 2011*).

The occurrence of secreted autotransporter protein (*sat*) was often found to be associated with cytotoxic effects on renal and bladder cells. In literature, the occurrence of *sat* gene was often associated with pyelonephritis, corresponding to the virulence potential of isolates possessing them (*Guyer et al., 2002*). In the present study, none of the pyelonephritis isolates and only one isolate of urosepsis was identified to harbour this gene. A lower distribution of this gene was also observed among the isolates of other cUTI categories, in contrast to the existing data on the prevalence of this gene among UPEC isolates (*Morales-Espinosa et al., 2016*).

The presence of protectins as polysaccharide capsules on the surface of UPEC is well known to aid UPEC attachment on various surfaces and protects the bacterium from the host immune system, bactericidal effects and also from antimicrobial agents (*Sarkar et al., 2014*). The occurrence of the outer membrane protein (*ompT*) gene among UPEC enables this bacterium to produce an enzyme with serine proteases activity, which helps the bacterium to cleave the cationic peptides with antibiotic activity. This subsequently promotes the bacteria to retain longer in the urinary tract by inhibiting the antimicrobial peptides produced by the host's innate immune system. The occurrence of *ompT* gene in this study was observed to be highest among the isolates of urosepsis followed by CAUTI and recurrent UTI.

The distribution of another protectins, a capsule-producing gene, *kpsMTII* was also observed highly distributed among the isolates of both urosepsis and pyelonephritis. The occurrence of *kpsMTII* gene in the UPEC is known to play an important role in the development of IBC as well as exhibiting resistance to antimicrobial agents. The presence of protectins could enhance the survival of UPEC in bloodstream, necessitating its existence in urosepsis strains.

Association of antimicrobial resistance among phylogroups

In most clinical circumstances, the inadequate treatment outcome from cUTI was often reported to be associated with increased MDR potential of the organism involved. The incidence of the emergence of MDR, especially in UPEC has become a major threat in UTI management. The data on antimicrobial resistance of UPEC isolated from cUTI in this study demonstrated higher resistance to all antimicrobials tested. Prior use of antibiotics, non-empirical antibiotic prescriptions, hospitalisation, age, genitourinary disturbances and recurrent infections (UTI) are known predisposing risk factors for emergence of MDR organisms (*Kot, 2019*). Data from the current study revealed that more than 40% of the isolates demonstrated resistance to nine antimicrobial agents tested (AMP, AMC, SAM, TP, CXM, CTX, FEP, AK, and GN). A similar trend was also reported by *Malekzadegan et al. (2018)* and *Tewawong et al. (2020)*. MDR UPEC reported by the present study (52.5%) was lower than previous reports of 77.8% by *Malekzadegan et al. (2018)*, 63.3% by *Ramírez-Castillo et al. (2018)* and 97% by *Paniagua-Contreras et al. (2017)*. The MDR and challenges in the management of these cUTI conditions in all these study settings probably represents a wider global situation, warranting urgent attention. It was observed that the AMR of cUTI UPEC isolates were comparatively higher than the AMR among ASB *E. coli* isolates from the same study setting (*Maniam et al., 2022*). The occurrence of MDR among ASB isolates could be regarded as a bigger public health threat, indicating the spread of MDR strains in the community.

Relational analysis of phylogroups, distribution of virulence genes and MDR among different cUTI categories and ASB *E. coli* isolates

UPEC are highly diverse pathotypes of ExPEC, identified to possess numerous virulence factors associated with adhesin, iron uptake system, toxin and capsule production among the isolates from various epidemiological backgrounds as reported in the literature (*Firoozeh et al., 2022*; *Halaji et al., 2022*; *Jahandeh et al., 2015*). The differences in clinical severity of cUTI are known to be multifactorial, depending on the virulence of the invading pathogen and the susceptibility of the host, especially if there is a concomitant urological illness. The diagnosis and treatment of any of the UTI categories depend on the clinical manifestations and a significant number of uropathogens isolated. Despite the known and well-studied association between the presence of virulence genes with UTI pathogenesis, the current diagnosis and prognosis of UTI still overlook the nature of the organism, including its virulence profile.

Based on the findings in this study, UPEC isolated from the five different cUTI categories (and the added category of cUTI with co-morbidities) were observed to be highly heterogeneous, possessing the virulence genes of adhesin, iron uptake system, toxin and capsule synthesis in different permutations and combinations. Acquisition of virulence genes from different functional groups could be collectively facilitating UPEC's ability to survive in the hostile environment of the urinary tract. The present study also provides valid evidence towards the relationship between the number of virulence genes possessed and cUTI disease severity. It was observed that UPEC isolates of upper cUTI categories such as urosepsis and pyelonephritis had higher aggregative virulence scores than UPEC

isolates of cystitis representing lower UTI category. Most of the UPEC isolates representing upper cUTI categories were identified to possess at least one virulence gene tested from each virulence functional category, demonstrating their increased fitness to survive in the upper urinary tract of the host. Studies by *Ruiz et al. (2002)* and *Johnson et al. (2005a)*, have reported UPEC isolated from non-invasive UTI, such as cystitis had less virulence score than those isolates causing more-invasive UPEC syndromes such as pyelonephritis. However, reports on virulence score among UPEC isolated from recurrent UTI and CAUTI were not available in the existing literature.

Analysis of co-occurrence of the virulence genes revealed that the co-occurrence of *sfa/cnf* and *iroN/hlyA* are common among UPEC and ASB, indicating the probability of a common origin for the virulence determinants. The varying clinical manifestations of cUTI and ASB could be considered as diverging ends of the clinical spectrum of UTI, and the isolates associated with ASB could probably cause clinically manifesting UTI. Previous reports by *Foxman et al. (1995)* and *Johnson et al. (2001b)* have shown that association between virulence factors vary and genetic linkages between virulence genes were not constant, indicating the possibility of horizontal transfer of virulence genes. The findings of higher average aggregative virulence scores for isolates causing invasive UTIs (pyelonephritis and urosepsis) in comparison to ASB possibly provides evidences towards horizontal gene acquisition leading to enhanced virulence and survival capabilities among the ExPEC (*Boyd & Hartl, 1998*; *Johnson, Russo & Donnenberg, 2018*). A comprehensive genetic profiling based on virulence determinants could be a promising tool for determining prognostic probability of severe cUTI diseases.

On analysis, it was found that the isolates with two virulence genes and cause clinically manifesting UTI had adhesins and iron uptake system genes, adhesins and toxins or adhesins and capsule synthesis genes together. The isolates with three virulence genes had adhesins, iron uptake systems and toxins or adhesins, iron uptake systems and capsule synthesis genes in combination. As they progressed to possess four genes, the isolates had a combination of adhesins, iron uptake systems, toxins and capsule synthesis genes together. As the number of virulence genes increased from four to 12 genes, each of the isolates started demonstrating various genes of the four virulence gene groups (adhesins, iron uptake systems, toxins and capsule synthesis).

There were a few UPEC strains isolated from low-severity UTI diseases, such as cystitis, recurrent UTI and CAUTI, which closely mimicked the genetic profile of UPEC of high-severity diseases. These isolates indicate a prognostic probability of the risk of development of high severity diseases in these cUTI patients. Despite the occurrence of a higher number of virulence genes among some of the isolates from cystitis, recurrent UTI and CAUTI, the probable reason behind their reduced disease severity could be due to the lack of expression of the virulence genes involved. Although a few UPEC isolates ($n = 42$) of various cUTI categories, were identified with the minimal number (1 to 3) virulence genes tested in this study, the occurrence of these isolates in high cUTI disease severity could probably be due to possession of other UPEC associated virulence genes which were not screened in this study.

The isolates of UPEC causing cUTI in this study demonstrated a similar trend in terms of occurrence of phylogroups and virulence genes in comparison to ASB *E. coli* isolates as reported by *Maniam et al. (2022)*, which is also from the same study setting as this study. However, in both *E. coli* pathotypes (cUTI and ASB), isolates from phylogroup B2 were observed higher in distribution and were found to possess a high number of virulence genes tested; thus, assumed to be highly pathogenic than isolates of other phylogroups. In a previous study by *Maniam et al. (2022)*, the relational analysis of the occurrence of *papC/sfa, iroN/fyuA, cnf/hlyA, kpsMTII,* and *usp* virulence genes among ASB strains was recommended to be prognostic towards the cumulative virulence potential of the isolates in causing clinically significant UTI cases. In agreement with this, results from this study revealed that cUTI UPEC isolates from phylogroup B2 had significantly different occurrence for *fyuA, chuA, cnf, kpsMTII* and *usp*. This evidence could be explored further with appropriate sample sizes and study design to understand the cumulative effect of combination of genes in virulence potential of the phylogroups.

Limitations of the study

The current study is explorative and relational in design and tested for the occurrence of the genes that were reported in the literature. There could be other virulence genes influencing the clinical outcome. The current study reports data based on a limited sample size if individual cUTI categories are considered. Due to non-comparable differences in the sample size for each cUTI category, the statistical comparison to check the significant association between the occurrences of virulence genes within the clinical categories could not be performed. Furthermore, the current study objective did not include the expression levels of virulence genes.

CONCLUSIONS

The cUTI isolates were distributed differentially among both ExPEC and non-ExPEC phylogroups. Phylogroup B2 isolates were observed to possess the highest average aggregative virulence score (7.17). In addition to that, the occurrence of MDR strains was observed across all the phylogroups, indicating an alarming situation in the emergence of MDR strains across *E. coli* of various pathotypes representing pathogens (ExPEC) and commensals (non-ExPEC). The observations of higher resistance against the common antibiotics used to treat UTI and the isolates of B2 phylogroup causing severe cUTI demonstrating higher MDR could be considered as warning signs to carefully relook at the diagnostic algorithm UTIs. Including the genetic profile of the pathogen isolated in the diagnostic algorithm will enable prognosis towards probable disease development and will ensure a better management of the patients.

Similar to the UPEC isolates in this study, ASB *E. coli* isolates reported from the same study setting were also highly heterogeneous in their virulence profile. Although these ASB isolates were also mostly distributed in phylogroup B2, generally they were identified with a reduced number of virulence genes and lower frequency of MDR potential.

Analysis of the occurrence of virulence genes among UPEC isolates of various disease categories revealed that the occurrence of the virulence genes tested was not distinctive

to any clinical cUTI categories. However, the data from the analysis of the average aggregative virulence score in this study indicated the probable relation between the increased cumulative actions of virulence genes with increased cUTI disease severity. The average aggregative virulence score of B2 phylogroup isolates from cUTI categories was higher than the average aggregative virulence score of B2 phylogroup isolates from ASB. The data strongly points towards the accumulation of virulence genes as a predicative indicator of disease severity. The strains with higher aggregative virulence scores could also be found among ASB isolates. This observation points towards possibility of higher expression of these genes among the isolates from clinically manifesting cUTI categories in comparison to ASB isolates, which could be explored further.

The data suggest that UPEC isolates which carry virulence genes from all four virulence genes groups studied (adhesins, iron uptake systems, toxins and capsule synthesis) and isolates from phylogroup B2 specifically could predispose to severe UTI categories involving the upper urinary tract. Therefore, specific exploration of the genotypic characteristics of UPEC could be further performed through analysis of the actions of combination of virulence genes, as a prognostic marker for predicting disease severity. Such an enhanced diagnostic algorithm which considers isolate characteristics will enable a more evidence driven treatment decision making, ensuring reduced complications and recurrence. This will go a long way in enhancing favourable therapeutic outcomes and reducing the antimicrobial resistance burden among UTI patients.

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Competing Interests

Cindy Shuan Ju Teh is an Academic Editor for PeerJ.

Author Contributions

- Lalitha Maniam conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Kumutha Malar Vellasamy conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Teng Aik Ong conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the article, sample collection, and approved the final draft.
- Cindy Shuan Ju Teh conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Kartini Abdul Jabar conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the article, sample collection, and approved the final draft.
- Vanitha Mariappan conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Vallikkannu Narayanan conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the article, sample collection, and approved the final draft.
- Jamuna Vadivelu conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Vinod Pallath conceived and designed the experiments, performed the experiments, analyzed the data, authored or reviewed drafts of the article, and approved the final draft.

Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

Ethical approval for this study was obtained from the Universiti Malaya Medical Centre (UMMC) Medical research ethics committee (MRECID 201765-5313).

Data Availability

The raw data measurements are available in the Supplementary File.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.15305#supplemental-information.

REFERENCES

Amarsy R, Guéret D, Benmansour H, Flicoteaux R, Berçot B, Meunier F, Mougari F, Jacquier H, Pean de Ponfilly G, Clermont O, Denamur E, Teixeira A, Cambau E.

2019. Determination of *Escherichia coli* phylogroups in elderly patients with urinary tract infection or asymptomatic bacteriuria. *Clinical Microbiology and Infection* **25**:839–844 DOI 10.1016/j.cmi.2018.12.032.

Barber AE, Norton JP, Wiles TJ, Mulvey MA. 2016. Strengths and limitations of model systems for the study of urinary tract infections and related pathologies. *Microbiology and Molecular Biology Reviews: MMBR* **80**:351–367 DOI 10.1128/MMBR.00067-15.

Beghain J, Bridier-Nahmias A, Le Nagard H, Denamur E, Clermont O. 2018. Clermon-Typing: an easy-to-use and accurate in silico method for Escherichia genus strain phylotyping. *Microbial Genomics* **4**:7 DOI 10.1099/mgen.0.000192.

Belmont-Monroy L, Ribas-Aparicio RM, González-Villalobos E, Pérez-Ramos JA, Aparicio-Ozores G, Eslava-Campos CA, Hernández-Chiñas U, Aquino-Andrade A, Balcázar JL, Molina-López J. 2022. Molecular typification of *Escherichia coli* from community-acquired urinary tract infections in Mexico. *International Journal of Antimicrobial Agents* **60**:106667 DOI 10.1016/j.ijantimicag.2022.106667.

Bonkat G, Pickard R, Bartoletti R, Bruyère F, Geerlings S, Wagenlehner F, Wullt B. 2017. EAU guidelines on urological infections. *European Association of Urology* **18**:22–26.

Boyd EF, Hartl DL. 1998. Chromosomal regions specific to pathogenic isolates of *Escherichia coli* have a phylogenetically clustered distribution. *Journal of Bacteriology* **180**:1159–1165 DOI 10.1128/jb.180.5.1159-1165.1998.

Chegini Z, Khoshbayan A, Vesal S, Moradabadi A, Hashemi A, Shariati A. 2021. Bacteriophage therapy for inhibition of multi drug-resistant uropathogenic bacteria: a narrative review. *Annals of Clinical Microbiology and Antimicrobials* **20**:30–30 DOI 10.1186/s12941-021-00433-y.

Clermont O, Christenson JK, Denamur E, Gordon DM. 2013. The clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environmental Microbiology Reports* **5**:58–65 DOI 10.1111/1758-2229.12019.

Clinical and Laboratory Standards Institute (CLSI). 2018. *Performance standards for antimicrobial susceptibility testing*. 28th edition. Wayne: Clinical and Laboratory Standards Institute.

Dadgostar P. 2019. Antimicrobial resistance: implications and costs. *Infection and Drug Resistance* **12**:3903–3910 DOI 10.2147/idr.S234610.

Dadi BR, Abebe T, Zhang L, Mihret A, Abebe W, Amogne W. 2020. Distribution of virulence genes and phylogenetics of uropathogenic *Escherichia coli* among urinary tract infection patients in Addis Ababa, Ethiopia. *BMC Infectious Diseases* **20**:108–108 DOI 10.1186/s12879-020-4844-z.

Davari Abad E, Khameneh A, Vahedi L. 2019. Identification phenotypic and genotypic characterization of biofilm formation in *Escherichia coli* isolated from urinary tract infections and their antibiotics resistance. *BMC Research Notes* **12**:796 DOI 10.1186/s13104-019-4825-8.

Desvaux M, Dalmasso G, Beyrouthy R, Barnich N, Delmas J, Bonnet R. 2020. Pathogenicity factors of Genomic Islands in intestinal and extraintestinal escherichia coli. *Frontiers in Microbiology* **11**:2065 DOI 10.3389/fmicb.2020.02065.

Dhakal BK, Mulvey MA. 2012. The UPEC pore-forming toxin α-hemolysin triggers proteolysis of host proteins to disrupt cell adhesion, inflammatory, and survival pathways. *Cell Host & Microbe* **11**:58–69 DOI 10.1016/j.chom.2011.12.003.

Eghbalpour F, Vahdat S, Shahbazi R, Mohebi S, Kholdi S, Hadadi M, Javadi K, Hashemizadeh Z, Motamedifar M. 2022. Pathogenic features of urinary *Escherichia coli* strains causing asymptomatic bacteriuria during pregnancy. *Gene Reports* **27**:101559 DOI 10.1016/j.genrep.2022.101559.

Ejrnæs K. 2011. Bacterial characteristics of importance for recurrent urinary tract infections caused by *Escherichia coli*. *Danish Medical Bulletin* **58**:B4187.

Eliakim-Raz N, Babitch T, Shaw E, Addy I, Wiegand I, Vank C, Torre-Vallejo L, Joan-Miquel V, Steve M, Grier S, Stoddart M, Nienke C, Leo vdH, Vuong C, MacGowan A, Carratalà J, Leibovici L, Pujol M, RS Group. 2019. Risk factors for treatment failure and mortality among hospitalized patients with complicated urinary tract infection: a multicenter retrospective cohort study (Rescuing study group). *Clinical Infectious Diseases* **68**:29–36 DOI 10.1093/cid/ciy418.

Er DK, Dundar D, Uzuner H, Osmani A. 2015. Relationship between phylogenetic groups, antibiotic resistance and patient characteristics in terms of adhesin genes in cystitis and pyelonephritis isolates of *Escherichia coli*. *Microbial Pathogenesis* **89**:188–194 DOI 10.1016/j.micpath.2015.10.014.

Firoozeh F, Zibaei M, Badmasti F, Khaledi A. 2022. Virulence factors, antimicrobial resistance and the relationship between these characteristics in uropathogenic *Escherichia coli*. *Gene Reports* **27**:101622 DOI 10.1016/j.genrep.2022.101622.

Foroogh N, Rezvan M, Ahmad K, Mahmood S. 2021. Structural and functional characterization of the FimH adhesin of uropathogenic *Escherichia coli* and its novel applications. *Microbial Pathogenesis* **161**:105288 DOI 10.1016/j.micpath.2021.105288.

Foxman B, Zhang L, Palin K, Tallman P, Marrs CF. 1995. Bacterial virulence characteristics of *Escherichia coli* isolates from first-time urinary tract infection. *The Journal of Infectious Diseases* **171**:1514–1521 DOI 10.1093/infdis/171.6.1514.

Gao Q, Wang X, Xu H, Xu Y, Ling J, Zhang D, Gao S, Liu X. 2012. Roles of iron acquisition systems in virulence of extraintestinal pathogenic *Escherichia coli*: salmochelin and aerobactin contribute more to virulence than heme in a chicken infection model. *BMC Microbiology* **12**:143 DOI 10.1186/1471-2180-12-143.

Gatya Al-Mayahie SM, Al-Guranie DRdT, Hussein AA, Bachai ZA. 2022. Prevalence of common carbapenemase genes and multidrug resistance among uropathogenic Escherichia coli phylogroup B2 isolates from outpatients in Wasit Province/ Iraq. *PLOS ONE* **17(1)**:e0262984 DOI 10.1371/journal.pone.0262984.

Guyer DM, Radulovic S, Jones F-E, Mobley HLT. 2002. Sat, the secreted autotransporter toxin of uropathogenic *Escherichia coli*, is a vacuolating cytotoxin for bladder and kidney epithelial cells. *Infection and Immunity* **70**:4539–4546 DOI 10.1128/IAI.70.8.4539-4546.2002.

Halaji M, Fayyazi A, Rajabnia M, Zare D, Pournajaf A, Ranjbar R. 2022. Phylogenetic group distribution of uropathogenic *Escherichia coli* and related antimicrobial resistance pattern: a meta-analysis and systematic review. *Frontiers in Cellular and Infection Microbiology* **12**:790184–790184 DOI 10.3389/fcimb.2022.790184.

Hancock V, Klemm P. 2007. Global gene expression profiling of asymptomatic bacteriuria *Escherichia coli* during biofilm growth in human urine. *Infection and Immunity* **75**:966–976 DOI 10.1128/IAI.01748-06.

Huijsdens XW, Linskens RK, Mak M, Meuwissen SG, Vandenbroucke-Grauls CM, Savelkoul PH. 2002. Quantification of bacteria adherent to gastrointestinal mucosa by real-time PCR. *Journal of Clinical Microbiology* **40**:4423–4427 DOI 10.1128/jcm.40.12.4423-4427.2002.

Idress M, Mussarat U, Badshah Y, Qamar R, Bokhari H. 2010. Virulence factors profile of drug-resistant *Escherichia coli* isolates from urinary tract infections in Punjab, Pakistan. *European Journal of Clinical Microbiology & Infectious Diseases* **29**:1533–1537 DOI 10.1007/s10096-010-1036-6.

Iranpour D, Hassanpour M, Ansari H, Tajbakhsh S, Khamisipour G, Najafi A. 2015. Phylogenetic groups of *Escherichia coli* strains from patients with urinary tract infection in iran based on the new clermont phylotyping method. *BioMed Research International* **2015**:846219 DOI 10.1155/2015/846219.

Jahandeh N, Ranjbar R, Behzadi P, Behzadi E. 2015. Uropathogenic *Escherichia coli* virulence genes: invaluable approaches for designing DNA microarray probes. *Central European Journal of Urology* **68**:452–458 DOI 10.5173/ceju.2015.625.

Johnson JR, Delavari P, Kuskowski M, Stell AL. 2001a. Phylogenetic distribution of extraintestinal virulence-associated traits in *Escherichia coli*. *The Journal of Infectious Diseases* **183**:78–88 DOI 10.1086/317656.

Johnson JR, Kuskowski MA, Gajewski A, Soto S, Horcajada JP, de Anta MTJ, Vila J. 2005a. Extended virulence genotypes and phylogenetic background of *Escherichia coli* isolates from patients with cystitis, pyelonephritis, or prostatitis. *The Journal of Infectious Diseases* **191**:46–50 DOI 10.1086/426450.

Johnson JR, Kuskowski MA, Gajewski A, Soto S, Horcajada JP, Jimenez de Anta MT, Vila J. 2005b. Extended virulence genotypes and phylogenetic background of *Escherichia coli* isolates from patients with cystitis, pyelonephritis, or prostatitis. *The Journal of Infectious Diseases* **191**:46–50 DOI 10.1086/426450.

Johnson JR, Russo TA, Donnenberg MS. 2018. Molecular epidemiology of extraintestinal pathogenic *escherichia coli*. *EcoSal Plus* **8**:1 DOI 10.1128/ecosalplus.ESP-0004-2017.

Kot B. 2019. Antibiotic resistance among uropathogenic *escherichia coli*. *Polish Journal of Microbiology* **68**:403–415 DOI 10.33073/pjm-2019-048.

Kotłowski R, Grecka K, Kot B, Szweda P. 2020. New approaches for *Escherichia coli* genotyping. *Pathogens* **9**:73 DOI 10.3390/pathogens9020073.

Kudinha T, Johnson JR, Andrew SD, Kong F, Anderson P, Gilbert GL. 2013. Distribution of phylogenetic groups, sequence type ST131, and virulence-associated

traits among *Escherichia coli* isolates from men with pyelonephritis or cystitis and healthy controls. *Clinical Microbiology and Infection* **19**:E173–E180 DOI 10.1111/1469-0691.12123.

Lara FBM, Nery DR, de Oliveira PM, Araujo ML, Carvalho FRQ, Messias-Silva LCF, Ferreira LB, Faria-Junior C, Pereira AL. 2017. Virulence markers and phylogenetic analysis of *Escherichia coli* strains with hybrid EAEC/UPEC genotypes recovered from sporadic cases of extraintestinal infections. *Frontiers in Microbiology* **8**:146 DOI 10.3389/fmicb.2017.00146.

Lavigne J-P, Boutet-Dubois A, Laouini D, Combescure C, Bouziges N, Marès P, Sotto A. 2011. Virulence potential of *Escherichia coli* strains causing asymptomatic bacteriuria during pregnancy. *Journal of Clinical Microbiology* **49**:3950–3953 DOI 10.1128/JCM.00892-11.

Lodise TP, Chopra T, Nathanson BH, Sulham K, Rodriguez M. 2022. Epidemiology of complicated urinary tract infections due to enterobacterales among adult patients presenting in emergency departments across the United States. *Open Forum Infectious Diseases* **9**:ofac315 DOI 10.1093/ofid/ofac315.

Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection* **18**:268–281 DOI 10.1111/j.1469-0691.2011.03570.x.

Malekzadegan Y, Khashei R, Sedigh Ebrahim-Saraie H, Jahanabadi Z. 2018. Distribution of virulence genes and their association with antimicrobial resistance among uropathogenic *Escherichia coli* isolates from Iranian patients. *BMC Infectious Diseases* **18**:572–572 DOI 10.1186/s12879-018-3467-0.

Maniam L, Vellasamy KM, Jindal HM, Narayanan V, Danaee M, Vadivelu J, Pallath V. 2022. Demonstrating the utility of *Escherichia coli* asymptomatic bacteriuria isolates' virulence profile towards diagnosis and management—a preliminary analysis. *PLOS ONE* **17**:e0267296 DOI 10.1371/journal.pone.0267296.

Mann R, Mediati DG, Duggin IG, Harry EJ, Bottomley AL. 2017. Metabolic Adaptations of Uropathogenic E. coli in the Urinary Tract. *Frontiers in Cellular and Infection Microbiology* **7**:241–241 DOI 10.3389/fcimb.2017.00241.

Momtaz H, Karimian A, Madani M, Safarpoor Dehkordi F, Ranjbar R, Sarshar M, Souod N. 2013. Uropathogenic *Escherichia coli* in Iran: Serogroup distributions, virulence factors and antimicrobial resistance properties. *Annals of Clinical Microbiology and Antimicrobials* **12**:8 DOI 10.1186/1476-0711-12-8.

Morales-Espinosa R, Hernandez-Castro R, Delgado G, Mendez JL, Navarro A, Manjarrez A, Cravioto A. 2016. UPEC strain characterization isolated from Mexican patients with recurrent urinary infections. *Journal of Infection in Developing Countries* **10**:317–328 DOI 10.3855/jidc.6652.

Norinder BS, Köves B, Yadav M, Brauner A, Svanborg C. 2012. Do *Escherichia coli* strains causing acute cystitis have a distinct virulence repertoire? *Microbial Pathogenesis* **52**:10–16 DOI 10.1016/j.micpath.2011.08.005.

Odonkor ST, Ampofo JK. 2013. Escherichia coli as an indicator of bacteriological quality of water: an overview. *Microbiology Research* **4**:e2 DOI 10.4081/mr.2013.e2.

Paniagua-Contreras GL, Monroy-Pérez E, Rodríguez-Moctezuma JR, Domínguez-Trejo P, Vaca-Paniagua F, Vaca S. 2017. Virulence factors, antibiotic resistance phenotypes and O-serogroups of *Escherichia coli* strains isolated from community-acquired urinary tract infection patients in Mexico. *Journal of Microbiology, Immunology and Infection* **50**:478–485 DOI 10.1016/j.jmii.2015.08.005.

Ramírez-Castillo FY, Moreno-Flores AC, Avelar-González FJ, Márquez-Díaz F, Harel J, Guerrero-Barrera AL. 2018. An evaluation of multidrug-resistant *Escherichia coli* isolates in urinary tract infections from Aguascalientes, Mexico: cross-sectional study. *Annals of Clinical Microbiology and Antimicrobials* **17**:34 DOI 10.1186/s12941-018-0286-5.

Rezatofighi SE, Mirzarazi M, Salehi M. 2021. Virulence genes and phylogenetic groups of uropathogenic *Escherichia coli* isolates from patients with urinary tract infection and uninfected control subjects: a case-control study. *BMC Infectious Diseases* **21**:361–361 DOI 10.1186/s12879-021-06036-4.

Robinson AE, Heffernan JR, Henderson JP. 2018. The iron hand of uropathogenic Escherichia coli: the role of transition metal control in virulence. *Future Microbiology* **13**:745–756 DOI 10.2217/fmb-2017-0295.

Roos V, Ulett GC, Schembri MA, Klemm P. 2006. The asymptomatic bacteriuria *Escherichia coli* strain 83972 outcompetes uropathogenic *E. coli* strains in human urine. *Infection and Immunity* **74**:615–624 DOI 10.1128/IAI.74.1.615-624.2006.

Ruiz J, Simon K, Horcajada JP, Velasco M, Barranco M, Roig G, Moreno-Martínez A, Martínez JA, Jiménez de Anta T, Mensa J, Vila J. 2002. Differences in virulence factors among clinical isolates of Escherichia coli causing cystitis and pyelonephritis in women and prostatitis in men. *Journal of Clinical Microbiology* **40(12)**:4445–4449 DOI 10.1128/JCM.40.12.4445-4449.2002.

Sabih A, Leslie SW. 2021. Complicated urinary tract infections. In: *StatPearls*. Treasure Island: StatPearls Publishing Copyright ©2021, StatPearls Publishing LLC.

Sarkar S, Ulett GC, Totsika M, Phan MD, Schembri MA. 2014. Role of capsule and O antigen in the virulence of uropathogenic *Escherichia coli*. *PLOS ONE* **9**:e94786 DOI 10.1371/journal.pone.0094786.

Schifano E, Marazzato M, Ammendolia MG, Zanni E, Ricci M, Comanducci A, Goldoni P, Conte MP, Uccelletti D, Longhi C. 2019. Virulence behavior of uropathogenic *Escherichia coli* strains in the host model Caenorhabditis elegans. *MicrobiologyOpen* **8**:e00756 DOI 10.1002/mbo3.756.

Schwartz DJ, Kalas V, Pinkner JS, Chen SL, Spaulding CN, Dodson KW, Hultgren SJ. 2013. Positively selected FimH residues enhance virulence during urinary tract infection by altering FimH conformation. *Proceedings of the National Academy of Sciences of the United States of America* **110**:15530–15537 DOI 10.1073/pnas.1315203110.

Shahbazi S, Asadi Karam MR, Habibi M, Talebi A, Bouzari S. 2018. Distribution of extended-spectrum β-lactam, quinolone and carbapenem resistance genes, and genetic diversity among uropathogenic *Escherichia coli* isolates in Tehran, Iran. *Journal of Global Antimicrobial Resistance* **14**:118–125 DOI 10.1016/j.jgar.2018.03.006.

Shreya Basu SKM, Hazra Avijit, Mukherjee Mandira. 2013. Molecular characterization of uropathogenic escherichia coli: nalidixic acid and ciprofloxacin resistance. *Virulent Factors and Phylogenetic Background. Journal of Clinical and Diagnostic Research* **7**:2727–2731.

Stork C, Kovács B, Rózsai B, Putze J, Kiel M, Dorn Á, Kovács J, Melegh S, Leimbach A, Kovács T, Schneider G, Kerényi M, Emödy L, Dobrindt U. 2018. Characterization of asymptomatic bacteriuria *Escherichia coli* isolates in search of alternative strains for efficient bacterial interference against uropathogens. *Frontiers in Microbiology* **9**:214 DOI 10.3389/fmicb.2018.00214.

Subashchandrabose S, Mobley HLT. 2015. Virulence and fitness determinants of uropathogenic *escherichia coli*. *Microbiology Spectrum* **3**:4 DOI 10.1128/microbiolspec.UTI-0015-2012.

Tabasi M, Karam MRA, Habibi M, Mostafavi E, Bouzari S. 2016. Genotypic characterization of virulence factors in *Escherichia coli* isolated from patients with acute cystitis, pyelonephritis and asymptomatic bacteriuria. *Journal of Clinical and Diagnostic Research* **10**:DC01-DC07 DOI 10.7860/JCDR/2016/21379.9009.

Tanabe RHS, Dias RCB, Orsi H, de Lira DRP, Vieira MA, Dos Santos LF, Ferreira AM, Rall VLM, Mondelli AL, Gomes TAT, Camargo CH, Hernandes RT. 2022. Characterization of uropathogenic *Escherichia coli* reveals hybrid isolates of uropathogenic and diarrheagenic (UPEC/DEC) E. coli. *Microorganisms* **10**:645 DOI 10.3390/microorganisms10030645.

Tarchouna M, Ferjani A, Ben-Selma W, Boukadida J. 2013. Distribution of uropathogenic virulence genes in *Escherichia coli* isolated from patients with urinary tract infection. *International Journal of Infectious Diseases* **17**:e450-e453 DOI 10.1016/j.ijid.2013.01.025.

Terlizzi ME, Gribaudo G, Maffei ME. 2017. UroPathogenic *Escherichia coli* (UPEC) Infections: virulence factors, bladder responses, antibiotic, and non-antibiotic antimicrobial strategies. *Frontiers in Microbiology* **8**:1566 DOI 10.3389/fmicb.2017.01566.

Tewawong N, Kowaboot S, Pimainog Y, Watanagul N, Thongmee T, Poovorawan Y. 2020. Distribution of phylogenetic groups, adhesin genes, biofilm formation, and antimicrobial resistance of uropathogenic *Escherichia coli* isolated from hospitalized patients in Thailand. *PeerJ* **8**:e10453 DOI 10.7717/peerj.10453.

Vejborg RM, Hancock V, Schembri MA, Klemm P. 2011. Comparative genomics of *Escherichia coli* strains causing urinary tract infections. *Applied and Environmental Microbiology* **77**:3268–3278 DOI 10.1128/AEM.02970-10.

Wiles TJ, Mulvey MA. 2013. The RTX pore-forming toxin α-hemolysin of uropathogenic Escherichia coli: progress and perspectives. *Future Microbiology* **8**:73–84 DOI 10.2217/fmb.12.131.

Yun KW, Kim HY, Park HK, Kim W, Lim IS. 2014. Virulence factors of uropathogenic *Escherichia coli* of urinary tract infections and asymptomatic bacteriuria in children. *Journal of Microbiology, Immunology and Infection* **47**:455–461 DOI 10.1016/j.jmii.2013.07.010.