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Virulence-associated traits and antimicrobial resistance of the uropathogenic *Escherichia coli* (UPEC) strains in relation to phylogenetic background and host factors from Malaysian patients

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ABSTRACT

Aims: Hundreds of uropathogenic *Escherichia coli* (UPEC) lineages with diverse virulence-associated traits have been reported worldwide. However, the complex interactions between the bacterial traits and host factors remain largely unexplored. This study was aimed to determine the distribution of virulence-associated traits, phylogenetic background and antimicrobial resistance profiles of the UPEC isolates in relation to the host factors.
Methodology and results: Polymerase chain reactions (PCRs) were conducted to determine the prevalence of 32 virulence genes (VGs), eight pathogenicity island (PAI) markers and phylogroups of 105 UPEC isolates. The antimicrobial susceptibility testing was performed using the disc diffusion method. Results suggest that the virulence-associated traits and antimicrobial resistance profiles of the UPEC strains were associated with phylogenetic background, host age and gender. Most of the virulence-associated traits tested were distributed prevalently in phylogroup B2, age group 40-59 and male gender, including *papC*, *papG* II_III, *papG* allele III, *sfa/focDE*, *yfcV*, *hlyA*, *cnf1*, *cdtB*, *malX*, PAI I_{536}, PAI II_{536}, PAI I_{CFT073}, PAI II_{CFT073} and PAI II_{J96}. Besides, higher rates of multidrug resistance (MDR) were significantly associated with non-phylogroup B2 (25/38; 65.8%; $p<0.05$) and age group 60-79 (29/42; 69.0%; $p<0.05$) but not with male gender (14/25; 56.0%; $p=0.527$). The UPEC strains collected from males and age 60-79 were significantly resistant to cefuroxime, cefotaxime and ceftazidime (all $p<0.05$).
Conclusion, significance and impact of study: Research findings elucidate the key molecular characteristics of the UPEC strains in relation to the host age and gender.

Keywords: Age, multidrug resistance, urinary tract infection, uropathogenic *Escherichia coli*, virulence traits

INTRODUCTION

Urinary tract infection (UTI) represents the second most common cause of bacterial infections, with approximately 405 million cases reported globally in 2019 (García *et al.*, 2021; Yang *et al.*, 2022; Zeng *et al.*, 2022). UTI is a serious public health issue in terms of morbidity (e.g., infant boys, older men and females of all ages) and mortality (secondary bloodstream infection for catheter-associated UTI patients) (Flores-Mireles *et al.*, 2015). From 1990 to 2019, the age-standardised incidence rate of UTI increased by 1.11 times, from 4715.0 to 5229.3 per 100 000 population, whereas the age-standardised death rate also increased by 1.72 times, from 1.8 to 3.1 per 100 000 population (Zeng *et al.*, 2022).

Uropathogenic *Escherichia coli* (UPEC) is the primary aetiological agent that accounts for almost 80% of UTI cases (Klein and Hultgren, 2020). A successful UTI infection often requires bacterial adherence, internalisation, propagation and dissemination in the host (Terlizzi *et al.*, 2017). Therefore, the UPEC strains usually confer various virulence genes (VGs) that offer pathogenic strategies, such as adhesins, toxins, protectins, iron uptake and miscellaneous (Johnson and Russo, 2018). Some VGs are encoded by a particular region of bacterial chromosome known as pathogenicity

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islands (PAIs) (Ostblom *et al.*, 2011; Desvaux *et al.*, 2020). The PAIs contain mobile elements, such as bacteriophages, plasmids and insertion sequences, which permit the horizontal transfer of VGs (Desvaux *et al.*, 2020). Among the UPEC strains, PAI I-IV of UPEC 536, PAI I and II of UPEC CFT073 and PAI I and II of UPEC J96 are the most studied PAIs (Sabaté *et al.*, 2006).

Extensive literature has developed on unravelling the crucial roles of the VGs in UTI pathogenesis or determining the antimicrobial resistance genes that facilitate the dissemination of multidrug-resistant (MDR) UPEC strains (Biggel *et al.*, 2020; Biggel *et al.*, 2022). Little research emphasised the interactions between the bacterial traits and the host factors, especially for host age (Lin *et al.*, 2021a). Although recent studies have demonstrated that the UPEC strains isolated from elderly patients showed greater antimicrobial resistance and conferred less VGs (Lin *et al.*, 2021a; 2021b), these works were constrained to a specific geographical area. More literature is needed to clarify the relationships between the bacterial traits of the UPEC strains and the host factors. In the present study, we highlight the host age and gender differences in association with the virulence-associated characteristics and antimicrobial resistance profiles of the UPEC strains collected from Malaysian patients.

MATERIALS AND METHODS

Ethical approval

This research project was reviewed and approved by the Medical Research and Ethics Committee of the Ministry of Health Malaysia, with the reference number KKM/NIHSEC/P21-31(4). Besides, prior consent was obtained for collecting the UPEC samples and patient data from Raja Permaisuri Bainun Hospital.

Biological sample

A total of 105 UPEC clinical strains were consecutively collected from Raja Permaisuri Bainun Hospital in Perak, Malaysia, between August 2020 and January 2021. All bacterial isolates were isolated from the urine specimens, with a colony count of more than 100,000 colony-forming units/mL and without mixed growth of two or more microbes (Wilson and Gaido, 2004). All isolates were verified as *E. coli* through the Microflex® LT/SH MALDI-TOF biotyper (Bruker, Germany) and preserved in 15% (v/v) glycerol at -80 °C.

DNA extraction

The fast-boiling method was used for extracting all the template deoxyribonucleic acids (DNAs) (Kor *et al.*, 2013). The concentration and purity of the template DNAs were assessed using the NanoDrop™ 1000 spectrophotometer (Thermo Scientific, United States) before storing at -20 °C.

Detection of virulence genes

A total of 32 VGs, including *papAH*, *papC*, *papEF*, *papG* I, *papG* II_III, *papG* allele I, *papG* allele II, *papG* allele III, *sfa/focDE*, *sfaS*, *focG*, *afa/draBC*, *gafD*, *bmaE*, *fimH*, *yfcV*, *hlyA*, *cnf1*, *cdtB*, *vat*, *kpsMT* II, K1, K5, *kpsMT* III, *rfc*, *fyuA*, *chuA*, *iutA*, *cvaC*, *ibeA*, *traT* and *malX* were tested in this study (Johnson and Stell, 2000; Spurbeck *et al.*, 2012). The multiplex polymerase chain reaction (PCR) assay was separated into six groups, in which the amplification conditions were followed as described by Johnson and Stell (2000). The positive control strains used in the assay were UPEC isolates J96, 2H16, 2H25, V27, PM9 and L31.

Detection of pathogenicity island markers

The presence of 8 PAI markers in the UPEC prototype strains 536 (PAI I_{536}, PAI II_{536}, PAI III_{536} and PAI IV_{536}), CFT073 (PAI I_{CFT073} and PAI II_{CFT073}) and J96 (PAI I_{J96} and PAI II_{J96}) were determined through the multiplex PCR assays (Sabaté *et al.*, 2006), with slight modifications to the concentration of the primers and the PCR reagents. The positive control strains used in the assay were UPEC isolates 536 and J96.

Clermont phylotyping

All UPEC isolates were subjected to the Clermont phylotyping for phylogroup classifications using the GoTaq®G2 DNA Polymerase (Promega, United States) (Clermont *et al.*, 2013). All PCR products were loaded on 2.5% (w/v) agarose gel pre-stained with the EtB“Out” nucleic acid stain (Yeastern Biotech, Taiwan). Then, the agarose gel was visualised under ultraviolet light and captured through the ChemiDoc™ XRS+ with Image Lab™ software (Bio-Rad, United States).

Antimicrobial susceptibility testing

A total of 15 antimicrobials were assessed in this study, including ampicillin, 10 µg; ampicillin-sulbactam, 10/10 µg; amoxicillin-clavulanic acid, 20/10 µg; cefuroxime, 30 µg; cefotaxime, 30 µg; ceftazidime, 30 µg; ertapenem, 10 µg; meropenem, 10 µg; imipenem, 10 µg; gentamicin, 10 µg; nitrofurantoin, 300 µg; trimethoprim-sulfamethoxazole, 1.25/23.75 µg; levofloxacin, 5 µg; ciprofloxacin, 5 µg; fosfomycin, 200 µg. The antimicrobial susceptibility testing was performed using the disc diffusion method as described by the Clinical Laboratory Standard Institute (CLSI) guidelines 2021 (CLSI, 2021). For analysis, results interpreted as intermediate using the CLSI criteria were considered resistant. MDR isolates referred to the isolates that were resistant to at least one antimicrobial in 3 or more different antimicrobial categories (Magiorakos *et al.*, 2012).

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Table 1: Phylogenetic distribution of virulence genes.

VG	No. (%) of UPEC isolates carrying VGs							
	A (n=3)	B1 (n=13)	B2 (n=67)	C (n=1)	D (n=13)	E (n=1)	F (n=7)	Total (n=105)
Adhesin								
papAH	0(0.0)	1 (7.7)[a]	41(61.2)[a]	0(0.0)	2(15.4)[a]	0(0.0)	4(57.1)	48(45.7)
papC	0(0.0)	1(7.7)[a]	38(56.7)[a]	0(0.0)	2(15.4)[a]	0(0.0)	4(57.1)	45(42.9)
papEF	0(0.0)	1(7.7)[a]	38(56.7)[a]	0(0.0)	6(46.2)	0(0.0)	4(57.1)	49(46.7)
papG II_III	0(0.0)	1(7.7)[a]	38(56.7)[a]	0(0.0)	2(15.4)[a]	0(0.0)	3(42.9)	44(41.9)
papG allele II	0(0.0)	1(7.7)	20(29.9)	0(0.0)	2(15.4)	0(0.0)	3(42.9)	26(24.8)
papG allele III	0(0.0)	1(7.7)	23(34.3)[a]	0(0.0)	0(0.0)[a]	0(0.0)	0(0.0)	24(22.9)
sfa/focDE	0(0.0)	1(7.7)	23(34.3)[a]	0(0.0)	0(0.0)[a]	0(0.0)	0(0.0)	24(22.9)
sfaS	0(0.0)	0(0.0)	13(19.4)[a]	0(0.0)	0(0.0)	0(0.0)	0(0.0)	13(12.4)
focG	0(0.0)	1(7.7)	6(9.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	7(6.7)
afa/draBC	0(0.0)	0(0.0)	7(10.4)	0(0.0)	1(7.7)	0(0.0)	0(0.0)	8(7.6)
fimH	2(66.7)	13(100.0)	67(100.0)[a]	1(100.0)	13(100.0)	0(0.0)[a]	6(85.7)	102(97.1)
yfcV	0(0.0)[a]	0(0.0)[a]	65(97.0)[a]	0(0.0)	0(0.0)[a]	0(0.0)	5(71.4)	70(66.7)
Toxin								
hlyA	0(0.0)	0(0.0)[a]	24(35.8)[a]	0(0.0)	1(7.7)	0(0.0)	0(0.0)	25(23.8)
cnf1	0(0.0)	0(0.0)[a]	26(38.8)[a]	0(0.0)	0(0.0)[a]	0(0.0)	0(0.0)	26(24.8)
cdtB	0(0.0)	0(0.0)	1(1.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(1.0)
vat	0(0.0)	2(15.4)[a]	49(73.1)[a]	1(100.0)	1(7.7)[a]	0(0.0)	1(14.3)	54(51.4)
Protectin								
kpsMT II	0(0.0)[a]	1(7.7)[a]	64(95.5)[a]	0(0.0)	12(92.3)	1(100.0)	5(71.4)	83(79.0)
K1	0(0.0)	0(0.0)[a]	26(38.8)[a]	0(0.0)	3(23.1)	1(100.0)	3(42.9)	33(31.4)
K5	0(0.0)	1(7.7)[a]	32(47.8)	0(0.0)	8(61.5)	0(0.0)	2(28.6)	43(41.0)
kpsMT III	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(7.7)	0(0.0)	0(0.0)	1(1.0)
rfc	0(0.0)	1(7.7)	2(3.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(2.9)
Iron-uptake								
fyuA	2(66.7)	5(38.5)[a]	67(100.0)[a]	0(0.0)	11(84.6)	1(100.0)	4(57.1)	90(85.7)
chuA	0(0.0)[a]	0(0.0)[a]	67(100.0)[a]	0(0.0)	13(100.0)	1(100.0)	7(100.0)	88(83.8)
iutA	1(33.3)	5(38.5)	33(49.3)	1(100.0)	9(69.2)	0(0.0)	5(71.4)	54(51.4)
Miscellaneous								
cvaC	1(33.3)	3(23.1)	5(7.5)	1(100.0)	2(15.4)	0(0.0)	0(0.0)	12(11.4)
ibeA	0(0.0)	0(0.0)	5(7.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	5(4.8)
traT	2(66.7)	10(76.9)	42(62.7)	1(100.0)	9(69.2)	0(0.0)	4(57.1)	68(64.8)
malX	1(33.3)	0(0.0)[a]	66(98.5)[a]	0(0.0)	1(7.7)[a]	0(0.0)	3(42.9)	71(67.6)
Median (range)	3(2-4)[a]	4(1-13)[a]	14(5-21)[a]	-	8(4-13)[a]	-	8(6-13)	

papG I, *papG* allele I, *gafD*, *bmaE* were not detected among the UPEC isolates.
VG: Virulence genes, UPEC: Uropathogenic *E. coli*.
[a]$p<0.05$ considered as statistically significant.

Statistical analysis

Statistical analysis was performed using the SPSS version 26 statistical software (IBM, United States). Pearson's chi-square test or Fisher's exact test was used to analyse the categorical variables. The Mann-Whitney *U* test was used to analyse the continuous variables. All the tests were two-tailed and a *p*-value of <0.05 was considered statistically significant.

RESULTS

Prevalence of virulence genes and pathogenicity island markers

The prevalence of 32 VGs among the 105 UPEC isolates ranged from 0% (*papG* I, *papG* allele I, *bmaE* and *gafD*) to 97.1% (*fimH*), as illustrated in Table 1. Among the adhesion genes, *fimH* (102/105; 97.1%) was the most prevalent among the UPEC strains, followed by *yfcV* (70/105; 66.7%), *papEF* (49/105; 46.7%) and *papAH* (48/105; 45.7%). For the toxic genes, *vat* (54/105; 51.4%) was more common than *cnf1* (26/105; 24.8%) and *hlyA* (25/105; 23.8%). All the iron-uptake genes (*fyuA*, *chuA* and *iutA*) were present in over 50.0% of the UPEC isolates. *kpsMT* II (83/105; 79.0%) was widely distributed among the UPEC isolates, in contrast to other protections such as *rfc* (3/105; 2.9%) and *kpsMT* III (1/105; 1.0%). Of the studied miscellaneous genes, *malX* (71/105; 67.6%) and *traT* (68/105; 64.8%) were more commonly distributed as compared to *cvaC* (12/105; 11.4%) and *ibeA* (5/105; 4.8%).

Most of the UPEC isolates (93/105; 88.6%) harboured at least 1 PAI marker, and 11.4% of them (12/105) had 7

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Table 2: Phylogenetic distribution of pathogenicity island markers.

PAI marker	No. (%) of UPEC isolates carrying PAI markers							
	A (n=3)	B1 (n=13)	B2 (n=67)	C (n=1)	D (n=13)	E (n=1)	F (n=7)	Total (n=105)
I_{536}	1(33.3)	1(7.7)	23(34.3)[a]	0(0.0)	0(0.0)[a]	0(0.0)	0(0.0)	25(23.8)
II_{536}	0(0.0)	0(0.0)	23(34.3)[a]	0(0.0)	0(0.0)	0(0.0)	0(0.0)	23(21.9)
III_{536}	0(0.0)	0(0.0)	13(19.4)[a]	0(0.0)	0(0.0)	0(0.0)	0(0.0)	13(12.4)
IV_{536}	2(66.7)	6(46.2)[a]	67(100.0)[a]	0(0.0)	11(84.6)	1(100.0)	4(57.1)[a]	91(86.7)
malX / I_{CFT073}	1(33.3)	0(0.0)[a]	66(98.5)[a]	0(0.0)	1(7.7)[a]	0(0.0)	3(42.9)	71(67.6)
II_{CFT073}	1(33.3)	0(0.0)[a]	53(79.1)[a]	0(0.0)	0(0.0)[a]	0(0.0)	1(14.3)	55(52.4)
II_{J96}	1(33.3)	0(0.0)[a]	24(35.8)[a]	0(0.0)	0(0.0)[a]	0(0.0)	0(0.0)	25(23.8)
Median (range)	1(0-5)	0(0-2)[a]	3(1-7)[a]	-	1(0-2)[a]	-	1(0-2)[a]	

Both primer sets used for detecting the *malX* and PAI I_{CFT073} were from the same reference.
PAI I_{J96} was not detected among the UPEC isolates.
PAI: Pathogenicity island marker, UPEC: Uropathogenic *E. coli*.
[a]$p<0.05$ is considered statistically significant.

Table 3: Prevalence of antimicrobial resistance among different phylogroups.

Antimicrobial	No. (%) of resistant isolates							
	A (n=3)	B1 (n=13)	B2 (n=67)	C (n=1)	D (n=13)	E (n=1)	F (n=7)	Total (n=105)
Ampicillin	3(100.0)	11(84.6)	41(61.2)[a]	1(100.0)	11(84.6)	0(0.0)	5(71.4)	72(68.6)
Amoxicillin-clavulanic acid	1(33.3)	8(61.5)[a]	19(28.4)	0(0.0)	6(46.2)	0(0.0)	1(14.3)	35(33.3)
Ampicillin-sulbactam	1(33.3)	1(7.7)	4(6.0)	0(0.0)	1(7.7)	0(0.0)	0(0.0)	7(6.7)
Cefuroxime	0(0.0)	5(38.5)	16(23.9)	1(100.0)	4(30.8)	0(0.0)	3(42.9)	29(27.6)
Cefotaxime	0(0.0)	5(38.5)	16(23.9)	1(100.0)	3(23.1)	0(0.0)	3(42.9)	28(26.7)
Ceftazidime	0(0.0)	5(38.5)	11(16.4)	1(100.0)	2(15.4)	0(0.0)	3(42.9)	22(21.0)
Ciprofloxacin	3(100.0)[a]	6(46.2)	20(29.9)	1(100.0)	1(7.7)	0(0.0)	1(14.3)	32(30.5)
Levofloxacin	3(100.0)[a]	5(38.5)	22(32.8)	1(100.0)	4(30.8)	0(0.0)	1(14.3)	36(34.3)
Gentamicin	1(33.3)	4(30.8)	11(16.4)	0(0.0)	2(15.4)	0(0.0)	1(14.3)	19(18.1)
Nitrofurantoin	0(0.0)	0(0.0)	1(1.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(1.0)
Trimethoprim-sulfamethoxazole	3(100.0)[a]	6(46.2)	19(28.4)	1(100.0)	3(23.1)	0(0.0)	3(42.9)	35(33.3)
Fosfomycin	0(0.0)	1(7.7)	1(1.5)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(1.9)
Multidrug resistance	3(100.0)	10(76.9)[a]	28(41.8)[a]	1(100.0)	7(53.8)	0(0.0)	4(57.1)	53(50.5)

All isolates were susceptible to ertapenem, imipenem and meropenem.
[a]$p<0.05$ is considered statistically significant.

PAI markers. PAI IV_{536} (91/105; 86.7%) was the most prevalent PAI marker, followed by PAI I_{CFT073} (71/105; 67.6%), PAI II_{CFT073} (55/105; 52.4%) and PAI I_{536} (25/105; 23.8%) (Table 2). Meanwhile, PAI I_{J96} was not detected among the UPEC isolates.

Phylogenetic distribution of the virulence-associated traits and antimicrobial resistance

The UPEC isolates were assigned into seven different phylogroups, in which the phylogroup B2 (67/105; 63.8%) was the most prominent, followed by the phylogroup B1 (13/105; 12.4%) and phylogroup D (13/105; 12.4%) (Table 1 and Table 2). Phylogroup F (7/105; 6.7%), phylogroup A (3/105; 2.9%), phylogroup C (1/105; 0.9%) and phylogroup E (1/105; 0.9%) were less common (Table 1 and Table 2).

Out of the 7 phylogroups, the phylogroup B2 exhibited the highest number of VGs and PAI markers, as shown in Table 1 and Table 2. In addition, 17 VGs (e.g., *papAH*, *papC*, *papEF*, *papG* II_III, *papG* allele III, *sfa/focDE*, *sfaS*, *fimH*, *yfcV*, *hlyA*, *cnf1*, *vat*, *kpsMT* II, K1, *fyuA*, *chuA* and *malX*) and 7 PAI markers (e.g., PAI I_{536}, PAI II_{536}, PAI III_{536}, PAI IV_{536}, PAI I_{CFT073}, PAI II_{CFT073} and PAI II_{J96}) were significantly more prevalent among the phylogroup B2 (Table 1 and Table 2). Remarkably, a consensus virulence-associated traits profile of *fimH*, *yfcV*, *kpsMT* II, *fyuA*, *chuA*, *malX*, PAI IV_{536} and PAI I_{CFT073} was exclusively detected in the phylogroup B2 (63/67; 94.0%).

Overall, the UPEC isolates showed the highest resistance against ampicillin (72/105; 68.6%), followed by levofloxacin (36/105; 34.3%), trimethoprim-sulfamethoxazole (35/105; 33.3%) and amoxicillin-clavulanic acid (35/105; 33.3%) (Table 3). A low prevalence of antimicrobial resistance was observed in ampicillin-sulbactam (7/105; 6.7%), fosfomycin (2/105; 1.9%) and nitrofurantoin (1/105; 1.0%). All UPEC isolates were susceptible to ertapenem, imipenem and meropenem.

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Table 4: Distribution of virulence-associated traits among multidrug-resistant isolates.

Virulence-associated trait	MDR (n=53)	Non-MDR (n=52)	Total (n=105)	*p*-value
VG				
papAH	16(30.2)	32(61.5)	48(45.7)	0.001
papC	14(26.4)	31(59.6)	45(42.9)	0.001
papEF	17(32.1)	32(61.5)	49(46.7)	0.002
papG II_III	13(24.5)	31(59.6)	44(41.9)	<0.0001
papG allele II	13(24.5)	13(25.0)	26(24.8)	0.955
papG allele III	3(5.7)	21(40.4)	24(22.9)	<0.0001
sfa/focDE	5(9.4)	19(36.5)	24(22.9)	0.001
sfaS	0(0.0)	13(25.0)	13(12.4)	<0.0001
focG	4(7.5)	3(5.8)	7(6.7)	1.000
afa/draBC	5(9.4)	3(5.8)	8(7.6)	0.716
fimH	52(98.1)	50(96.2)	102(97.1)	0.618
yfcV	29(54.7)	41(78.8)	70(66.7)	0.009
hlyA	5(9.4)	20(38.5)	25(23.8)	<0.0001
cnf1	5(9.4)	21(40.4)	26(24.8)	<0.0001
cdtB	0(0.0)	1(1.9)	1(1.0)	0.495
vat	19(35.8)	35(67.3)	54(51.4)	0.001
kpsMT II	37(69.8)	46(88.5)	83(79.0)	0.019
K1	14(26.4)	19(36.5)	33(31.4)	0.264
K5	17(32.1)	26(50.0)	43(41.0)	0.062
kpsMT III	0(0.0)	1(1.9)	1(1.0)	0.495
rfc	2(3.8)	1(1.9)	3(2.9)	1.000
fyuA	42(79.2)	48(92.3)	90(85.7)	0.056
chuA	39(73.6)	49(94.2)	88(83.8)	0.004
iutA	35(66.0)	19(36.5)	54(51.4)	0.002
cvaC	6(11.3)	6(11.5)	12(11.4)	0.972
ibeA	1(1.9)	4(7.7)	5(4.8)	0.205
traT	35(66.0)	33(63.5)	68(64.8)	0.782
malX	32(60.4)	39(75.0)	71(67.6)	0.109
Median (range)	9(1-18)	15(1-21)		<0.0001
PAI marker				
I_{536}	7(13.2)	18(34.6)	25(23.8)	0.010
II_{536}	4(7.5)	19(36.5)	23(21.9)	<0.0001
III_{536}	0(0.0)	13(25.0)	13(12.4)	<0.0001
IV_{536}	43(81.1)	48(92.3)	91(86.7)	0.092
I_{CFT073}	32(60.4)	39(75.0)	71(67.6)	0.109
II_{CFT073}	25(47.2)	30(57.7)	55(52.4)	0.280
II_{J96}	6(11.3)	19(36.5)	25(23.8)	0.002
Median (range)	2(0-6)	3(0-7)		0.008

VG: Virulence gene, PAI: Pathogenicity island marker, MDR: Multidrug resistance.

As shown in Table 3, the phylogroup B2 isolates were significantly less MDR (39/67; 58.2%) but displayed a higher rate of resistance towards ampicillin (41/67; 61.2%). In contrast, phylogroup B1 (10/13; 76.9%) was significantly associated with the MDR phenotype (10/13; 76.9%) and amoxicillin-clavulanic acid (8/13; 61.5%) (Table 3).

Association between virulence-associated traits against antimicrobial resistance

In this study, the non-MDR isolates exhibited significantly more virulence-associated traits than the MDR isolates (Table 4). Among the 32 VGs investigated, 13 VGs (e.g., *papAH*, *papC*, *papEF*, *papG* II_III, *papG* allele III, *sfa/focDE*, *sfaS*, *yfcV*, *hlyA*, *cnf1*, *vat*, *kpsMT* II and *chuA*) were significantly more prevalent among the non-MDR isolates, as illustrated in Table 4. Furthermore, the non-MDR isolates also carried more PAI markers, including PAI I_{536}, PAI II_{536}, PAI III_{536} and PAI II_{J96} (Table 4). *iutA* (35/53; 66.0%) was the only exception that was frequently detected among the MDR isolates.

Characteristics of the UPEC strains in relation to host age and gender

Among the five age groups, the UPEC strains isolated from age group 60-79 (42/105; 40.0%) exhibited fewer virulence-associated traits, including *papAH*, *papC*, *papEF*, *papG* II_III, *papG* allele III, *sfa/focDE*, *cnf1*,

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Table 5: Prevalence of virulence-associated traits among different age groups.

Virulence-associated trait	Host age group (years old) (No. (%) of UPEC isolates)					Total
	≤19 (n=9)	20-39 (n=20)	40-59 (n=26)	60-79 (n=42)	≥80 (n=8)	(n=105)
VG						
papAH	3(33.3)	11(55.0)	16(61.5)	13(31.0)[a]	5(62.5)	48(45.7)
papC	3(33.3)	10(50.0)	16(61.5)[a]	11(26.2)[a]	5(62.5)	45(42.9)
papEF	3(33.3)	11(55.0)	16(61.5)	13(31.0)[a]	6(75.0)	49(46.7)
papG II_III	3(33.3)	10(50.0)	16(61.5)[a]	10(23.8)[a]	5(62.5)	44(41.9)
papG allele II	2(22.2)	6(30.0)	7(26.9)	7(16.7)	4(50.0)	26(24.8)
papG allele III	1(11.1)	5(25.0)	12(46.2)[a]	5(11.9)[a]	1(12.5)	24(22.9)
sfa/focDE	1(11.1)	4(20.0)	12(46.2)[a]	5(11.9)[a]	2(25.0)	24(22.9)
sfaS	1(11.1)	3(15.0)	6(23.1)	2(4.8)	1(12.5)	13(12.4)
focG	0(0.0)	1(5.0)	3(11.5)	2(4.8)	1(12.5)	7(6.7)
afa/draBC	2(22.2)	0(0.0)	1(3.8)	5(11.9)	0(0.0)	8(7.6)
fimH	9(100.0)	20(100.0)	25(96.2)	41(97.6)	7(87.5)	102(97.1)
yfcV	6(66.7)	13(65.0)	20(76.9)	26(61.9)	5(62.5)	70(66.7)
hlyA	1(11.1)	5(25.0)	10(38.5)[a]	6(14.3)	3(37.5)	25(23.8)
cnf1	1(11.1)	4(20.0)	12(46.2)[a]	6(14.3)[a]	3(37.5)	26(24.8)
cdtB	0(0.0)	0(0.0)	1(3.8)	0(0.0)	0(0.0)	1(1.0)
vat	6(66.7)	11(55.0)	17(65.4)	18(42.9)	2(25.0)	54(51.4)
kpsMT II	8(88.9)	18(90.0)	23(88.5)	28(66.7)[a]	6(75.0)	83(79.0)
K1	5(55.6)	9(45.0)	8(30.8)	10(23.8)	1(12.5)	33(31.4)
K5	3(33.3)	8(40.0)	14(53.8)	14(33.3)	4(50.0)	43(41.0)
kpsMT III	1(11.1)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(1.0)
rfc	0(0.0)	0(0.0)	1(3.8)	2(4.8)	0(0.0)	3(2.9)
fyuA	8(88.9)	16(80.0)	23(88.5)	35(83.3)	8(100.0)	90(85.7)
chuA	9(100.0)	18(90.0)	22(84.6)	32(76.2)	7(87.5)	88(83.8)
iutA	7(77.8)	7(35.0)	10(38.5)	24(57.1)	6(75.0)	54(51.4)
cvaC	2(22.2)	3(15.0)	2(7.7)	4(9.5)	1(12.5)	12(11.4)
ibeA	0(0.0)	2(10.0)	1(3.8)	2(4.8)	0(0.0)	5(4.8)
traT	6(66.7)	13(65.0)	15(57.7)	29(69.0)	5(62.5)	68(64.8)
malX	6(66.7)	13(65.0)	19(73.1)	28(66.7)	5(62.5)	71(67.6)
Median (range)	9(4-18)	11.5(2-18)	14.5(1-21)[a]	9(1-18)[a]	11.5(5-18)	
PAI marker						
I_{536}	1(11.1)	4(20.0)	10(38.5)[a]	8(19.0)	2(25.0)	25(23.8)
II_{536}	1(11.1)	4(20.0)	11(42.3)[a]	5(11.9)[a]	2(25.0)	23(21.9)
III_{536}	1(11.1)	3(15.0)	6(23.1)	2(4.8)	1(12.5)	13(12.4)
IV_{536}	8(88.9)	16(80.0)	24(92.3)	35(83.3)	8(100.0)	91(86.7)
I_{CFT073}	6(66.7)	13(65.0)	19(73.1)	28(66.7)	5(62.5)	71(67.6)
II_{CFT073}	4(44.4)	6(30.0)[a]	17(65.4)	24(57.1)	4(50.0)	55(52.4)
II_{J96}	1(11.1)	4(20.0)	11(42.3)[a]	7(16.7)	2(25.0)	25(23.8)
Median (range)	2(0-7)	2(0-7)	3(0-7)[a]	3(0-7)	2.5(1-7)	

VG: Virulence genes, PAI: Pathogenicity island marker, UPEC: Uropathogenic *E. coli*.
[a]$p<0.05$ is considered statistically significant.

kpsMT II and PAI II_{536} (Table 5). In contrast, nine virulence-associated traits (e.g., *papC*, *papG* II_III, *papG* allele III, *sfa/focDE*, *hlyA*, *cnf1*, PAI I_{536}, PAI II_{536} and PAI II_{J96}) were significantly prevalent among age group 40-59, ranging from 38.5% to 61.5% (Table 5). The UPEC strains isolated from male patients (25/105; 23.8%) exhibited more virulence-associated traits as compared to female patients, including *yfcV* (21/25; 84.0%), *chuA* (25/25; 100.0%), *malX* (22/25; 88.0%), PAI I_{CFT073} (22/25; 88.0%) and PAI II_{CFT073} (18/25; 72.0%) (Table 6).

The UPEC strains isolated from age group 60-79 had the highest rate of MDR (29/42; 69.0%) and were more resistant towards cefuroxime (18/42; 42.9%), cefotaxime (18/42; 42.9%), ceftazidime (15/42; 35.7%), levofloxacin (21/42; 50.0%) and gentamicin (12/42; 28.6%) (Table 7). However, the UPEC strains from the age group 40-59 were highly susceptible to cefuroxime (23/26; 88.5%) and cefotaxime (23/26; 88.5%). The UPEC strains isolated from male patients had a higher prevalence of MDR (14/25; 56.0%) than those collected from female patients (Table 7). All the antimicrobials tested showed greater resistance among male patients, except for fosfomycin (Table 7). Cefuroxime (11/25; 44.0%), cefotaxime (11/25; 44.0%) and ceftazidime (9/25; 36.0%) were significantly associated with the male gender, as illustrated in Table 7.

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Table 6: Prevalence of virulence-associated traits among different genders.

Virulence-associated trait	Host gender (No. (%) of UPEC isolates)		Total	*p*-value
	Female (n=80)	Male (n=25)	(n=105)	
VG				
papAH	34(42.5)	14(56.0)	48(45.7)	0.237
papC	32(40.0)	13(52.0)	45(42.9)	0.290
papEF	35(43.8)	14(56.0)	49(46.7)	0.284
papG II_III	31(38.8)	13(52.0)	44(41.9)	0.241
papG allele II	17(21.3)	9(36.0)	26(24.8)	0.136
papG allele III	18(22.5)	6(24.0)	24(22.9)	0.876
sfa/focDE	17(21.3)	7(28.0)	24(22.9)	0.483
sfaS	10(12.5)	3(12.0)	13(12.4)	1.000
focG	4(5.0)	3(12.0)	7(6.7)	0.353
afa/draBC	5(6.3)	3(12.0)	8(7.6)	0.392
fimH	78(97.5)	24(96.0)	102(97.1)	0.562
yfcV	49(61.3)	21(84.0)	70(66.7)	0.035
hlyA	18(22.5)	7(28.0)	25(23.8)	0.573
cnf1	18(22.5)	8(32.0)	26(24.8)	0.337
cdtB	0(0.0)	1(4.0)	1(1.0)	0.238
vat	42(52.5)	12(48.0)	54(51.4)	0.694
kpsMT II	60(75.0)	23(92.0)	83(79.0)	0.068
K1	26(32.5)	7(28.0)	33(31.4)	0.672
K5	30(37.5)	13(52.0)	43(41.0)	0.198
kpsMT III	1(1.3)	0(0.0)	1(1.0)	1.000
rfc	2(2.5)	1(4.0)	3(2.9)	0.562
fyuA	66(82.5)	24(96.0)	90(85.7)	0.112
chuA	63(78.8)	25(100.0)	88(83.8)	0.011
iutA	40(50.0)	14(56.0)	54(51.4)	0.600
cvaC	10(12.5)	2(8.0)	12(11.4)	0.727
ibeA	4(5.0)	1(4.0)	5(4.8)	1.000
traT	51(63.7)	17(68.0)	68(64.8)	0.698
malX	49(61.3)	22(88.0)	71(67.6)	0.013
Median (range)	9(1-19)	11(4-21)		0.077
PAI marker				
I_{536}	19(23.8)	6(24.0)	25(23.8)	0.980
II_{536}	17(21.3)	6(24.0)	23(21.9)	0.772
III_{536}	10(12.5)	3(12.0)	13(12.4)	1.000
IV_{536}	67(83.8)	24(96.0)	91(86.7)	0.179
I_{CFT073}	49(61.3)	22(88.0)	71(67.6)	0.013
II_{CFT073}	37(46.3)	18(72.0)	55(52.4)	0.024
II_{J96}	19(23.8)	6(24.0)	25(23.8)	0.980
Median (range)	2(0-7)	3(0-7)		0.045

VG: Virulence genes, PAI: Pathogenicity island marker, UPEC: Uropathogenic *E. coli*.

DISCUSSION

This study gives a representative picture of the molecular epidemiology of the UPEC strains in Perak, Malaysia. Among the seven phylogroups, phylogroup B2 was the most prominent phylogroup that accounted for 63.8% of the UPEC isolates (Table 1 and Table 2). This observation resembled the previous findings in Southeast Asia countries (e.g., Thailand and Vietnam), East Asia countries (e.g., Taiwan, Mongolia and Korea) and European countries (e.g., Romania, Germany and France) (Ramos *et al.*, 2012; Toval *et al.*, 2014; Lavigne *et al.*, 2016; Munkhdelger *et al.*, 2017; Cristea *et al.*, 2019; Tewawong *et al.*, 2020; Hyun *et al.*, 2021; Lin *et al.*, 2021b).

Our results demonstrated that the antimicrobial resistance of the UPEC strains was inversely associated with the prevalence of virulence-associated traits and a B2 phylogenetic background. The phylogroup B2 isolates harboured the highest prevalence of virulence-associated traits but were significantly less MDR (28/67; 41.8%) as compared to other phylogroups (Table 1, Table 2 and Table 3). An extensive repertoire of virulence-associated traits presumably provides diverse pathogenic strategies for this phylogroup to establish a more successful infection. For example, *fyuA*, *chuA*, *yfcV* and *vat*, which

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Table 7: Antimicrobial resistance among different age groups and genders.

Antimicrobial	No. (%) of resistant isolates							*p*-value	Total
	≤19 (n=9)	20-39 (n=20)	40-59 (n=26)	60-79 (n=42)	≥80 (n=8)	Female (n=80)	Male (n=25)		(n=105)
Ampicillin	7(77.8)	15(75.0)	14(53.8)	32(76.2)	4(50.0)	54(67.5)	18(72.0)	0.672	72(68.6)
Amoxicillin-clavulanic acid	2(22.2)	7(35.0)	8(30.8)	17(40.5)	1(12.5)	26(32.5)	9(36.0)	0.746	35(33.3)
Ampicillin-sulbactam	0(0.0)	2(10.0)	0(0.0)	5(11.9)	0(0.0)	4(5.0)	3(12.0)	0.353	7(6.7)
Cefuroxime	1(11.1)	5(25.0)	3(11.5)[a]	18(42.9)[a]	2(25.0)	18(22.5)	11(44.0)	0.036	29(27.6)
Cefotaxime	1(11.1)	5(25.0)	3(11.5)[a]	18(42.9)[a]	1(12.5)	17(21.3)	11(44.0)	0.025	28(26.7)
Ceftazidime	1(11.1)	4(20.0)	2(7.7)	15(35.7)[a]	0(0.0)	13(16.3)	9(36.0)	0.034	22(21.0)
Ciprofloxacin	2(22.2)	4(20.0)	7(26.9)	17(40.5)	2(25.0)	22(27.5)	10(40.0)	0.236	32(30.5)
Levofloxacin	3(33.3)	4(20.0)	6(23.1)	21(50.0)[a]	2(25.0)	24(30.0)	12(48.0)	0.098	36(34.3)
Gentamicin	3(33.3)	1(5.0)	3(11.5)	12(28.6)[a]	0(0.0)	13(16.3)	6(24.0)	0.384	19(18.1)
Nitrofurantoin	0(0.0)	0(0.0)	0(0.0)	1(2.4)	0(0.0)	0(0.0)	1(4.0)	0.238	1(1.0)
Trimethoprim-sulfamethoxazole	3(33.3)	8(40.0)	9(34.6)	14(33.3)	1(12.5)	26(32.5)	9(36.0)	0.746	35(33.3)
Fosfomycin	0(0.0)	0(0.0)	0(0.0)	2(4.8)	0(0.0)	2(2.5)	0(0.0)	1.000	2(1.9)
Multidrug resistance	3(33.3)	9(45.0)	10(38.5)	29(69.0)[a]	2(25.0)	39(48.8)	14(56.0)	0.527	53(50.5)

[a]$p<0.05$ is considered statistically significant.

provide a significant advantage in urinary tract colonisation (Spurbeck *et al.*, 2012), were present in over 70% of the phylogroup B2 (Table 1). PAI markers (e.g., PAI I_{536}, PAI II_{536}, PAI III_{536}, PAI IV_{536}, PAI II_{CFT073} and PAI II_{J96}) and VGs (e.g., *papC*, *papG* allele II, *iutA*, K1, K5, *hlyA* and *malX*) that are associated with the persistence of *E. coli* (Nowrouzian *et al.,* 2001a; 2001b; 2003; Ostblom *et al.*, 2011), were also frequently detected among the B2 isolates. Notably, distinct virulence-associated traits that included *fimH*, *yfcV*, *kpsMT* II, *fyuA*, *chuA*, *malX*, PAI IV_{536} and PAI I_{CFT073} were overrepresented among the phylogroup B2 (63/67; 94.0%). Although some of these VGs (e.g., *fimH*, *kpsMT* II, *fyuA* and *malX*) were observed in other pandemic UPEC lineages (Riley, 2014), their co-occurrence has not been reported. Considering the widespread presence of these virulence-associated traits among the phylogroup B2 isolates, where most of them were not MDR (Table 3), we speculate that these consensus virulence-associated traits may confer a niche-specific selective advantage.

In this study, most virulence-associated traits tested were significantly more prevalent among the non-MDR isolates, except for *iutA*, which was commonly shared among the MDR isolates (35/53; 66.0%; p=0.002) (Table 4). *iutA* can be chromosomally encoded by pathogenicity islands or plasmids (Kudinha, 2017). The high prevalence of *iutA* among the MDR UPEC isolates in the present study suggests that the widespread of *iutA* may be mediated through the MDR plasmids instead of the pathogenicity islands (PAI I_{CFT073}) (Zurfluh *et al.*, 2018).

Recent studies demonstrated that the UPEC strains isolated from elderly patients above 80 years exhibited higher antimicrobial resistance and fewer VGs, including *papG* allele II and *cnf1* (Lin *et al.*, 2021a; 2021b). Here, we observed that the UPEC strains isolated from age group 60-79 showed the highest rate of MDR and exhibited fewer *papAH*, *papC*, *papEF*, *papG* II_III, *papG* allele III, *sfa/focDE*, *cnf1*, *kpsMT* II and PAI II_{536} as compared to other age groups (Table 5 and Table 7). These observations may be attributed to the higher frequency of antimicrobial usage and the decline of the immune system among the elderly age group, which increase the risks of being infected by MDR and low virulence UPEC strains (Lin *et al.*, 2021a; 2021b). Conversely, the high occurrence of virulence-associated traits, such as *papC*, *papG* II_III, *papG* allele III, *sfa/focDE*, *hlyA*, *cnf1*, PAI I_{536}, PAI II_{536} and PAI II_{J96} were observed among the age group 40-59 (Table 5). While the underlying mechanism behind this incident remains to be clarified, these virulence-associated traits have been described to mediate colonisation, internalisation and dissemination of the UPEC strains into deeper layers of the urothelium, which are essential in persisting in the hosts (Ostblom *et al.*, 2011; Lüthje and Brauner, 2014).

Our findings further reinforce the hypothesis that males are less likely to develop UTIs due to low virulence or non-MDR UPEC strains (Kudinha *et al.*, 2013). In the current investigation, the UPEC strains collected from male patients exhibited a higher prevalence of virulence-associated traits and MDR when compared to those collected from female patients (Table 6 and Table 7). Likewise, prior research demonstrated that the UPEC strains isolated from male patients with febrile UTI were relatively virulent, despite the presence of most compromising conditions (e.g., urinary tract instrumentation, diabetes mellitus and renal cortical scarring) (Johnson *et al.*, 2005; Kudinha *et al.*, 2013). Besides, extensive research also revealed that the male gender was significantly associated with the MDR phenotype (Benaissa *et al.*, 2021; Shakya *et al.*, 2021).

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Several limitations were present in this study. The current investigation may encounter type II errors (failure to detect a difference exists) due to the small sample size, or uneven distribution of UPEC isolates among different phylogroups, age groups and gender. While positive PCR results may indicate the presence of the corresponding virulence-associated traits, negative PCR results may not necessarily be equivalent to their absence (Rezatofighi *et al.*, 2021). Although this study focused on the patient demographics such as age and gender, other factors (e.g., urinary catheters, sexual intercourse and anatomical abnormalities of urinary tracts) that predispose to UTI were not investigated (Foxman and Brown, 2003).

CONCLUSION

In conclusion, the UPEC strains showed different characteristics depending on the phylogenetic background, host age and gender. Phylogroup B2 was the most prominent phylogroup that significantly correlated with a higher prevalence of virulence-associated traits but a lower prevalence of MDR. The UPEC strains collected from the age group 40-59 and male gender conferred more virulence-associated traits, whereas those collected from the age group 60-79 and male gender displayed higher rates of antimicrobial resistance.

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