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Phylogenetic background, drug susceptibility and virulence factors of uropathogenic *E. coli* isolate in a tertiary university hospital in central Thailand

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Abstract. Uropathogenic *Escherichia coli* (UPEC) is an important pathogen of the urinary tract infection (UTI) and is most commonly associated with human disease among the extraintestinal pathogenic *E. coli* (ExPEC). The aim of this study is to determine drug susceptibility pattern, virulence factors and the distribution of phylogenetic groups of UPEC in clinical isolates of a tertiary university hospital in central Thailand. A total of 119 UPEC isolates were tested for antimicrobial drug susceptibility. The prevalence of hemolysin, biofilm and the presence of *papC* and *fimH* genes were analyzed. The phylogenetic groups were determined by detection of *yjaA* and *chuA* genes and TspE4.C2 fragment. Antimicrobial susceptibility tests showed that the resistance of UPEC isolates to ampicillin, trimethoprim/sulfamethoxazole, ciprofloxacin, and norfloxacin were 89.1, 60.5, 59.7 and 57.1%, respectively. Moreover, 38.7 and 62.2% of UPEC were ESBL producing and multidrug-resistant strains (MDR), respectively. The prevalence of hemolysin production, biofilm formation, and the presence of *papC* and *fimH* virulence genes were 5.9, 44.5, 29.4 and 82.4%, respectively. Phylogenetic classification revealed that 54.6% of UPEC belonged to group B2, followed by groups D (19.3%), A (15.1%) and B1 (10.9%). When compared to other groups, UPEC belongs to group B2 that harbored a battery of virulence factors and had a high rate of multidrug resistance ($p<0.05$). Stratified according to ciprofloxacin susceptibility, the prevalence of virulence of group B2 resistant strains was lower than the susceptible strains. MDR strains among groups B2 and D showed lower prevalence of virulence than non-MDR isolates. This study showed that multidrug resistance of UPEC and treatment of UTI with antimicrobial agents in this area should be highlighted. Since the relationship between virulence factor and drug susceptibility of UPEC is complex, further studies are required.

INTRODUCTION

Urinary tract infection (UTI) is a major public health problem worldwide. Uropathogenic *Escherichia coli* (UPEC) are classified as extraintestinal pathogenic *E. coli* (ExPEC) and were found to be the most common causative agent of UTI. (Naveen & Mathai, 2005; Merçon *et al.*, 2010). By targeting three genetic markers including *chuA* (heme binding protein), *yjaA* (unknown function) genes and DNA fragment TSPE4.C2, *E. coli* were divided into four phylogenetic groups that included A, B1, B2 and D (Clermont *et al.*, 2000). Phylogenetic groups B2 and D were found to be the predominant groups causing extraintestinal infection which included UTI and carries several virulence genes (Rijavec *et al.*, 2008, Vagarali *et al.*, 2008).

Previous studies have shown that UPEC expressed vast virulence factors involved in the pathogenesis of UTI. The most important of these factors were adhesins or fimbriae,

biofilm formation, and toxins such as hemolysin (Rijavec *et al*., 2008; Vagarali *et al*., 2008). Though a large number of adhesins subclasses have been reported, Type I and P fimbriae are the most common fimbriae found in UPEC strains. They play role in binding and invading bladder epithelial cells (Mulvey, 2002) and kidney epithelial cells, triggering an inflammatory response (Wullt *et al*., 2000; Mabbett *et al*., 2009). UPEC strains were also found to produce a labile pore-forming toxin known as α-hemolysin which is able to lyse erythrocytes and nucleated host cells and induce apoptosis of host cells, leading to bacterial invasion through the mucosal barrier of the urinary tract (Johnson *et al*., 2002, Laura *et al*., 2012; Justin & Hunstad, 2012). Moreover, biofilm, a form of a group of bacteria with the extracellular matrix, promoted growth and persistence of bacteria and protected them from antimicrobial substances, resulting in resistance to antibiotics (Hancock *et al*., 2010; Ponnusamy & Nagappan, 2013). A previous investigation in the southern part of Thailand reported that more than 50% of UPEC belonged to phylogenetic group D carrying a number of virulence factors and having high levels of antimicrobial resistance, especially to the fluoroquinolones. In addition, most of the UPEC isolates produced extended-spectrum β-lactamases (ESBLs) (Themphachana *et al*., 2015). However, the characteristics of UPEC and their antimicrobial susceptibility patterns in Thailand are not well known. Knowledge of antibiotic resistance trends and the etiological agents are important for optimizing the use of effective antibiotics for appropriate treatment of UTI patients. In this study, we aimed to determine the drug susceptibility, virulence factor and a phylogenetic group of UPEC responsible for UTI patients in a tertiary university hospital in central Thailand.

MATERIALS AND METHODS

E. coli isolates

A total of one hundred and nineteen non-duplicate *E. coli* obtained from a urine sample in pure culture at $>10^4$ CFU/ml were collected at Microbiology Laboratory Unit, Thammasat University Hospital between March and April 2013. Bacteria were identified with standard biochemical assays. UPEC were cultured on Mueller-Hinton agar (Oxoid, England) at 37°C for 18-24 hour before use.

Antimicrobial susceptibility testing

Antimicrobial susceptibility test was performed by the Kirby-Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2010). After incubating the inoculated plates aerobically at 37°C for 18–24 h in an aerobic atmosphere, the susceptibility of the *E. coli* isolates to each antimicrobial agent was measured. The results were interpreted. *E. coli* ATCC 25922 was used as quality control. Extended-spectrum β-lactamases (ESBLs) production was determined by using combination disk method. Briefly, antimicrobial disk involved ceftazidime (CAZ, 30 µg), cefotaxime (CTX, 30 µg), ceftazidime/clavulanic acid (CAZ, 30 µg/CLA, 10 µg) and cefotaxime/clavulanic acid (CTX, 30 µg/CLA, 10 µg) (BD, England) were used. The diameter of inhibition zone around antimicrobial disk was measured and ESBLs producing strain was interpreted according to CLSI guideline (CLSI, 2010). Addition, the multidrug-resistant isolate was defined as an isolate with resistance to at least 1 agent in three or more different classes of antibiotics (Magiorakos *et al*., 2012).

Hemolysin

To detect the ability of hemolysin production of UPEC, the bacteria were inoculated into 5% sheep blood agar and incubated at 37°C for 16 hours. Hemolysin production was detected by the presence of a complete clear zone of the erythrocytes around the bacterial colonies (Tabasi *et al*., 2015).

Biofilm formation

The biofilm formation of UPEC was assayed in microtiter plates by using crystal violet based method according to the method of Sato and colleague (2006) with some modification. Bacteria were grown in MHB at 37°C for 24 hours. A 25 µl of bacterial

suspension was dispensed into the well of 96-well plates containing 75 µl LB medium and incubated at 37°C for 24 hr. Supernatant was removed and biofilm was stained with 0.2% (W/V) crystal violet for 15 min. The excess dye was washed 3 times with 200 µl distilled water. To elute dye, 200 µl of absolute ethanol was added. Then, 125 µl of the solution was transferred to the new plate. The optical density at 570 nm was measured by ELISA reader (Dynex, Opsys MR, USA). Each assay was performed in triplicate. A strain was considered to be positive for biofilm production when the absorbance was greater than four-fold the value for a control well without bacteria.

Phylogenetic study and virulence genes detection by PCR

Phylogenetic group and virulence genes were determined by PCR. The primers used in this study are listed in Table 1. Phylogenetic groups were analyzed by detecting *chuA*, *yjaA*, and TSPE4.C2 with 281, 216 and 152 bp, respectively using multiplex PCR as described previously (Doumit *et al.*, 2012). The *papC* and *fimH* genes were detected by PCR as described elsewhere (Sato *et al.*, 2011; Johnson *et al.*, 2000). A *gadA* gene was used as an internal amplification control in all reactions. PCR amplicon was analyzed using 2% agarose gel electrophoresis technique. Gel was stained with ethidium bromide and DNA was observed under UV light with gel documentation system (Biosens SC810, China).

Statistic analysis

For statistical analysis, isolates were regarded as independent samples. Correlation between the prevalence of antimicrobial resistance and virulence factors among UPEC belonging to phylogenetic group B2 and non-group B2 were tested used Fisher's exact test. The virulence factor (VF) score was calculated according to the method previously described elsewhere (Lou *et al.*, 2012). The differences in VF score between phylogenetic groups were analyzed by the Kruskal-Wallis test. A *p*-value of 0.05 was considered statistically signicant.

RESULTS

Antimicrobial susceptibility pattern among UPEC isolates

The antimicrobial susceptibility pattern of UPEC isolates are shown in Figure 1. The prevalence of ampicillin resistance was the highest (89.1%), followed by trimethoprim/

Table 1. Primer sequences used in this study

Gene/Marker	Direction	Primer sequence (5'–3')	Product length (bp)	Reference
Phylogenetic				
chuA	Forward	ATGATCATCGCGGCGTGCTG	281	Doumith *et al.*, 2012
	Reverse	AAACGCGCTCGCGCCTAAT		
yjaA	Forward	TGTTCGCGATCTTGAAAGCAAACGT	216	Doumith *et al.*, 2012
	Reverse	ACCTGTGACAAACCGCCCTCA		
TSPE4.C2	Forward	GCGGGTGAGACAGAAACGCG	152	Doumith *et al.*, 2012
	Reverse	TTGTCGTGAGTTGCGAACCCG		
Adhesin genes				
fimH	Forward	TGCAGAACGGATAAGCCGTGG	508	Johnson *et al.*, 2000
	Reverse	GCAGTCACCTGCCCTCCGGTA		
papC	Forward	GACGGCTGTACTGCAGGGTGTGGCG	328	Sato *et al.*, 2011
	Reverse	ATATCCTTTCTGCAGGGATGCAATA		
Internal control				
gadA	Forward	GATGAAATGGCGTTGGCGCAAG	373	Doumith *et al.*, 2012
	Reverse	GGCGGAAGTCCCAGACGATATCC		

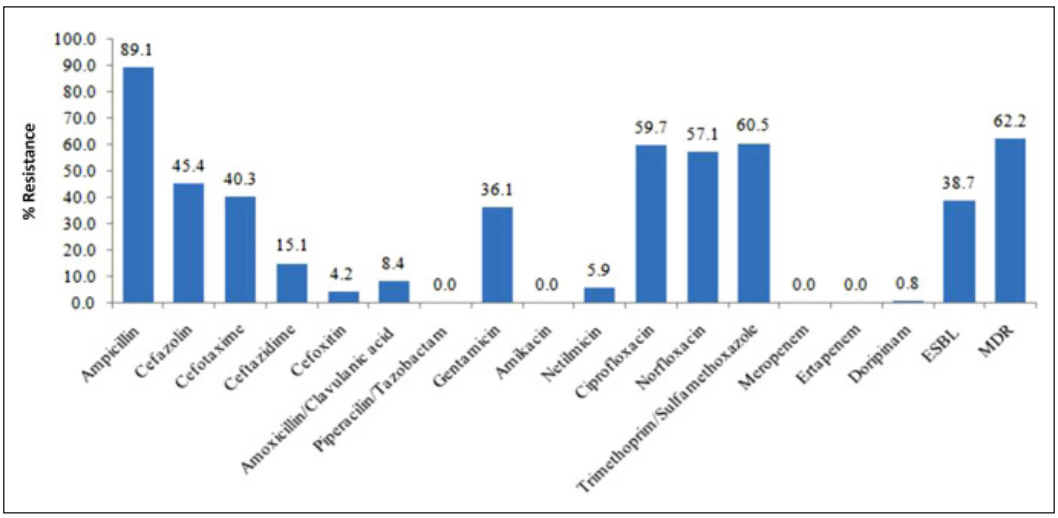

Figure 1. Antimicrobial resistance of UPEC isolates in tertiary university hospital in central Thailand.

Table 2. Distribution of virulence factor of UPEC in different phylogenetic groups

Virulence	Phylogenetic groups [No. of isolates (%)]					*p*-value[a]
	Total (n=119)	A (n=18)	B1 (n=13)	B2 (n=65)	D (n=23)	
Hemolysis	7 (5.9)	0	0	7 (10.8)	0	0.016
Biofilm	53 (44.5)	7 (38.9)	3 (23.1)	34 (52.3)	9 (39.1)	
fimH	98 (82.4)	13 (72.2)	9 (69.2)	57 (87.7)	19 (82.6)	
papC	35 (29.4)	3 (16.7)	1 (7.7)	23 (35.4)	8 (34.8)	
VF≥2	63 (52.9)	6 (33.3)	3 (23.1)	43 (66.2)	12 (52.2)	0.006
VF≥3	22 (18.5)	1 (5.66)	1 (7.7)	18 (27.7)	3 (13.0)	0.019

[a]*p*-values (by Fisher's exact test) are for comparison of indicated virulence factor between group B2 versus other groups.
Only *p*-values of <0.05 are shown.
VFs; number of virulence factor.

sulfamethoxazole (60.5%), and both second-generation fluoroquinolones ciprofloxacin (59.7%) and norfloxacin (57.1%). The isolates showed the highest sensitivity to piperacillin/tazobactam (100%) and carbapenem drug group including ertapenem (100%), meropenem (100%) and doripenem (99.2%). Combination disk method showed that 38.7% of UPEC were ESBL producing strains. In addition, 62.2% were multidrug resistance (Figure 1).

The phylogenetic background and prevalence of virulence factor among UPEC isolates

Of the 119 UPEC isolates, we found that 65 (54.6%), 23 (19.3%), 18 (15.1%) and 13 (10.9%) isolates were classified as group B2, D, A and B1, respectively. Hemolysin production was observed in 7 isolates (5.9%) of UPEC. The remaining isolates showed non-hemolysis. There were 53 isolates (44.5%) able to produce biofilm. From the result of *fimH* and *papC* gene detection obtained by PCR, ninety-eight isolates (82.4%) carried *fimH* and 35 (29.4%) *papC* genes. Comparison of virulence determinant among four phylogenetic groups revealed that the prevalence of virulence determinants involved biofilm, hemolysis, and virulence genes included *fimH* and *papC* gene of group B2 were highest (52.3, 10.8, 87.7 and 35.4%, respectively) followed by group D, A, and B1 (Table 2). All isolate producing hemolysis

belonged to group B2 (p<0.05). Most of the UPEC (52.9%) carried at least 2 types of virulence determinant tested in this study. The presence of virulence factor at least 2 markers (VF≥2) in group B2 was higher than that in non-group B2. The comparison of the VF score revealed that there was significantly different among four phylogenetic groups (p<0.05). The VF scores were highest in group B2, intermediate in group D, and lowest in groups A and B1.

The correlation of phylogenetic groups and virulence factors with ciprofloxacin resistance and MDR among UPEC

When the prevalence of ciprofloxacin resistance and MDR was correlated with phylogenetic groups and virulence factor, the following results were obtained (Table 3). Although group B2 was the most frequent group among both the susceptible and the resistant bacteria. The prevalence of B2 strains was significantly higher among ciprofloxacin-resistant strains and MDR than among susceptible isolates (62.0 vs. 43.8%, respectively; p=0.006) and non-MDR isolates (64.9 vs. 37.8%, respectively; p<0.001). With regard to the distribution of virulence factor, the prevalence of hemolysin and *fimH* did not differ according to the antimicrobial susceptibility. A slightly higher capacity to form biofilms were determined among ciprofloxacin resistant isolates, 50.7%, compared to 35.4% among susceptible isolates, but not statistically significant. We found that a prevalence of UPEC harboring *papC* among ciprofloxacin susceptible and non-MDR isolate was significantly higher (43.8 and 42.2%, respectively) than in ciprofloxacin resistance and MDR (19.7 and 21.6%, respectively) (p<0.05). Ciprofloxacin susceptible and non-MDR isolates exhibited a slightly higher number of virulence factor (VF) than resistant and MDR isolates, but not signicant.

The correlation of drug susceptibility and virulence within phylogenetic group

To determine whether the presence of virulence factors also specifically related to phylogenetic groups, we evaluated the capability to produce hemolysin and form biofilm and the frequency of *fimH* and *papC* of UPEC within each phylogenetic group among susceptible or resistant (Table 4 and 5). Within each group, the prevalence of biofilm formation and *fimH* gene among susceptible and resistant *E. coli* isolates were not significantly different, as found in the total strains (Table 3). The *papC* gene was almost absent in group A and B1 (Table 2). While, hemolysin production were not

Table 3. Correlation of phylogenetic group and virulence factor with ciprofloxacin resistance and MDR among 119 UPEC in a tertiary university hospital in central Thailand

Group or VF	No. (%) of *E. coli* isolates					
	Ciprofloxacin			MDR		
	Susceptible (n=48)	Resistant (n=71)	p-value[a]	non-MDR (n=45)	MDR (n=74)	p-value[a]
A	9 (18.8)	9 (12.7)		11 (24.4)	7 (9.5)	
B1	6 (12.5)	7 (9.9)		8 (17.8)	5 (6.8)	
B2	21 (43.8)	44 (62.0)	0.006	17 (37.8)	48 (64.9)	<0.001
D	12 (25.0)	11 (15.5)		9 (20.0)	14 (18.9)	
Hemolysis	5 (10.4)	2 (2.8)		4 (8.9)	3 (4.1)	
Biofilm	17 (35.4)	36 (50.7)		19 (42.2)	34 (45.9)	
fimH	41 (85.4)	57 (80.3)		37 (82.2)	61 (82.4)	
papC	21 (43.8)	14 (19.7)	0.005	19 (42.2)	16 (21.6)	0.022
VF≥2	27 (56.3)	36 (50.7)		26 (57.8)	37 (50.0)	
VF≥3	12 (25.0)	10 (14.1)		12 (26.7)	10 (13.5)	

[a]Only p-value of <0.05 (by the Fisher exact test) are shown.
VFs; number of virulence factor.

Table 4. Distribution of VFs within each phylogenetic group according to ciprofloxacin resistance phenotype among 119 *Escherichia coli* isolates from UTIs

Virulence	No. (%) of group A isolates (n=18)		No. (%) of group B1 isolates (n=13)		No. (%) of group B2 isolates (n=65)		*p*-value[a]	No. (%) of group D isolates (n=23)	
	Susceptible (n=9)	Resistant (n=9)	Susceptible (n=6)	Resistant (n=7)	Susceptible (n=21)	Resistant (n=44)		Susceptible (n=12)	Resistant (n=11)
Hemolysis	0	0	0	0	5 (23.8)	2 (4.5)	0.031	0	0
Biofilm	3 (33.3)	4 (44.4)	1 (16.7)	2 (28.6)	8 (38.1)	26 (59.1)		5 (41.7)	4 (36.4)
fimH	6 (66.7)	7 (77.7)	6 (100)	3 (42.9)	19 (90.5)	38 (86.4)		10 (83.3)	9 (81.8)
papC	1 (11.1)	2 (22.2)	0	1 (14.3)	14 (66.7)	9 (20.5)	0.001	6 (50.0)	2 (18.2)
VF≥2	3 (33.3)	3 (33.3)	1 (16.7)	2 (28.6)	15 (71.4)	27 (61.4)		8 (66.7)	4 (36.4)
VF≥3	0	1 (1.1)	0	1 (14.3)	10 (47.6)	7 (15.9)	0.014	2 (16.7)	1 (9.1)

[a]*p*-values (by Fisher's exact test) are for comparison of indicated virulence factor between susceptible versus resistant isolates in each phenotypic group. Only *p*-values of <0.05 are shown.
VFs; number of virulence factor.

Table 5. Distribution of VFs within each phylogenetic group according to multidrug resistance (MDR) phenotype among 119 *Escherichia coli* isolates from UTIs

Virulence	No. (%) of group A isolates (n=18)		No. (%) of group B1 isolates (n=13)		No. (%) of group B2 isolates (n=65)		*p*-value[a]	No. (%) of group D isolates (n=23)		*p*-value[a]
	Non-MDR (n=11)	MDR (n=7)	Non-MDR (n=8)	MDR (n=5)	Non-MDR (n=17)	MDR (n=48)		Non-MDR (n=9)	MDR (n=14)	
Hemolysis	0	0	0	0	4 (23.5)	3 (6.3)		0	0	
Biofilm	4 (36.4)	3 (42.9)	2 (25.0)	1 (20.0)	8 (47.1)	26 (54.2)		5 (55.6)	4 (28.6)	
fimH	7 (63.6)	6 (85.7)	6 (75.0)	3 (60.0)	16 (94.1)	44 (91.7)		8 (88.9)	11 (78.6)	
papC	0	3 (42.9)	1 (12.5)	0	12 (70.6)	11 (22.9)	0.001	6 (66.7)	2 (14.3)	0.023
VF≥2	2 (18.2)	4 (57.1)	2 (25.0)	1 (20.0)	14 (82.4)	28 (58.3)		8 (88.9)	4 (28.6)	0.009
VF≥3	0	1 (14.3)	1 (12.5)	0	9 (52.9)	8 (16.7)	0.008	2 (22.2)	1 (7.1)	

[a]*p*-values (by Fisher's exact test) are for comparison of indicated virulence factor between non-MDR versus MDR isolates in each phenotypic group. Only *p*-values of <0.05 are shown.
VFs; number of virulence factor.

found in group A, B1, and D (Table 2). Thus, it was possible to compare the frequencies of hemolysin production and *papC* according to the ciprofloxacin and multidrug resistance phenotype within group B2 and the frequency of *papC* within group D. Among ciprofloxacin susceptible *E. coli* B2 strains, the incidences of hemolysin production and *papC* were 23.8 and 66.7% respectively, whereas, in resistant B2 isolates, the incidence was 4.5% for hemolysin production (p=0.031) and 20.5% for *papC* (p=0.001).

According to multidrug resistance phenotype, the frequency of *papC* among non-MDR isolates within group B2 was significantly higher than among MDR isolate (70.6 vs. 22.9%, p=0.001). The similar results were also found in group D, 66.7% of *papC* among non-MDR vs. 14.3% among MDR (p=0.023). Additional, the presence of virulence factors (VF) among resistance phenotypes in each group were compared. Our results indicate that, within group B2, ciprofloxacin susceptible and non-MDR isolates exhibited the higher number of virulence factor (VF≥3) than susceptible isolates (p<0.05). Within group D, the presence of virulence factor at least 2 markers (VF≥2) in non-MDR isolate was also higher than that in MDR (p=0.009).

DISCUSSION

In this study, the prevalence of antimicrobial resistance was relatively high among drugs commonly used as empirical therapy such as ampicillin, trimethoprim/sulfamethoxazole, and both second-generation fluoroquinolones. Moreover, most of them were MDR strain. Our findings are consistent with previous studies reported in Thailand and another country (Polwichai *et al.*, 2009; Themphachana *et al.*, 2015; Tabasi *et al.*, 2015). This may be due to the high rates of prescription of these drugs, unprescribed and inappropriate use in our country and also the transfer of resistant isolates (Lina *et al.*, 2007).UPEC have different types of fimbriae which promote bacterial attachment to host tissue within urinary tract (Bien *et al.*, 2012). From the result of *fimH* and *papC* gene detection obtained by PCR, ninety-eight isolates (82.4%) carried *fimH* and 35 (29.4%) *papC* genes. This was almost similar with previously reported data (Sato *et al.*, 2006; Er *et al.*, 2015; Lee *et al.*, 2016). It may indicate a crucial role of *fimH* in the pathogenesis of UPEC. Whereas, 29.4% possessed P fimbriae. It is in agreement with the previous study which showed that P fimbriae were expressed by only a limited number of *E. coli* strains (Hagan & Mobley, 2007). However, P fimbriae play role in binding to kidney epithelial cells and trigger specic signaling pathways leading to mucosal inflammation and surrounding tissue damage (Wullt *et al.*, 2000; Mabbett *et al.*, 2009). Biofilm, a structured community of bacterial cells embedded in an extracellular polymeric matrix, allows the UPEC to persist a long time in the urinary tract and interfere with bacterial eradication (Sato, 2014). In our study, biofilm formation was found in 53 isolates (44.5%). α-hemolysin is responsible for promoting bacterial invasion through the epithelial barrier (Laura *et al.*, 2012; Justin & Hunstad, 2012). The hemolysin was produced in 7 isolates (5.9%) of UPEC tested.

E. coli strains can be classified into four main phylogenetic groups including A, B1, B2 and D (Clermont *et al.*, 2000; Doumit *et al.*, 2012). The predominate extraintestinal pathogenic strains belong to phylogenetic groups B2 and D (Rijavec *et al.*, 2008; Vagarali *et al.*, 2008). As an aspect, we found that 65 (54.6%) of the isolates tested belonged to group B2, 23 (19.3%) to group D, 18 (15.1%) to group A and 13 (10.9%) to the B1 group. The distribution of a phylogenetic group of UPEC in our study is discordance to those obtained in the southern part of Thailand which reported the predominant of UPEC group D carrying a number of virulence factors (Themphachana *et al.*, 2015). In addition to the geographic variation, differences in host population characteristics and differences in sampling methods, we believe that the disagreement may be due to the difference DNA primers used in the phylogenetic study. Since the assay with primers originally described by Clemont *et al.* (2000) used in

the previous study had low amplification efficiencies and can give the inconsistent PCR amplification result. It led to some isolates being assigned to a seemingly anomalous phylogenetic group. In this study, the assay modified by Doumit *et al.* (2012) with new primers designed to accommodate sequence variations in the three targeted markers was used. This modified assay has been found to be more sensitive and reliable and better congruence with MLST data (Doumith *et al.*, 2012).

According to our result, the prevalence of virulence factor involved biofilm and virulence genes included *fimH* and *papC* gene were highest for group B2 followed by group D, A, and B1. All UPEC strains producing hemolysin belonged to phylogenetic group B2. Moreover, the comparison revealed the highest incidence of virulence factor in group B2 ($p<0.05$). The results emphasize the fact that group B2 is the most virulent group (Rijavec *et al.*, 2008, Vagarali *et al.*, 2008).

Despite our investigation found that the prevalence of ciprofloxacin resistance and MDR were associated to groups B2 (62.0 and 64.9%, respectively). Disagreement to previous studies demonstrated the association of ciprofloxacin resistance and MDR towards the non-B2 phylogenetic group (Johnson *et al.*, 2005; Moreno *et al.*, 2006; Rijavec *et al.*, 2008). The association of ciprofloxacin susceptibility and MDR with certain virulence factors of UPEC including *papC*, *hlyA* and *cnf*1 has been reported (Johnson *et al.*, 2005; Rijavec *et al.*, 2008; Piatti *et al.*, 2008; Harwalkar *et al.*, 2014). In this study, the prevalence of *papC* gene among ciprofloxacin resistant and MDR were significantly lower than ciprofloxacin susceptible and non-MDR strains. We also found that the prevalence of virulence of group B2 with ciprofloxacin resistant including *papC* and hemolysin was lower than the susceptible strains. While, MDR strain among groups B2 and D showed lower prevalence of *papC* than non-MDR isolates. This results confirmed the association of susceptibility to antibiotics and virulence in UPEC. The principal basis of that phenomenon is still unclear whether a low presence of certain virulence factor precedes resistance (Johnson *et al.*, 2005) or the low present of certain virulence factor follows the acquisition of a certain type of resistance (Piatti *et al.*, 2008) or both mechanism coexist. Since the relationship between virulence factor and drug susceptibility is complex, further study is required.

In summary, UPEC isolated in the central Thailand were highly resistance to ampicillin, trimethoprim/sulfamethoxazole, and fluoroquinolones. A number of antimicrobial agents such as β-lactam/clavulanic acid, cefoxitin, ceftazidime, and carbapenem can still be used in the therapy. In this study, most of the UPEC belonged to phylogenetic groups B2 which possessed a battery of virulence factors and have a high rate of multidrug resistance, making them a serious and challenging health problem in this area. This study augments the need for public awareness on multidrug resistance and the proper and careful use of antimicrobial agents.

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