Prevalence, antibiogram and virulence gene profiles of uropathogenic *Escherichia coli* from pregnant women with urinary tract infection

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Urinary tract infection (UTI) is commonly caused by *Escherichia coli* and occurs when bacteria colonize the bladder. Pregnant women have a higher risk of contracting UTI, a situation which may lead to birth complications. UTI treatment is complicated by drug resistance which limits treatment options for pregnant women. The objectives of this study were to determine the prevalence, antibiogram and virulence gene profiles of uropathogenic *E. coli* (UPEC) among pregnant women visiting the outpatient department of a tertiary hospital in Metro Manila. Culture methods were utilized to determine significant bacteriuria and to isolate UPEC, while PCR was performed to confirm the presence of UPEC and other UTI-causing bacteria, and to detect the presence of virulence genes. Disk diffusion assay was done to determine the antibiogram profiles of the isolates. Twenty-one percent of the samples were positive for UTI, 42% of which were UPEC-positive. Sixty three percent of UPEC isolates were resistant to ampicillin, 50% to amoxicillin-clavulanate and 25% to cephalothin. Multidrug resistance phenotypes were observed and genes for P fimbriae, Type 1 fimbriae adhesion, and Dr II adhesin were detected from the UPEC isolates. The results confirmed the increase as well as high incidence of drug resistance among UPEC isolates, through antibiotic susceptibility tests (AST). It is recommended that AST be done prior to prescription of drugs for UTI and that fosfomycin be used as an alternative to the treatment of UTI in pregnant women.

KEYWORDS

Urinary tract infection, pregnant women, *Escherichia coli*, antibiotic resistance, virulence genes

INTRODUCTION

Urinary tract infection (UTI) occurs when bacteria enter the urinary tract through the urethra and multiply in the bladder. It occurs mainly in women and is commonly caused by *Escherichia coli* (Matuszkiewicz-Rowińska et al. 2015). Pregnant women have a higher risk of acquiring UTI due to physiological and mechanical changes during pregnancy which include glycosuria and urinary stasis that encourage bacterial growth (Bánhidy et al. 2007). If left untreated, UTI may lead to preterm birth and low birth weight (LBW) which occur more frequently in mothers with UTI, whether asymptomatic or symptomatic (Bánhidy et al. 2007, Colgan et al. 2006).

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Table 1: Category and break points of the antimicrobial agents for Enterobacteriaceae. List of the antimicrobial agents used, their antimicrobial category, US FDA drug category, and breakpoints for Kirby- Bauer disk diffusion assay (CLSI, 2014).

Antimicrobial category	Antimicrobial agent	Drug category	Concentration (µg)	Zone diameter in mm		
				S	I	R
Penicillin	Ampicillin	B	10	≥ 17	14-16	≤ 13
ß-lactam/ ß-lactamase inhibitor combination	Amoxicillin-clavulanate	B	20 or 10	≥ 18	14-17	≤ 13
Cephems	Cephalothin	B	30	≥ 18	15-17	≤ 14
Fosfomycin	Fosfomycin	B	200	≥ 16	13-15	≤ 12
Nitrofurans	Nitrofurantoin	B	300	≥ 17	15-16	≤ 14
Folate pathway inhibitor	Trimethoprim-sulfamethoxazole	C	1.25 or 23.75	≥ 16	11-15	≤ 10

Treatment options suitable for pregnant women are limited (Ghouri et al. 2018, Krcmery et al. 2001, Delzell and Lefevre, 2000). Pregnant women in their first trimester (1-12 weeks) are discouraged from taking medications due to increased risk of teratogenicity to the fetus (Thorpe et al. 2013). Some antibiotics have been shown to cause miscarriage or fetal abnormalities (Gilstrap and Ramin, 2001). Medicines are classified into different categories by the United States Food and Drug Administration (US FDA) according to their safety for introduction during pregnancy, where Category A and Category B drugs are the safest for use (Matuszkiewicz-Rowińska et al. 2015). Uropathogens, detected in UTI, have become more resistant to antibiotics. High rates of resistance to amoxicillin and ampicillin have been documented in *E. coli* isolates from pregnant women with UTI (Demilie et al. 2012).

Virulence factors also play key roles in the persistence of uropathogenic *E. coli* (UPEC). These factors, such as those for adhesion, toxin production, and iron-sequestering system enable UPEC to successfully establish and colonize the urinary tract and cause symptoms associated with UTI (Lüthje and Brauner, 2014). Adhesins are important virulence factors since the first step for the establishment of UPEC is through its tight adherence to urothelial cells. Examples of adhesins are Type 1 fimbriae, Dr adhesins, and P fimbriae. Type 1 fimbriae, which is encoded by the *fimH* gene, is an established virulence factor in UPEC that is needed for successful infection. In spite of the mechanical stress present in the urinary tract, Type 1 fimbria is able to bind tightly to the urothelial surface (Lüthje and Brauner, 2014; Bien et al. 2012). Dr adhesins are often associated with diarrhea and UTI in children and pregnant women and is encoded by *draE2* gene (Nowicki et al. 2001). P fimbria, encoded by the *pap* gene (Bien et al. 2012), is second to Type 1 fimbriae as the most common virulence factor in UPEC. It adheres to the mucosal and tissue matrix and produces cytokines. It is associated with pyelonephritis and ascending UTI as it allows for the early colonization of the tubular epithelium.

In the Philippines, little is known about the antibiogram and virulence gene profiles of UPEC. Since antimicrobial resistance complicates treatment options, it is important to determine which drugs remain effective in treating UTI. The objective of this study was to determine the prevalence, antibiogram, and virulence gene profiles of UPEC among pregnant women visiting the outpatient department of a tertiary hospital in Metro Manila.

METHODOLOGY

Ethical consideration

The study involved pregnant women. Permit to conduct sample and data collection was obtained from the OB-GYN Outpatient Department and Head of the tertiary hospital where sampling was conducted. The details of the study protocol, which was approved by the Far Eastern University-Nicanor Reyes Medical Foundation Institutional Ethics Review Committee (FEU-NRMF IERC 2016-0126), were explained to the participants individually. It was made clear that participation is voluntary and can be discontinued at any time. Written consent forms were obtained and all data gathered were treated as highly confidential with the identities of the participants being kept anonymous.

Sample and data collection

From an average of 1,032 pregnant patients per month, a minimum sample size of 88 patients was determined using Survey Systems (Creative Research Systems, nd) with a confidence level of 95% and a confidence interval of 10. Random sampling was done among pregnant women who were not on antibiotic treatment at least one week before sample collection. Socio-demographic and clinical data were obtained using questionnaires. Each participant was instructed to collect a clean-catch midstream urine specimen with a sterile screw-capped wide-mouth container. Samples were then coded, sealed, and stored at 4°C to prevent false positive results in bacteriuria and were transported, thereafter, to the Medical Microbiology Laboratory (MML), Institute of Biology, University of the Philippines Diliman, within 24 hours of collection, for processing (Delanghe and Speeckaert 2014; Aspevall et al. 2002). Sampling was done for one month.

Culture and bacteriuria determination

Urine samples were diluted to 10^{-2} with sterile 0.1% peptone. Approximately 0.1 mL of the diluted samples was spread on Blood Agar (BAP), MacConkey Agar (MCA), and Eosin-

Table 2: Socio-demographics and clinical variables of pregnant women (n=90) visiting the OB-GYN OPD of a tertiary hospital in Manila

Risk Factors	Number (n=90)	Percentage (%)
Age		
14-20	24	26.7
21-27	30	33.3
28-34	27	30
35-41	9	10
Gravidity		
Primigravida	36	40
Multigravida	46	51.1
Grand multigravida	8	8.9
Parity		
Nullipara	6	6.7
Primipara	36	40
Multipara	42	46.7
Grand multipara	6	6.7
Gestational age		
First trimester	9	10
Second trimester	18	20
Third trimester	63	70
Livelihood		
None	51	56.7
Employed	26	28.9
Self-employed	13	14.4
Educational status		
<Grade School	4	4.4
High School	28	31.1
Vocational	8	8.9
College	50	55.6
Sexually active[a]		
Yes	40	44.4
No	50	55.6
UTI History		
Yes	60	66.7
No	30	33.3

[a] state of being sexually active is during the time of pregnancy

Table 3: Prevalence of *E. coli*, *Klebsiellasp.*, and S. *saprophyticus* based on PCR amplicons from all urine samples (n=90), and from UTI positive samples (n=19). Samples were determined to be UTI positive through colony count.

Bacteria	All samples n (%)	UTI positive samples n (%)
Escherichia coli	29 (32.2%)	8 (42.1%)
Klebsiellasp	16 (17.8%)	2 (10.5%)
Staphylococcus saprophyticus	3 (3.3%)	0 (0%)

n=number of samples positive for the specified bacteria

Methylene Blue Agar (EMBA) plates and incubated at 37°C for 24 hours (Delost 2014). Urine samples that had $\geq 10^5$ CFU/mL for BAP and/or MCA were considered as having significant bacteriuria (Wilson and Gaido 2004). Colonies with a metallic green sheen on EMBA were considered as putative *E. coli* isolates and were purified through streak plate method.

Bacterial identification

Bacterial DNA was extracted using the boiling method (Binet et al. 2014). Polymerase chain reaction (PCR) procedure was used to determine the presence of *E. coli*, *Klebsiella* sp., and *Staphylococcus saprophyticus* using primers and PCR conditions adapted from published literature (Liu et al. 2008;

Table 4: Chi- square value (P), Odd's ratio (OR), and confidence interval (CI) results for the association of risk factors with UTI (UTI-positive cultures n=19; UTI-negative culture n=71)

	Culture results		
Risk Factors	**UTI**	**Non UTI**	**P, OR, (95%CI)**
Age			
14-20	3	21	
21-27	8	22	0.199, 2.545 (0.594-10.910)
28-34	6	21	0.697, 0.786 (0.233-2.65)
35-41	2	7	1, 1 (0.233-2.65)
Gravidity			
Primigravida	5	31	
Multigravida	12	34	0.176, 0.457 (0.145-1.445)
Grand multigravida	2	6	0.948, 1.059 (0.188-5.974)
Parity			
Nullipara	0	6	
Primipara	7	29	0.237,0.00 (1.057-1.457)
Multipara	12	30	0.349, 0.603 (0.208-1.747)
Grand multipara	0	6	0.131, 0.00 (1.156-1.695)
Gestational age			
First trimester	0	9	
Second trimester	5	13	0.08, 1.385 (1.04-1.844)
Third trimester	14	49	0.624, 0.743 (0.226-2.442)
Livelihood			
None	13	38	
Employed	3	23	0.154, 2.623 (0.675-10.198)
Self-employed	3	10	0.346, 2.30 (0.394-13.424)
Educational status			
<Grade School	0	4	
High School	5	23	0.358, 0.00 (1.024-1.447)
Vocational	1	7	0.72, 0.657 (0.065-6.605)
College	13	37	0.407, 0.407 (0.046-3.628)
Sexually active[a]			
Yes	6	34	
No	13	37	0.204, 0.502 (0.172-1.47)
UTI History			
Yes	15	45	
No	4	26	0.201, 2.167 (0.65-7.222)

[a] where the state of being sexually active is during the time of pregnancy

Higgins et al. 2001; Martineau et al. 2000). These three bacteria are the leading causes of UTI with *E. coli* as the most common (Behzadi et al. 2010; Delzell and Lefevre, 2000). PCR evaluation of *Klebsiella* sp. and *S. saprophyticus* was done to determine whether *E. coli* is still the most common cause of UTI. DNA templates from *E. coli* (BIOTECH 1634), *Klebsiella sp.* (BIOTECH 1754), and *S. saprophyticus* (BIOTECH 1802) were used as positive controls while PCR amplification of the 16S rRNA gene was used as the internal control.

Virulence gene profile

PCR was used to determine the presence of the *fimH*, *pap*, and *draE2* genes by using primers and PCR conditions adapted from published literature (Johnson et al. 2013; Tiba et al. 2008; Matar et al. 2005; Birošova et al. 2004).

Antibiotic susceptibility test

Standard procedures from the Clinical and Laboratory Standards Institute (CLSI 2014) were followed in disk diffusion assay for *E. coli* isolates. Briefly, isolates were diluted to match a 0.5 McFarland standard using 0.9% saline solution and were streaked on Mueller Hinton Agar (MHA) plates using a sterile cotton swab. Antibiotic disks were placed 24 mm apart from center to center after which three antibiotic disks were placed on the MHA plates. The antibiotics used were ampicillin (AMP), amoxicillin-clavulanate (AMC), cephalothin (CEP), fosfomycin (FOS), nitrofurantoin (NIT), and trimethoprim-sulfamethoxazole (COT). The plates were incubated at 37°C for 18 hours (CLSI 2014; Schwalbe et al. 2007) after which the zones of inhibition were measured. The antibiogram profile was determined for each isolate using breakpoint from CLSI (Table 1).

Table 5: Prevalence of virulence factors: Type 1 fimbriae, P fimbriae, and Dr II adhesin, in all *E. coli*-positive samples (n=29), and in UTI-positive *E. coli* samplesor UPEC (n=8)

Virulence factor	All *E. coli* positive samples n_1 (%)	UTI positive *E. coli* samples n_2 (%)
Type 1 fimbriae	24 (82.7%)	6 (75%)
P fimbriae	20 (68.9%)	4 (50%)
Dr II adhesin	1 (3.4%)	1 (12.5%)

n_1=number of samples positive for *E. coli*
n_2=number of samples positive for UTI and *E. coli*

Table 6: Antibiogram profile of all *E. coli* positive samples (n=29) based on the incidence of susceptible, intermediate, resistant, and multiple drug resistant isolates (and the number of antibiotics they are resistant to).

Antibiogram profile	Incidence/total	Percentage (%)
Susceptible to all	2/29	6.9
Intermediate to at least one	7/29	24.1
Resistant to at least one antibiotic	20/29	69
MDR (at least 3 antibiotics)	7/29	24.1
MDR (of those resistant to at least one)	7/20	35
MDR (resistant to 4 antibiotics)	1/29	3.4
MDR (resistant to 5 antibiotics)	2/29	6.9

Table 7: Antibiogram profile (S-susceptible, I-intermediate, R-resistant) of all *E. coli* positive samples (n=29) to different antibiotics and the percentage of *E. coli* positive samples resistant to the antibiotics based on CLSI (2014) standards

Antibiotic	S	I	R	% Resistant
Ampicillin	10	0	19	65.6
Amoxicillin-clavulanate	11	1	17	58.6
Cephalothin	9	13	7	24.1
Nitrofurantoin	24	1	4	13.7
Trimethoprim-sulfamethoxazole	25	1	3	10.3
Fosfomycin	28	1	0	0

Data analysis
Chi-square test and odds ratio were used to determine significant statistical association between culture results, UTI or non-UTI, and risk factors considered in the study. Results were deemed significant if $p < 0.05$ (Emiru et al. 2013; Szumillas, 2010).

RESULTS

Socio-demographic and clinical characteristics
Of 90 pregnant women who participated in the study, 37% were 21-27 years old, 55.60% had college education, 56.70% were unemployed, 55.60% were not sexually active during pregnancy, and 66.70% had a history of UTI (66.7%). Many of them, at 46.70%, were multiparous while more than half, at 51.10%, are multigravidous. Many of them, at 70%, were in their third trimester of pregnancy (Table 2).

Prevalence of UTI and bacterial profile
Colony counts, at $\geq 10^5$ CFU/mL revealed the presence of UTI, while PCR confirmed the presence of *E. coli, Klebsiella* sp., and *S. saprophyticus.* Nineteen, or 21.1%, of the participants had significant bacteriuria ($\geq 10^5$CFU/ mL) while 29, or 32.2%, were positive for *E. coli*. Sixteen samples, or 17.8%, were positive for *Klebsiella* sp., while only 3, 3.3%, samples were positive for *S. saprophyticus*. Eight samples, or 8.9%, were positive for both *E. coli* and *Klebsiella* sp. All UTI-positive samples were negative for *S. saprophyticus* while 9, or 47.4%, that were positive for bacteriuria were negative for *E. coli*, *Klebsiella* sp., and *S. saprophyticus*. Eight, or 42.1%, samples that were UTI-positive were likewise positive for *E. coli* (Table 3).

Associated risk factor for UTI
The assessment of potential risk factors of UTI showed that: age (using 14-20 years as a reference, 21-27 years; P=0.199, OR=2.545, CI= 0.594,10.910, 28-34 years; P=0.697, OR=0.786, CI=0.233, 2.65, 35-41 years; P=1.00, OR=1.000, CI=0.233, 2.65), gravidity (taking primigravida as a reference: multigravida; P= 0.176, OR= 0.457, CI=0.145,1.445, grand multigravida; P= 0.948, OR= 1.059, CI= 0.1888, 5.974), parity (taking nullipara as a reference: primipara; P=0.237, OR=0.000, CI=1.057, 1.457, multipara; P=0.349, OR= 0.603, CI=0.208, 1.747, grand multipara; P=0.131, OR= 0.000, CI=1.156,1.695), gestational age (taking first trimester as a reference, second trimester; P=0.08, OR= 1.385, CI: 1.04, 1.844, third trimester; P=0.624, OR=0.743, CI=0.226, 2.442), livelihood (taking none as reference, employed; P=0.154, OR=2.623, CI= 0.675, 10.198, self-employed; P= 0.346, OR= 2.30, CI= 0.394,13.424), educational status (taking grade school or lower as reference, high school; P=0.358, OR=0.000, CI=1.024,1.447, vocational; P=0.72, OR=0.657, CI=0.065, 6.605, college ; P=0.407; OR=0.407; CI= 0.046, 3.628), state of being sexually active (P=0.204, OR=0.502, CI=0.172, 1.47), and history of UTI (P=0.201, OR= 0.502, CI=0.172, 1.47) were not significantly associated with UTI (Table 4).

Virulence gene profile
Out of 29 *E. coli*-positive samples, 24 or 82.75% were positive for *fimH* while 20 or 68.9% samples were positive for *pap*, and

Table 8: Antibiogram profile (S-susceptible, I-intermediate, R-resistant) of all UTI-positive *E. coli*-positive samples or UPEC (n=8) to different antibiotics and the percentage of UPEC samples resistant to the antibiotics based on CLSI (2014) standards

Antibiotic	S	I	R	% Resistant
Ampicillin	3	0	5	62.5
Amoxicillin-clavulanate	4	0	4	50
Cephalothin	2	4	2	25
Nitrofurantoin	7	1	0	0
Trimethoprim-sulfamethoxazole	7	1	0	0
Fosfomycin	7	1	0	0

Table 9: Antibiogram profile of all UTI positive- *E. coli* positive samples (n=8) based on the incidence of susceptible, intermediate, resistant, and multiple drug resistant isolates (and the number of antibiotics they are resistant to).

Antibiogram profile	incidence/total	Percentage (%)
Susceptible to all	0/8	0
Intermediate to at least one	3/8	37.5
Resistant to at least one antibiotic	5/8	62.5
MDR (at least 3 antibiotics)	1/8	12.5

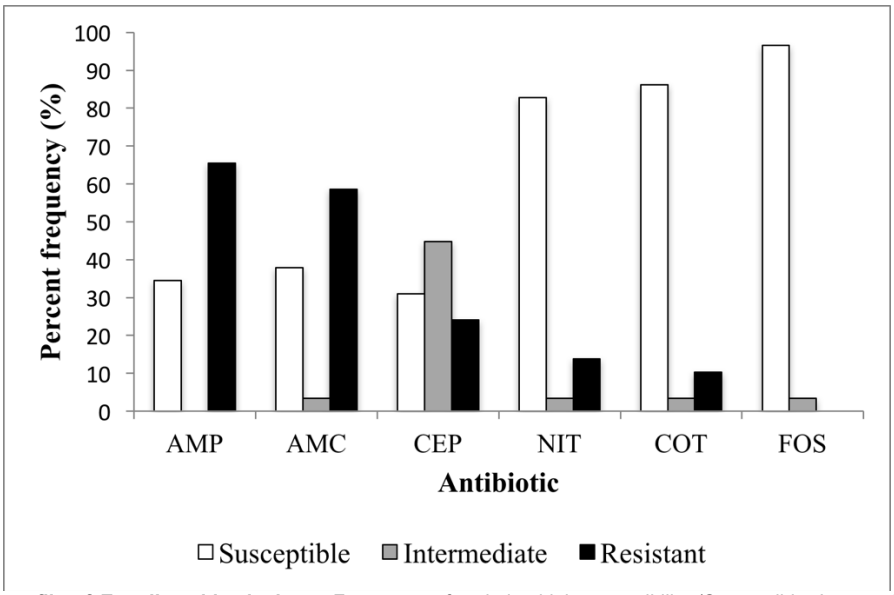

Figure 1: Antibiogram profile of *E. coli* positive isolates. Frequency of antimicrobial susceptibility (Susceptible, Intermediate, or Resistant) of E. coli isolates from all samples (n=29). AMP: ampicillin, AMC: amoxicillin-clavulanate, CEP: cephalothin, NIT: nitrofurantoin, COT: trimethoprim-sulfamethoxazole, and FOS: fosfomycin)

1 or 3.4% was positive for *draE2*. Out of the 8 UPEC samples, 75, 50 and 12.5% were positive for *fimH*, *pap*, and/or *draE2*, respectively (Table 5).

Antibiogram profile

From the 29 *E. coli*-positive isolates, only 2, or 6.9%, were susceptible to all the antibiotics tested. Seven, or 24.1%, isolates exhibited intermediate susceptibility to at least one antibiotic while 20, or 69.0%, were resistant to at least one antibiotic. Seven, or 35%, of those resistant to at least one antibiotic were multidrug resistant (MDR), or resistant to three or more antibiotics. The MDR isolates that were resistant to three antibiotics were all resistant to ampicillin and amoxicillin-clavulanate with one isolate resistant to cephalothin, nitrofurantoin, or trimethoprim-sulfamethoxazole. One, or 3.45%, isolate was resistant to four antibiotics including nitrofurantoin and cephalothin. The remaining two isolates were resistant to five antibiotics but not to fosfomycin (Table 6). The isolates were most susceptible to ampicillin, amoxicillin-clavulanate, and cephalothin at percentages of 65.6, 58.6, and 24.1, respectively (Table 7). No isolate was resistant to fosfomycin although one isolate exhibited an intermediate reaction to the drug (Fig 1).

E. coli isolates from UTI-positive samples were resistant to ampicillin at 62.5%, amoxicillin-clavulanate at 50%, and cephalothin at 25% (Table 8). Five isolates, or 62.5%, were resistant to at least one antibiotic while 3 isolates, or 37.5%, were intermediate to at least one. No UTI-positive *E. coli* isolate was susceptible to all antibiotics. Only one, or 12.5%, isolate was MDR. Another isolate was resistant to ampicillin, amoxicillin-clavulanate, and cephalothin (Table 9, Fig 2).

DISCUSSION

Prevalence of UTI and bacterial profile

In the present study, 21.1% of the samples had significant bacteriuria. Although a nationwide data on UTI prevalence in pregnant women in the Philippines is currently unavailable (DOH 2014), a similar study on asymptomatic UTI prevalence in pregnant women visiting the OB-GYN OPD of the Philippine General Hospital (PGH) reported a lower prevalence of 4.3%

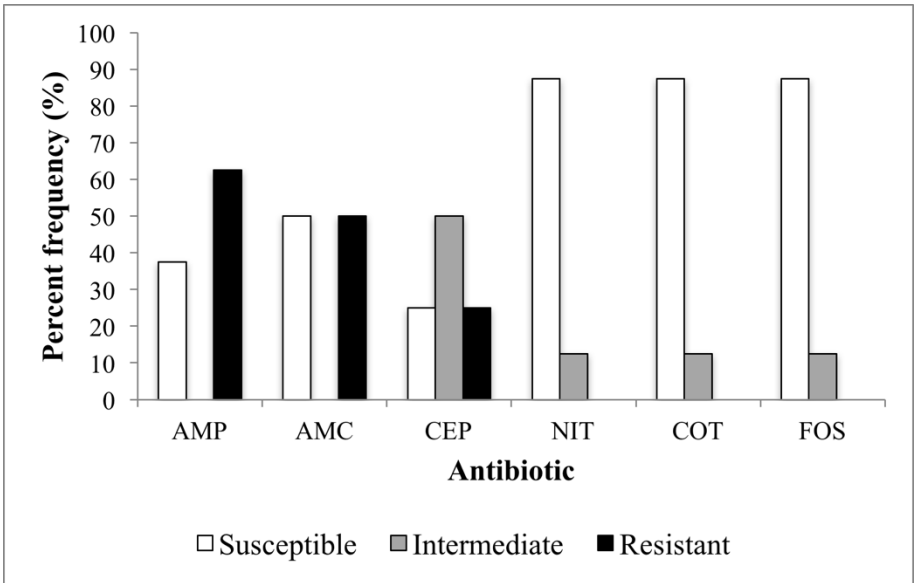

Figure 2: Antibiogram profile of *E. coli* positive isolates. Frequency of antimicrobial susceptibility (Susceptible, Intermediate, or Resistant) of UPEC (n=8). AMP: ampicillin, AMC: amoxicillin-clavulanate, CEP: cephalothin, NIT: nitrofurantoin, COT: trimethoprim-sulfamethoxazole, and FOS: fosfomycin)

(Sescon et al. 2003). In other countries, the prevalence varies greatly but generally, the observed rates are lower in developed countries at 7.7-8.0% than developing countries at about 30% (Tamalli et al. 2013). In neighboring developing countries, the incidence of UTI in pregnant women ranges from 26 to 48% (Demilie et al. 2012; Okonko et al. 2009).

While *E. coli* is the leading cause of UTI, *Klebsiella* sp. and *S. saprophyticus* are also known to cause the disease. *E. coli* accounts for 75-90% of UTI cases while *S. saprophyticus* is responsible for 5-15% particularly in uncomplicated cystitis (Jhora and Paul 2011; Drekonja and Johnson 2008). *Klebsiella* sp., on the other hand, together with *Proteus mirabilis* and *Citrobacter*, account for 5% of UTI cases (Drekonja and Johnson 2008).

Forty-two percent of the samples positive for bacteriuria (n=19) were due to UPEC. *Klebsiella* sp., on the other hand, was responsible for 10.5% of the bacteriuria. Interestingly, none of the UTI samples were positive for *S. saprophyticus*. The prevalence of *E. coli* and *Klebsiella* sp. in this study were similar to the data of Sescon and associate (2003), where 50% was caused by *E. coli* and 16.7% was due to *K. pneumoniae* and *K. ozanae*. In contrast to the data obtained in this study, 11.8% of their samples positive for bacteriuria were due to *S. saprophyticus*. A recent study conducted in a private tertiary hospital in the Philippines of uncomplicated UTI in women showed that 75.8% of UTI was due to *E. coli*, while 8.9% was due to *S. saprophyticus* (Gangcuangco et al 2015). The prevalence of UPEC reported in this study, however, was within the 42-87% range observed in other developing countries (Parveen et al. 2011; Okonko et al. 2009).

Associated risk factor for UTI

Several risk factors predispose individuals to UTI. Females, for instance, are more likely to suffer from it than men because of the short lengths of their urethra and its close proximity to the opening of the genital and intestinal tracts (Harrington and Hooton 2000). The incidence of UTI in women also increases with age with majority of symptomatic UTI cases occurring in women over the age of 50 (Rowe and Juthani-Mehta 2013). Sexual intercourse is likewise a common cause of UTI among women as bacteria in the vaginal area could be transported into the urethra during sexual intercourse (Moore et al 2008). Other risk factors include, gravidity, parity, and history of UTI. In this study, however, none of the risk factors examined was significantly associated with UTI (Table 4). Odds ratio, however, showed a difference in likelihood of acquiring UTI to most of the factors. This implies that people with certain risk factors are more likely to acquire UTI than those without the risk factors. Apparently, participants who were not sexually active and those who had a history of UTI had higher chances of acquiring UTI.

Virulence gene profile

The gene for Type 1 fimbriae had the highest frequency among the virulence genes examined, which is consistent with data in published literature. Type 1 fimbria is an established virulence factor common to UPEC-caused UTI (Bien et al. 2012). However, it was not found in two UPEC samples, indicating that some UPEC isolates may have other virulence factors that mediate their pathogenesis. Although *fimH* had the highest frequency, related studies have shown that not all UPEC necessarily have this virulence factor and that instead other UPEC may have other adhesion-related factors such as Afa adhesin, P fimbriae, Dr adhesin, S fimbriae, and/or FC1 fimbriae (Bien et al. 2012). The high prevalence of *pap* in the UPEC isolates can be associated with pyelonephritis infection which occurs at a higher rate among pregnant women (Gilstrap and Ramin 2001). Twenty, or 68.9%, of *E. coli* isolates were positive for both *fimH* and *pap*, a condition that enables the isolates to be more infective as this allows better adhesion to urothelial cells (Firoozeh et al. 2014; Bien et al. 2012). Only one sample was positive for *draE2,* a rate much lower compared to an observed 100% presence in a similar study (Matar et al. 2005). The low prevalence of the virulence factor reported here could be due to their association with gestational-pyelonephritis, which was not considered in this study. The presence and occasional occurrence of these virulence factors in *E. coli* from samples negative for bacteriuria indicate the potential of the bacterium to infiltrate the urothelial cells in cases where these factors are expressed in the presence of other virulence factors (Bien et al. 2012).

Antibiogram profile

There is an increasing incidence of antibiotic resistance among UTI-causing bacteria due to misuse of antibiotics (Tejada 2014). Constant exposure to a drug, particularly to first-line antibiotics, induces a need for higher dosages as efficacy declines due to resistance (Costelloe et al. 2010). *E. coli*, in general, has been

found to be moderately resistant to ampicillin and amoxicillin-clavulanate. Increasing degree of resistance to trimethoprim-sulfamethoxazole has also been noted in recent studies (Turnidge et al. 2013). Multidrug resistance was observed in 12% of UPEC isolates while *E. coli* isolates from UTI-negative urine samples were only susceptible to fosfomycin and were resistant to at least one of the remaining five antibiotics. In a local study of UPEC from uncomplicated UTI, the highest degrees of resistance were noted against ampicillin at 64.3% and trimethoprim-sulfamethoxazole at 41.3% while those for amoxicillin-clavulanate and nitrofurantoin were 11.7% and 5.1%, respectively (Gangcuangco et al. 2015). In the present study and in other local studies, resistance to ampicillin was found to be moderately high. Comparable data for cephalothin and fosfomycin are not available.

E. coli is reported to be susceptible to fosfomycin, with resistance rates of less than 5% (Alrowais et al. 2015). In UTI treatment, fosfomycin may be used, however, it is not the drug of choice in prescriptions. Clinicians normally recommend nitrofurantoin if beta-lactams is not prescribed (Task Force on UTI 2013). While the antibiogram profiles of UPEC isolates vary among developing countries, it is notable that resistance to ampicillin and amoxicillin-clavulanate has always been relatively high (Tamalli et al. 2013; Demilie et al. 2012). In the present study, 24% of the isolates were MDR, which is lower than a rate of 58% estimated in a similar study (Alemu et al. 2012). Although the prevalence of MDR isolates observed here is lower than those reported in previous studies, it is crucial to note that the existence of MDR uropathogen may lead to complications in UTI treatment if not addressed (Delzell and Lefevre 2000).

In the Clinical Practice Guidelines of the Philippines provided by the Task Force on UTI, limited number of antibiotics are recommended with nitrofurantoin as the first drug with certain limitations depending on the gestational age (Task Force on UTI, 2004). Beta-lactams, such as ampicillin and amoxicillin, are no longer recommended due to reported high incidence of resistance (PHIC, 2016). Nonetheless, usage of these antibiotics still persists in the country based on a survey in the present study. In outpatient care, control of which antibiotics the patient self-administers is not strictly monitored as compared to conditions under inpatient care. This situation may have contributed to the observed high resistance to ampicillin and amoxicillin-clavulanate.

CONCLUSION

The overall prevalence of UTI was 21.1% with no risk factor significantly associated with infection. *E. coli* was the predominant organism, which had high resistance to ampicillin, amoxicillin-clavulanate, and cephalothin. It was found that *E. coli* isolates from samples without significant bacteriuria were resistant to drugs commonly used to treat UTI and can cause UTI due to the presence of UPEC-related virulence factors. This study recommends that antimicrobial susceptibility tests be done prior to prescription of drugs for UTI and that fosfomycin be used as an alternative drug in the treatment of UTI in pregnant women.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

GLS and GMP conceptualized the study design. GLS conducted the data and sample collection, laboratory experiments and data analyses, and prepared the manuscript. GMP supervised the study, provided materials and reagents for the experiments, and edited the manuscript. The authors have read and approved the final version of the manuscript.

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