VIRULENCE CHARACTERISTICS AND ANTIMICROBIAL SUSCEPTIBILITY OF UROPATHOGENS FROM PATIENTS ON PHUKET ISLAND, THAILAND

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Abstract. Urinary tract infection (UTI) is among the most common infections in human. *Escherichia coli* and *Klebsiella pneumoniae* are common uropathogens found to cause UTI. In this study, 113 *E. coli* and 52 *K. pneumoniae* isolates were collected from three hospitals on Phuket Island, Thailand. The majority of *E. coli* and *K. pneumoniae* isolates were from elderly females. Antimicrobial susceptibility testing demonstrated that most of *E. coli* isolates (77%) were resistant to tetracycline while cotrimoxazole was ranked second (65%) and nitrofurantoin was the least resistant (1%). *K. pneumoniae* isolates were also most resistant to tetracycline and cefotaxime (65%). The presence of extended spectrum-beta lactamase (ESBL) producers among *E. coli* isolates were 46% and 57% in *K. pneumoniae*. Twenty-seven *E. coli* isolates carried at least one of the common urovirulence genes (*pap*, *afa*, *hlyA*), the majority isolated from patients in the internal medicine ward. One rare *K. ozaenae* was isolated from a 45 year-old catheterized male patient from the orthopedics surgery ward. This isolate demonstrated resistance to all antimicrobial agents tested except imipenem. This study is the first of such kind conducted in southern Thailand and should be useful in treating UTI patients in this area of Thailand.

Keywords: *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella ozaenae*, ESBL, uropathogen

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INTRODUCTION

Escherichia coli is a normal microbiota in the intestinal tract of humans and animals (Bien *et al*, 2012). Generally, *E. coli* forms a mutual beneficial relationship with its host, but certain strains of *E. coli* have the characteristics diverged from their mutual cohorts, becoming more pathogenic. Pathogenic *E. coli* is classified into pathotypes: enteropathogenic *E. coli*

(EPEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and enterohemorrhagic *E. coli* (EHEC) (Nataro and Kaper, 1998). These pathotypes lead to diseases in the intestinal tract, but extra-intestinal *E. coli* (ExPEC), such as uropathogenic *E. coli* (UPEC), which also resides in the gut without causing symptoms, maintains an ability to disseminate and colonize other host organs (Wiles *et al*, 2008).

Urinary tract infection (UTI) caused by *E. coli* is among the most common human infections (Kunin, 1987) and at least 10% to 20% of women experience UTI once in their lives (Johnson and Stamm, 1989). UTI is classified into categories based on the organs infected: cystitis (infection at the bladder), pyelonephritis (infection at the kidney), and bacteriuria (infection in the urine) (Foxman, 2003). The most important process of bacterial pathogenesis is the adhesion of microorganisms to the uroepithelial cells. P fimbriae (P-blood group antigens-associated fimbriae) is one of the surface virulence factors of UPEC in humans, being involved in the pathogenesis of ascending UTI and pyelonephritis (Leffer and Svanborg-Eden, 1981; Vaisanen *et al*, 1981; Plos *et al*, 1995). P fimbriae is the major virulence factor (Yamamoto *et al*, 1995) enhancing early colonization of the tubular epithelium (Bien *et al*, 2012) and in renal transplant patients. In the upper UTI, acute allograft injury is due to P fimbriae-expressing UPEC (Rice *et al*, 2006).

Another surface virulence factor, Afa adhesin, belongs to the family of Afa proteins (Le Bouguénec *et al*, 1992). UPEC expressing Afa adhesin (encoded by *afa*) has the potential to establish chronic or recurrent infection (Le Bouguénec, 2005). UPEC invades uroepithelial cells by means of adhesins AfaD and AfaE, avoiding host immunosurveillance and antibiotic treatment, thereby, is capable of initiating a new round of relapse (Dhakal *et al*, 2008).

The most important secreted virulence factor is α-hemolysin (Hlya, encoded by *hlyA*), which is associated with upper UTI such as pyelonephritis (Johnson, 1991). HlyA acts as a pore-forming toxin classified as a member of RTX (repeat in toxin) toxin family. This type of toxin is wide spread among gram-negative bacterial pathogens, and at low concentrations HlyA induces apoptosis of neutrophils, T lymphocytes and renal cells, resulting in exfoliation of uroepithelial cells (Jonas *et al*, 1993; Russo *et al*, 2005; Chen *et al*, 2006). Furthermore, at high concentrations the toxin causes lysis of erythrocytes and nucleated cells, promoting the pathogens to cross the mucosal barriers, leading to destruction of immune cells (Johnson, 1991). Approximately 50% of cases of pyelonephritis renal complication are caused by HlyA (Bien *et al*, 2012). In particular, HlyA-producing *E. coli* is able to cause permanent renal scarring, a common consequent complication (Jakobsson *et al*, 1994).

To the best of our knowledge, reports of the prevalence of uropathogenic bacteria, urovirulence factors and antimicrobial resistant pattern are lacking in Phuket Island, Thailand. Therefore, this study gathered data regarding the prevalence of uropathogenic bacteria, urovirulence factors and antibiogram pattern of uropathogens collected from patients in Phuket Island.

MATERIALS AND METHODS

Bacterial strains

A total of 166 uropathogenic bacteria were collected from wards throughout

Vachira Phuket Hospital and from Talang and Patong Hospitals in Phuket Province, Thailand, between February and September, 2013. The strains were identified using standard biochemical reactions, and all strains were kept at -80ºC for further analysis. This study was approved by the Ethics Committee of the Faculty of Medicine, Prince of Songkla University, Thailand (EC code: REC57-0136-19-2).

Antimicrobial susceptibility test

Antimicrobial susceptibilities of the uropathogenic bacteria were performed using the disk diffusion method (CLSI, 2012), employing seven antimicrobial agents, namely, cefotaxime (30 μg), ceftazidime (30 μg), cotrimoxazole (25 μg), imipenem (10 μg), nitrofurantoin (300 μg), norfloxacin (10 μg), tetracycline (30 μg) (Oxiod, Hampshire, UK). Production of extended-spectrum β-lactamases (ESBL) was also determined by the disk diffusion method. A result of ≥5 mm increase in a zone diameter for amoxicillin in combination with clavulanic acid versus amoxicillin alone was considered an ESBL-producer.

***E. coli* virulence genes determination**

Bacteria were subcultured into 3 ml of LB broth at 37ºC with shaking. One ml aliquot of culture broth was boiled for 10 minutes, placed on ice for 5 minutes and centrifuged at 11,000*g* for 10 minutes. DNA for PCR was from a 10-fold dilution of the supernatant. For virulence genes detection, two target genes for surface virulence factors (*pap* and *afa*) and one target gene for secreted virulence factor (*hlyA*) of UPEC, were determined using primers shown in Table 1. PCR was performed in a 25 μl reaction mixture consisting of 0.4 μM each primer pair, 0.1 mM dNTPs, 1X Go*Taq* DNA polymerase buffer, 0.5 U Go*Taq* DNA polymerase (Promega, Madison, WI), and 2 μl of PCR template. Amplification reaction was performed as described previously (Le Bouguénec *et al*, 1992; Yamamoto *et al*, 1995). In brief, for surface virulence factors investigation, *pap* and *afa*, the thermal cycler (T100™ Thermal Cycler, Bio-rad, Hercules, CA) conditions were as follows: 95ºC for 3 minutes followed by 35 cycles of 94ºC for 1 minute, 60ºC for 40 seconds, and 72ºC for 1 minute. The reactions were finalized at 72ºC for 5 minutes. The condition for *hlyA* was the same as for *pap* and *afa* except the annealing temperature was 58ºC and the time for extension was 1.20 minutes. Amplicons were analyzed by 1.0% agarose gel-electrophoresis and visualized by ethidium bromide staining.

RESULTS

Bacterial strains

The 166 uropathogenic bacteria collected from three hospitals in Phuket Province, Thailand, between February and September, 2013, consisted of *E. coli* (113 isolates), *K. pneumoniae* (52 isolates), and *K. ozaenae* (1 isolate). Focusing on the two main uropathogenic species, *E. coli* and *K. pneumoniae*, the majority were obtained from elderly females at the Internal Medicine Department, Vachira Phuket Hospital (Table 2). The second most frequent was from patients aged ≤15 years old (13% *E. coli* and 11% *K. pneumoniae*). *E. coli* infection in pediatric wards was 12% while *K. pneumonia* infection was 13% in surgery wards (Table 2).

Antimicrobial susceptibility

Employing disk diffusion test for 7 antimicrobial agents, the majority (77%) of *E. coli* isolates were resistant to tetracycline, followed by cotrimoxazole resistance (68%) (Table 3). As regards *K. pneumoniae* isolates, resistance to tetracycline

Table 1
Oligonucleotide primers used in this study.

Gene	Primer name	Sequence (5′ to 3′)	Amplicon size (bp)	Reference
pap	pap3	GCAACAGCAACGCTGGTTGCATCAT	336	Yamamoto *et al*, 1995
	pap4	AGAGAGAGCCACTCTTATACGGACA		
afa	afa1	GCTGGGCAGCAAACTGATAACTCTC	750	Le Bouguénec *et al*, 1992
	afa2	CATCAAGCTGTTTGTTCGTCCGCCG		
hlyA	hly1	AACAAGGATAAGCACTGTTCTGGCT	1,177	Yamamoto *et al*, 1995
	hly2	ACCATATAAGCGGTCATTCCCGTCA		

and cefotaxime were the top two (65%), with imipenem being the least resistant antimicrobial agent tested (12%) (Table 3). Disc diffusion assays revealed that 52 of 133 (46%) and 30 of 52 (57%) of *E. coli* and *K. pneumoniae* isolates, respectively, were ESBL-producers (Fig 1).

Presence of *E. coli* virulence genes

Two surface virulence genes, P fimbriae-encoding gene (*pap*) and adhesin-encoding gene (*afa*), and one secreted virulence gene encoding α-hemolysin (*hlyA*), were investigated by PCR. Of the *E. coli* isolates, 27 of 113 carried at least one of these three virulence genes, with 11 (41%) isolates having both *pap* and *hlyA*, followed by isolates harboring only *afa* (Table 4). Neither *E. coli* isolates had the presence of all three virulence genes, nor the genotype pap^-, afa^+, $hlyA^+$.

DISCUSSION

The development of UTI is depended upon factors such as the patient's anatomical features, the integrity of host defense mechanism, and the virulence of the infecting organism (Nicolle, 2002). In this present study, it was not surprising that elderly females constituted the group with the highest infection rate as the female anatomy is more favorable for uropathogenic infection than male (Jung *et al*, 2012). Furthermore, the robustness of host defense mechanisms against microorganisms in elderly people is impaired owing to, for instance, the instability of cytokines production and/or the impairment of signaling pathways involved in neutrophil recruitment to the bladder (Bien *et al*, 2012). Although the majority of the patients in this study were female, about a quarter and one-third of *E. coli* and *K. pneumoniae* infection, respectively, were isolated from male. Based on considerations of the human anatomy, we hypothesized that the type of specimens collected from male patients would be from infected urine catheter rather than midstream urine. This notion was borne out by the presence of 32 of 50 isolates of both *E. coli* and *K. pneumoniae* from male patients being collected from urine catheterized patients (data not shown).

Among the three subspecies of *K. pneumoniae*, namely, subspecies *pneumoniae*, subspecies *rhinoscleromatis* and subspecies *ozaenae* (Farmer and Kelly, 1991), the latter causes a chronic inflammatory disease of the upper respiratory tract (Falkow and Mekalanos, 1990) and is a causative agent of ozena, an atrophic

Table 2
Demographic data of patients infected by uropathogens from Phuket Island, Thailand.

Category	Number of isolates (%)		
	Escherichia coli (*n* = 113)	*Klebsiella pneumoniae* (*n* = 52)	*Klebsiella ozaenae* (*n* = 1)
Sex			
Male	32 (28)	18 (35)	1 (100)
Female	81 (72)	34 (65)	-
Age, years			
≤15	15 (13)	6 (11)	-
16-30	6 (5)	1 (2)	-
31-45	4 (4)	2 (4)	1 (100)
46-60	13 (12)	5 (10)	-
≥ 61	75 (66)	38 (73)	-
Hospital unit			
Outpatient			
OPD	6 (5)	5 (10)	-
ER	7 (6)	2 (4)	-
Inpatient			
Internal medicine	76 (67)	32 (61)	-
ICU	2 (2)	3 (6)	-
Surgery	9 (8)	7 (13)	1 (100)
Pediatric	13 (12)	3 (6)	-
Specimen			
Midstream urine	63 (56)	28 (54)	-
Catheter urine	50 (44)	24 (46)	1 (100)

Table 3
Antimicrobial resistance of uropathogenic bacteria.

Bacterial species	Number of positive isolates/Total isolates (%)						
	CTX	CAZ	SXT	IPM	F	NOR	TE
Escherichia coli	57/110 (52)	54/110 (49)	76/111 (68)	5/111 (5)	1/113 (1)	65/113 (58)	85/110 (77)
Klebsiella pneumoniae	34/52 (65)	32/52 (62)	32/51 (63)	6/52 (12)	14/51 (27)	19/51 (37)	32/49 (65)
Klebsiella ozaenae	1/1 (100)	1/1 (100)	1/1 (100)	0/1 (0)	1/1 (100)	1/1 (100)	1/1 (100)

CTX, cefotaxime; CAZ, ceftazidime; SXT, cotrimoxazole; IPM, imipenem; F, nitrofurantoin; NOR, norfloxacin; TE, tetracycline.

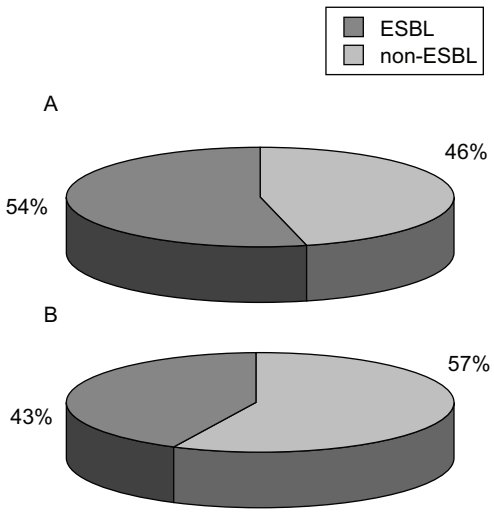

Fig 1–Proportion of ESBL-producing uropathogens among UTI patients, Phuket Island, Thailand. A, proportion of ESBL and non-ESBL *E. coli* isolates; B, proportion of ESBL and non-ESBL *K. pneumoniae* isolates.

rhinitis marked by a thick mucopurulent discharge, mucosal crusting and fetor (Malowany *et al*, 1972). Although this bacterial species is able to cause other symptoms such as meningitis, abscesses, otitis, and corneal ulcer, urinary tract infection are infrequent. Only twelve cases caused by *K. ozaenae* have been reported to date, most of which occurring in old male with immunocompromising conditions, such as the presence of cancer, leprosy, or diabetes mellitus (Kumar *et al*, 2013). In the present study, one isolate of *K. ozaenae* was obtained from a 45 year-old male hospitalized in the orthopedics surgery ward, obtained as the urine from catheter on June, 2013. This is the first report of UTI caused by *K. ozaenae* in southern Thailand.

The high rates of tetracycline and cotrimoxazole resistance obtained in this study have been reported in uropathogenic *E. coli* isolated from patients at Dhaka, Bangladesh, with tetracycline resistance of 74%-84% (Lina *et al*, 2007). However, in the Netherlands, reported uropathogenic *E. coli* resistant to cotrimoxazole was 16% and resistance to norfloxacin was only 3%. The high antibiotic prescription rates in developing countries and the patient's former intensive antibiotic exposure background, may explain in part for this phenomenon.

E. coli isolates in this study were most sensitive to nitrofurantoin, similar to a previous report from The Netherlands where the susceptibility to nitrofurantoin is 100% in urine samples from female patients with uncomplicated UTI although the rate of prescriptions for nitrofurantoin in 2004 and 2009 were 58% and 66%, respectively (den Heijer *et al*, 2010). More importantly, Liu *et al* (2011) reported that 79.1% of ESBL-producing *E. coli* isolated from a hospital in Taipei, Taiwan, were susceptible to nitrofurantoin. However, even if nitrofurantoin is still an effective antimicrobial agent for non-complicated cystitis, its usage is hindered by side effects and frequency of usage doses per day (Amábile-Cueras and Arredondo-García, 2011).

Although this study observed high resistance of *K. pneumoniae* to tetracycline and cotrimoxazole, only 27% of the bacteria in Dhaka, Bangladesh are resistant to tetracycline, but completely sensitive to imipenem. Bacterial antibiotic resistant characteristics are frequently found to be conferred by plasmids and the presence of antibiotic resistant plasmids among isolates is varied and found to be transferred in certain rates (Lina *et al*, 2007). Thus, the number of resistant bacteria in different countries can be diverged. In addition, the low level of imipenem resistance is not surprising because it is shown to be very active against gram-negative bacteria (Franklin *et al*, 2002).

Table 4
Virulence genes and antibiogram patterns of uropathogenic *Escherichia coli*.

Strain	Source of sample	Ward	Virulence gene pattern			Antibiogram pattern
			pap	*afa*	*hlyA*	
PSU90	MU	OPD	+	-	+	TE
PSU91	CU	Medicine	-	+	-	CTX, CAZ, SXT, NOR, TE
PSU92	CU	Medicine	-	+	-	SXT, TE
PSU93	MU	Pediatrics	+	-	+	SXT, TE
PSU94	CU	OPD	+	-	+	S
PSU95	MU	Medicine	+	-	-	SXT, TE
PSU96	CU	Medicine	+	-	+	CTX, CAZ, NOR
PSU139	MU	Medicine	-	-	+	CTX, CAZ, NOR, TE
PSU140	CU	Medicine	+	-	+	NOR, TE
PSU141	CU	ER	-	-	+	CTX, CAZ, SXT, IPM, NOR, TE
PSU142	MU	Medicine	-	+	-	CTX, CAZ, SXT, TE
PSU143	CU	Medicine	-	-	+	CTX, CAZ, SXT, NOR, TE
PSU144	MU	Pediatrics	-	-	+	S
PSU145	CU	Pediatrics	-	-	+	SXT, TE
PSU146	CU	Pediatrics	-	+	-	CTX, SXT
PSU147	MU	Medicine	-	+	-	SXT
PSU148	MU	Medicine	+	-	+	CAZ, SXT, TE
PSU149	MU	Medicine	+	-	+	CTX, CAZ, SXT, NOR, TE
PSU150	MU	Pediatrics	+	-	-	SXT
PSU151	MU	Medicine	+	-	+	CTX, CAZ, NOR, TE
PSU152	CU	Surgery	-	+	-	CTX, CAZ, SXT, NOR, TE
PSU153	MU	Medicine	+	-	-	SXT, NOR, TE
PSU154	CU	Pediatrics	+	-	+	CTX, CAZ, TE
PSU155	MU	Medicine	+	-	+	SXT
PSU156	CU	Medicine	-	+	-	CTX, CAZ, SXT, NOR, TE
PSU157	MU	Medicine	-	+	-	SXT, TE
PSU158	MU	Pediatrics	+	-	+	SXT, TE

MU, midstream urine; CU, catheter urine. CTX, cefotaxime; CAZ, ceftazidime; SXT, cotrimoxazole; IPM, imipenem; F, nitrofurantoin; NOR, norfloxacin; TE, tetracycline. S, susceptible to all antimicrobial agents tested.

Owing to the scarcity of information regarding uropathogenic prevalence and its antibiogram pattern, the correct therapeutic approach and the choice of antimicrobial agents used tend to vary, resulting in slow recuperation. This study, thus, provided the antibiogram profile of uropathogenic *E. coli* and *K. pneumoniae* isolated from number of hospitals on Phuket Island, and provides informations that would be useful for public health organizations in Thailand.

ACKNOWLEDGEMENTS

This research was funded in part by Prince of Songkla University. MT is a recipient of Prince of Songkla University grant, no. SCI560349S.

REFERENCES

Amábile-Cueras CF, Arredondo-García JL. Antimicrobial activity data in support of nitrofurantoin three times per day. *J Antimicrob Chemother* 2011; 66: 1652-3.

Bien J, Sokolova O, Bozko P. Role of uropathogenic *Escherichia coli* virulence factors in development of urinary tract infection and kidney damage. *Int J Nephrol* 2012; 2012: 681473.

Chen M, Tofighi R, Bao W, *et al*. Carbon monoxide prevents apoptosis induced by uropathogenic *Escherichia coli* toxins. *Pediatr Nephrol* 2006; 21: 382-9.

Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing; Twenty-second informational supplement M100-S22. Wayne: CLSI, 2012.

den Heijer CDJ, Donker GA, Maes J, Stobberingh EE. Antibiotic susceptibility of unselected uropathogenic *Escherichia coli* from female Dutch general practice patients: a comparison of two surveys with a 5 year interval. *J Antimicrob Chemother* 2010; 65: 2128-33.

Dhakal BK, Kulesus RR, Mulvey MA. Mechanisms and consequences of bladder cell invasion by uropathogenic *Escherichia coli*. *Eur J Clin Invest* 2008; 38: 2-11.

Falkow S, Mekalanos J. The enteric bacilli and vibrios. In: Davis BD, DuIbecco R, Eisen HN, Ginsberg HS, eds. Microbiology. 4th ed. Philadelphia: JB Lippincott, 1990: 561-87.

Farmer JJ, Kelly MT. *Enterobacteriaceae*. In: Balows A, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy HJ, eds. Manual of clinical microbiology. 5th ed. Washington DC: American Society for Microbiology 1991: 360-83.

Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Am J Med* 2002; 113 (supp 1A): S5-13.

Franklin GA, Moore KB, Synder JW, Polk HC Jr, Cheadle WG. Emergence of resistant microbes in critical care units in transient, despite an unrestricted formulary and multiple antibiotic trials. *Surg Infect* 2002; 3: 135-44.

Jakobsson B, Berg U, Svensson L. Renal scarring after acute pyelonephritis. *Arch Dis Child* 1994; 70: 111-5.

Johnson JR, Stamm WE. Urinary tract infections in women: diagnosis and therapy. *Ann Intern Med* 1989; 111: 906-17.

Johnson JR. Virulence factors in *Escherichia coli* urinary tract infection. *Clin Microbiol Rev* 1991; 4: 80-128.

Jonas D, Schultheis B, Klas C, Krammer PH, Bhakdi S. Cytocidal effects of *Escherichia coli* hemolysin on human T lymphocytes. *Infect Immun* 1993; 61: 1715-21.

Jung J, Ahn HK, Huh Y. Clinical and functional anatomy of the urethral sphincter. *Int Neurourol* 2012; 16: 102-6.

Kumar S, Alfaadhel T, AlBugami MM. *Klebsiella ozaenae* bacteremia in a kidney transplant recipient. *Case Rep Transplant* 2013; 2013: 493516.

Kunin CM. An overview of urinary tract infections. In: Kunin CM, eds. Detection, prevention and management of urinary tract infections. 4th ed. Philadelphia: Lea Febiger, 1987: 3-4.

Le Bouguénec C, Archambaud M, Labigne A. Rapid and specific detection of the *pap*, *afa*, and *sfa* adhesin-encoding operons in uropathogenic *Escherichia coli* strains by polymerase chain reaction. *J Clin Microbiol* 1992; 30: 1189-93.

Le Bouguénec C. Adhesins and invasions of pathogenic *Escherichia coli*. *Int J Med Microbiol* 2005; 295: 471-8.

Leffer H, Svanborg-Eden C. Glycolipid receptors for uropathogenic *Escherichia coli* on human erythrocytes and uroepithelial cells. *Infect Immun* 1981; 34: 920-9.

Lina TT, Rahman SR, Gomes DJ. Multiple-antibiotic resistance mediated by plasmids and integrons in uropathogenic *Escherichia coli* and *Klebsiella pneumoniae*. *Bangladesh J*

Microbiol 2007; 24: 19-23.

Liu HY, Lin HC, Lin YC, Yu SH, Wu WH, Lee YJ. Antimicrobial susceptibilities of urinary extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* to fosfomycin and nitrofurantoin in a teaching hospital in Taiwan. *J Microbiol Immunol Infect* 2011; 44: 364-8.

Malowany MS, Chester B, Allerhand J. Isolation and microbiologic differentiation of *Klebsiella rhinoscleromatis* and *Klebsiella ozaenae* in cases of chronic rhinitis. *Am J Clin Path* 1972; 58: 550-3.

Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* 1998; 11: 142-201.

Nicolle LE. Urinary tract infection in geriatric and institutionalized patients. *Curr Opin Urol* 2002; 12: 51-5.

Plos K, Connell H, Jodal U, *et al*. Intestinal carriage of P fimbriated *Escherichia coli* and the susceptibility to urinary tract infection in young children. *J Infect Dis* 1995; 171: 625-31.

Rice JC, Peng T, Kuo YF, *et al*. Renal allograft injury is associated with urinary tract infection caused by *Escherichia coli* bearing adherence factors. *Am J Transplant* 2006; 6: 2375-83.

Russo TA, Davidson BA, Genagon SA, *et al*. *E. coli* virulence factor hemolysin induces neutrophil apoptosis and necrosis/lysis in vitro and necrosis/lysis and lung injury in a rat pneumonia model. *Am J Physiol Lung Cell Mol Physiol* 2005; 289: L207-16.

Vaisanen V, Elo J, Tallgren LG. Mannose-resistant haemagglutination and P antigen recognition are characteristic of *Escherichia coli* causing primary pyelonephritis. *Lancet* 1981; 2: 8260-1.

Wiles TJ, Kulesus RR, Mulvey MA. Origins and virulence mechanisms of uropathogenic *Escherichia coli*. *Exp Mol Pathol* 2008; 85: 11-9.

Yamamoto S, Terai A, Yuri K, Kurazono H, Takeda Y, Yoshida O. Detection of urovirulence factors in *Escherichia coli* by multiplex polymerase chain reaction. *FEMS Immun Med Microbiol* 1995; 12: 85-90.