Global Advanced Research Journal of Medicine and Medical Science (ISSN: 2315-5159) Vol. 4(7) pp. 321-330, August, 2015
Available online http://garj.org/garjmms

Full Length Research Paper

Prevalence and risk factors of community-acquired urinary tract infections due to ESBL-producing Gram negative bacteria in an Armed Forces Hospital in Sothern Saudi Arabia

TA El-Kersh[1*], MA Marie[1], YA Al-Sheikh[1], and SA Al-Kahtani[2]

[1]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia
[2]Armed Forces Hospital Southern Region (AFHSR) Khamis Mushayt, Saudi Arabia

Accepted 04 August, 2015

This study investigated the prevalence and Antibiotic sensitivity profiles of Gram negative bacteria (GNB) isolated from urine specimens of out-patients with urinary tract infections (CA-UTI) in Armed Forces Hospital in southern Saudi Arabia, risk factors for infection, and extended spectrum β-lactamase production (ESBLs). The Vitek2 system (BioMe´rieux, France), was used for microbial identification, susceptibility testing, and ESBL detection, which was also confirmed by the Clinical and Laboratory Standards Institute double-disk synergy test. A total of 269 GNB were studied. *E. coli* accounted for 77 % (206/269), *Klebsiella pneumoniae* for 16 % (43/269) and other GNB 7% (20/269). Fifty four % (23/43) of *K. pneumoniae* and 44% (91/206) of *E coli* isolates, were ESBL positive. Females comprised 74% (200/269) of patients, and 14% (37/269) were pregnant. Patients aged ≤10 years and those aged 71-80 years showed significant high risk to CA-UTI at frequency of 20.4 % (55/269) and 24.9 %(67/269) respectively. Unlike pregnancy, diabetic, cardiac, and/or renal disease-patients showed also high risk to CA-UTI by *K. pneumonia. E. coli, K. pneumoniae* and other GNB susceptibility against meropenem, amikacin and gentamicin were 99%, 95 % and 85%; 88%, 88 % and 75%; and 75 %, 60 % and 70% respectively. While 53% and 25% of *E coli* isolates were resistant to TMP/SMZ and nitrofurantoin respectively. Seven Eterobacteriaceae isolates (*E coli, 2; K pneumonia, 2; Serratia marcescens, 2 and Citrobacter freundii,* 1) were resistant to all tested 14 drugs (pan-resistant isolates). Our findings identified risk factors which can be used to guide appropriate empiric therapy of CA-UTI, and targeted infection control measures.

Keywords: Community UTI, Risk factors, Vitek2, ESBL, pan-resistant isolates, *E. coli, K. pneumoniae*

INTRODUCTION

Community-acquired urinary tract infection (CA-UTI) endures the communal disease diagnosed in community practices, ranked only second to upper respiratory tract infections (Khawcharoenporn *et al.*, 2013). Its prevalence varies with gender and age, and obviously morbidity among adolescent females is much higher due to physical factors, absence of prostatic secretions, and anatomical urethral shortage (Alzohairy and Khadri, 2011). Although the frequency of different Gram-negative bacilli, enterococci and *Candida* spp., particularly in risk factors associated- complicated UTI, is increasing, *E. coli* is still the most frequent causative agent in more than 90% of UTI (Kurtaran *et al.*, 2010). While CA-UTI caused

*Corresponding Author E-mail: talatkersh@yahoo.com; tkersh@ksu.edu.sa; Tel: +966114693734; Fax: +966114693738

by *E. coli* are not usually associated with outbreaks, mounting evidence shows that *E. coli* may be responsible for community-wide epidemics (Alam *et al*., 2009; Vincent *et al*., 2010). Accurate and prompt diagnoses of UTI are important in shortening the disease course and hospital stay cost and for preventing the ascent of the infection to the upper urinary tract (pyelonephritis) sites.

Treatment of UTI is often started empirically (Kurtaran *et al*., 2010; Khawcharoenporn *et al*., 2013). Conveniently, the most frequently prescribed antibiotics are oral broad spectrum beta lactam antibiotics as amoxicillin, ampicillin/clavulanate, and oral cephalosporins, trimethoprim-sulfamethoxazole (TMP-SMZ), nitofurantoin, or quinolones for lower uncomplicated UTI (cystitis) while fluoroquinolones, 3rd and 4th generations cephalsporins, aminoglycosides, and carbapenems are appropriate for pyelonephritis and complicated UTI (Khawcharoenporn *et al*., 2013). The common misuse, underuse, and/or overuse, as well as often neglected local community susceptibility profiles of these agents, especially in developing countries including Saudi Arabia, invariably resulted in the emergence of multidrug resistant (MDR) isolates among all uropathogenic bacteria including *E coli*, thereby making treatment options very limited (Kurtaran *et al*., 2010; Khawcharoenporn *et al*., 2013; Sharma *et al*., 2013). Of different drug resistance mechanisms underlying such MDR isolates, the ESBLs including CTX-M type is currently known as the most widespread and threatening mechanism of β-lactam antibiotic-resistance, both in hospital and community settings (Al-Otaibi and Bukhari, 2013; Otajevwo, 2013; Fam *et al*., 2013; Ogutlu *et al*., 2014). Consequently, regular monitoring of susceptibility profiles in different regions of a country is necessary to improve local geographical guidelines for empirical antibiotic therapy (Fam *et al*., 2013; Ogutlu *et al*., 2014).

Accordingly, this study was conducted to determine prevalent Gram negative bacterial isolates from out-patients referred to an Armed Forces Hospital in Southern Saudi Arabia, associated risk factors, and their susceptibility pattern to 14 drugs, as well as detection of ESBL producers among studied uropathogens. To the best of our knowledge, this report is the first of its kind from this region of Saudi Arabia, dealing with the studied risk factors (Gender, age, diabetes mellitus, pregnancy, or chronic renal and/or heart diseases) in relation to ESBL-producing Gram negative bacteria in community.

MATERIALS AND METHODS

Design of the study

Urine specimens were obtained from outpatients admitted to Armed Forces Hospital Southern Region (AFHSR) Khamis Mushayt, Saudi Arabia from June to September, 2013.

The study protocol was approved by the research ethics committee of AFHSR and a written informed consent was filled up and taken from each of all subjects who agreed to participate in the study. After exclusion criteria (stated below), effectively 269 outpatients documented sequential cases with community acquired UTI were enrolled in this study. Their age varied from ≤1year to 80 years, and there were 69(26%) males, and 200(74%) females, pregnant 37(14%), diabetic 67(25%), and chronic renal 45(17%) or cardiac 18(7%) diseases.

Specimen- collection

The urine (mid-stream urine) specimens were aseptically collected in sterile containers from 2781 outpatients suspected of having a UTI (symptomatic) and routine checkup (asymptomatic). Patients who had have taken antibiotics 2 weeks prior to urine-specimen collections were excluded from the study. Urine specimens were transported to the microbiology laboratory within half hour of collection and immediately processed, or refrigerated at 4 C°. for only few hours, before processing. Based on macroscopic and microscopic analysis, only 1273 specimens were further processed for cultures and susceptibility testing. Of these only 34 specimens yielded Gram positive bacteria as etiological agents, and they were excluded from the study, whereas 269 specimens yielded Gram negative bacteria (other specimens yielded no growth, less than 10^5 CFU/ml urine, or just mere lactobacilli and/or alpha hemolytic streptococci). Each of 269 Gram negative isolates, single bacterial isolate-detected, was further processed for maintaining stock cultures, culture identity and susceptibility testing.

Bacterial culture and isolation

The urine specimen was mixed well (~6 times) to re-suspend solid particles. A sterile 1 µl loop (0.001ml.), was used to inoculate onto a half each of a CLED/blood agar bi-plate (Saudi Prepared Media Laboratory, Riyadh, KSA) by streaking down the center of the medium and then streaking across this line at a 90° angle, using a fresh inoculum for each half. The inoculated plates were aerobically incubated at 35 C° for 18-24 hours. All of the recovered 269 Gram-negative bacteria isolates were cultured in Brain Heart Infusion stock cultures containing 20% (v/v) glycerol (Saudi Prepared Media Laboratory, Riyadh, KSA) and maintained at (-80 C°). and as required, were revived on blood agar plates(Saudi Prepared Media Laboratory, Riyadh, KSA) , incubated overnight at 37 C° ,and single fresh colony of each isolate was further processed for culture identity and susceptibility testing.

Antimicrobial susceptibility testing

Vitek 2 ((BioMerieux France) is automated identification

Table 1. Frequency of Gram negative bacteria and ESBL producers among studied male and female subjects

Isolates	Total isolates n=269				ESBL(+) n=124				P value
	Male	Female	total	%	M	F	total	%	
E. coli	45	161	206	76	28	63	91	44	0.253
K. pneumoniae	12	31	43	16	7	16	23	54	0.289
Enterobacter cloacae and E. aerogenes	4	3	7	3.0	2	1	3	43	0.586
Serratia marcescens	3	1	4	2.0	2	0	2	50	0.628
Citrobacter freundii and C. koseri	1	2	3	1.0	1	0	1	33	0.559
Morganella morganii	0	1	1	0.3	0	1	1	100	0.461
Proteus mirabilis	1	0	1	0.3	1	0	1	100	0.461
Providencia stuartii	1	0	1	0.3	1	0	1	100	0.461
Pantoea spp.	0	1	1	0.3	0	0	0	0.0	0.539
Pseudomonas aeruginosa	2	0	2	0.7	1	0	1	50	0.710
Total	69(26%)	200(74)	269	100	43	81	124	100	

and susceptibility testing (Numanovic *et al.*, 2013). Each isolate of 269 Gram negative bacteria was tested with the Vitek2 system according to the manufacturer's instructions. Both a Gram negative identification card (ID-GNB) and an antimicrobial susceptibility testing card (AST-GN26) panels were inoculated with a bacterial colony suspension prepared in 0.45% saline equal to the turbidity of a 0.5 McFarland standard with the Densi-Chek 2 system (BioMerieux France). For quality control isolates, *E. coli (*ATCC 25922), *E. coli* (ATCC35218), *K. pneumoniae* (ATCC 700603), *P. aeruginosa* (ATCC 27853), were all used for comparison of identification and susceptibility testing, ESBL production and for monitoring the performance of the Vitek2 automated system.

Detection of extended spectrum β- lactamases (ESBL)

An expert system (AES) interprets the results obtained with Vitek2 by using nine different phenotypes relevant to β-lactam antibiotics, including the wild type, and ESBLs production. (Thomson *et al.*, 2007).

Conformation Test for extended spectrum β-Lactamases

Confirmatory test for ESBLs production was also confirmed by the double disk synergy test (DDST) Figure 1A. Thus DDST was used to confirm the presence of ESBL on each of all the isolates of enteric bacteria where, ceftazidime, and cefotaxime(30µg each) were placed around the amoxicillin-clavulanate disk at a center-to-center distance of 15 mm from the central disk, on a Muller-Hinton (Saudi Prepared Media Laboratory, Riyadh, KSA) agar plate (MHA). The ESBL was considered positive when decreased susceptibility to the broad-spectrum β-lactam agent on the outer disk is associated with synergy towards the central disk. Synergy was defined when the inhibition zone of the disk of cephalosporin plus clavulanic acid was ≥5 mm greater than that of the disk of cephalosporin alone (Numanovic *et al.*, 2013; Elsharkawy *et al.,* 2013).

Statistical methods

Data was stored and analyzed using SPSS 19 (Statistical Package for Social Science; release 19.0). Fishers exact test one way analysis variance (ANOVA), Kruskal-walis test (Non-parametric test), Chi-Square Liner trend, Chi-Square test were used and a P value< 0.05 was considered as significant.

RESULTS

Clinical specimens and isolates recovered

In the present study out of the examined 1273 urine specimens, 269 bacterial Gram negative isolates were recovered from the enrolled 269 outpatients who showed significant bactriuria (≥10^5 CFU/mL) of single type of Gram negative bacteria isolates. As presented in Table 1 *E. coli* (206/269, 76 %) was the most frequently isolated organism as expected, followed by *K. pneumoniae* (43/269, 16 %). Whereas *Enterobacter cloacae and E. aerogenes (*3%); *S. marcescens* (2%); *C. freundii and C. koseri* (1%) were rarely encountered . While *Morganella morganii*, *Proteus mirabilis, Providencia stuartii,* or *Pantoea* spp. , and the non-glucose fermenter *P. aeruginosa* constituted around 1% and 1% of total Gram negative bacteria isolates respectively. Table 1 also shows that the risk of UTI is much more common among females than males with all recovered Gram negative bacteria isolates, yet difference was not significant. Additionally, ESBL production was phenotypically confirmed (Figure, 1 A) by DDST. As presented in Table 1, ESBL producers were detected in 91 *E coli* (44 %), 23 *K. pneumoniae* (54%), 3*E. cloacae* (43%), 2*S.*

Table 2. Distribution of recovered Gram negative bacteria in relation to risk factors and their ESBLs positivity (%).

Risk factors subjects (n)	*E. coli* n=206		*K. pneumoniae* n=43		Other GNB n=20		Total Positive ESBL (%)
	n	ESBL (+)	n	ESBL (+)	n	ESBL (+)	
Diabetic 67	44 (65.7)	27 (61.4)*	18 (26.9)*	10 (55.6)	5 (7.5)	4 (80.0)	41(61.2)*
Non diabetic 202	162 (80.2)*	64 (39.5)	25 (12.4)	13 (52.0)	15 (7.4)	6 (40.0)	83(41.1)
Renal disease 45	29 (64.4)	16 (55.2)	14 (31.1)*	8 (57.1)	2 (4.4)	1 (50.0)	25 (55.6)
Non renal disease 224	177 (79.0)*	75 (42.4)	29 (12.9)	15 (51.7)	18 (8.0)	9 (50.0)	99 (44.2)
Cardiac 18	13 (72.2)	7 (53.8)	4 (22.2)	2 (50)	1 (5.6)	1 (100)	10 (55.6)
Non cardiac 251	193 (76.9)	84 (43.5)	39 (15.5)	21 (53.8)	19 (7.6)	9 (47.4)	114 (45.4)
Pregnant 37	32 (86.5)	9 (28.1)	4 (10.8)	0(0)	1(2.7)	0 (0)	9 (24.3)
Non pregnant 232	174 (75.5)	82 (47.1)*	39 (16.8)	23 (59.0)*	19 (8.2)	10 (52.6)	115 (49.6)*

*Significant p value ≤ 0.05 ; (Chi-square test or Fisher's exact test).
Other GNB: *Enterobacter cloacae* and *E aerogenes; Serratia marcescens; Citrobacter freundii and C. koseri; Pseudomonas aeruginosa, Morganella morganii; Proteus mirabilis, Providencia stuartii;* and *Pantoea* spp)

marcescens (50.0%), 1 *P. aeruginosa* (50.0%), 1 *C. freundii* (33%) and 1 for each of *M. morganii, P.mirabilis*, or *Providencia stuartii* (100 %), though their number was limited.

Analysis of studied risk factors upon the recovery of Gram negative bacteria and their ESBL- production

To assess the effect of age, on the prevalence of UTI, the enrolled patients in this study were categorized into different age-groups as presented in Figure 2. The subject's age varied from ≤1year to 80 years(y). The patients of the age group ≤1- 10 y and the age group 71-80 y, showed the highest prevalence of Gram negative bacteria causing UTI at frequency of 20.4 % and 29.4 % respectively ($P < 0.0001$). Whereas the patients of age group 31- 40 y and the age group of 41- 50 y exhibited the lowest risk of Gram negative bacteria causing UTI with frequency 9.7% and 7.1% respectively. This trend also holds true for the prevalence of positive ESBLs production among recovered Gram negative bacteria. Additionally, as presented in Table 2 diabetic (67) and non-diabetic (202) patients revealed that *E. coli* surprisingly was significantly frequently recovered from the latter group (162/202, 80.2 %) in comparison to those from diabetic ones (44/67, 65.7%). However, positive ESBLs isolates were significantly frequent (27/44, 61%) among those recovered from diabetic patients than those non-diabetic ones (64/162, 40%). Such trend holds also true for other Gram negative bacteria in the former group (4/5, 80%) and in the latter group (6/15, 40%). Results (Table 2) also indicated that diabetic patients were more vulnerable (18/67, 27%) to UTI by *K. pneumoniae* ($p=0.005$) as compared to non-diabetic ones (25/202, 12%), and also the ESBLs production among diabetic isolates (10/18, 56%) was only slightly higher than those of non-diabetic isolates (13/25, 52%). Table 2 also showed that the overall positive ESBLs production among all Gram negative bacteria was significantly higher (41/67, 61.2%) in diabetic patients versus non-diabetic ones (83/202, 41.1%). These trends regarding frequently recovered Gram negative bacteria and/or positive ESBLs production were also observed among the risk of renal disease and non-renal disease patients. Hence, positive ESBLs production among all Gram negative bacteria isolated from renal disease patients and from non-renal disease patients were (25/45, 55.6%) and (99/224, 44.2%) respectively. Likewise, the respective frequencies for cardiac disease and non-cardiac disease patients were (10/18, 55.6%) and (114/251, 45.4%) respectively. In contrast the risk of pregnancy exhibited different trends in both recoveries of the Gram negative bacteria as well as positive ESBLs production. Thus *E. coli* isolates were more frequently recovered from pregnant patients (32/37, 86.5%) than versus those from non-pregnant patients (174/232, 75.5%). However the prevalence of positive ESBLs production was significantly much less frequently encountered among isolates of the pregnant group (9/32, 28.1%) than those among isolates of the latter group (82/174, 47.1%).

Antimicrobial susceptibility pattern of tested Gram negative bacteria

Our susceptibility data of the recovered 269 Gram negative bacteria against the tested 14 antimicrobial agents are listed in Table 3. Generally all studied Gram negative bacteria exhibited high susceptibility against amoxicillin/clavulanic acid than to ampicillin alone, and also the same trend was observed with piperacillin/tazobactam, suggesting high prevalence of plasmid-mediated β-lactamases. *E. coli* was generally more susceptible to the tested antimicrobials than *K. pneumoniae*, and other Gram negative bacteria. Likewise most tested bacteria were far more susceptible to amikacin than that towards gentamicin. The *E coli* resistant rates to these drugs were 12% and25% versus 12% and39% or 25% and 30% with *K. pneumonia* or other Gram negative bacteria respectively. Additionally,

Table 3. Susceptibility rates (%) of *E. coli, K. pneumoniae*, and other Gram negative bacteria isolates against 14 tested drugs

Antimicrobial agents	*E coli* n=206	*K pneumoniae* n=43	Other GNB n=20
Ampicillin	41(20)	0(0.0)	0(0.0)
Amoxicillin/clavulanate	77(37)	16(37)	3(15)
Piperacillin/Tazobactam	135(66)	23(54)	10(50)
Cefuroxime	96(47)	18(42)	4(20)
Cefoxitin	147(71)	32(74)	4(20)
Cefotaxime	115(56)	18(42)	8(40)
Ceftazidime	115(56)	18(42)	9(45)
Cefepime	118(57)	19(44)	11(55)
Meropenem	204(99)	41(95)	17(85)
Amikacin	181(88)	38(88)	15(75)
Gentamicin	155(75)	26(61)	14(70)
Ciprofloxacin	115(56)	22(51)	13(65)
Nitrofurantoin	154(75)	6(14.0)	6(30)
Trimethoprim/ Sulafamethoxazole	96(47)	26(61)	13(65)

*Other GNB: *Enterobacter cloacae* and *E aerogenes; Serratia marcescens; Citrobacter freundiiandC. koseri; Pseudomonas aeruginosa, Morganella morganii; Proteus mirabilis, Providencia stuartii;* and *Pantoea* spp)

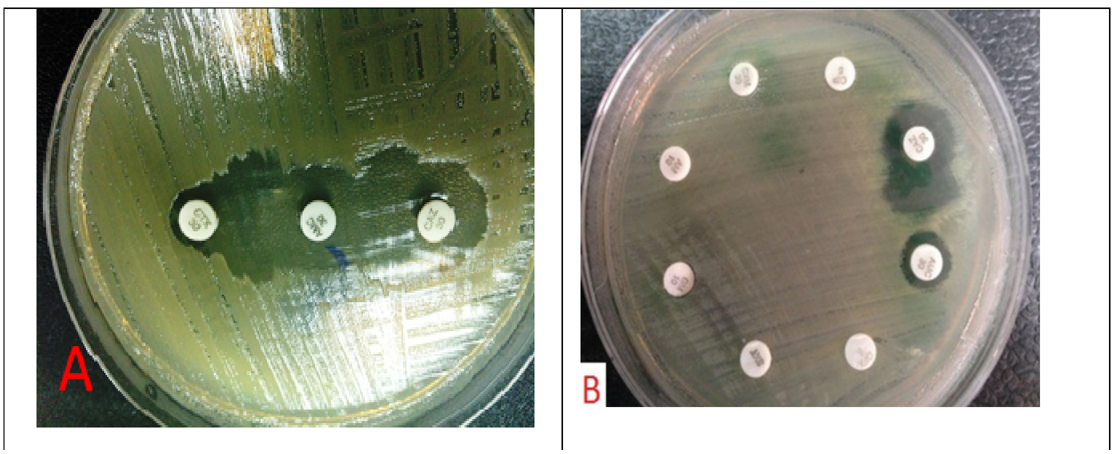

Figure1A. The double disk synergy test (DDST), ceftazidime, cefotaxime(30µg each) were placed around the amoxicillin-clavulanate disk at a center-to-center distance of 15 mm from the central disk., **B**. *E coli* strain that showed positive ESBL, and pan resistance to several tested drugs.

over 50% and 25% of *E coli* isolates were resistant to TMP/SMZ and nitrofurantoin, respectively. While ciprofloxacin showed moderate efficacy of 56%, 51% and 65% sensitivity rates against *E.coli, K.pneumoniae*,and other Gram negative bacteria isolates respectively, reflecting its moderate excessive empiric use.

The overall resistance rates to the 3rd generation cephalosporins (cefotaxime and ceftazidime) were 44 %; 58 %; and 60 % for *E. coli, K. pneumoniae*, and other Gram negative bacteria respectively, confirming the implication of ESBLs production, which also affected the 4th G cephalosporin cefepime efficacy (43%, 56%, and 45% resistance rates), but not cefoxtin which showed remarkable sensitivity efficacy of 71% and 74 % against *E.coli and K.pneumoniae* respectively. Other Gram negative bacteria isolates showed, however, less susceptibility (20%) towards this drug suggesting AmpC β-lactamases implication. As expected, meropenem exhibited the highest antimicrobial activity against all tested Gram negative bacteria isolates, with only 1 %, 5 % and 15% resistance rates for *E. coli, K. pneumonia* and other Gram negative bacteria isolates respectively. These isolates not only exhibited complete resistance to β-lactam antibiotics (Figure 1B) but also to all un-related antibiotic groups tested i.e. to all tested 14 drugs (pan-resistant isolates).These resistant isolates comprised *E coli* (2 isolates), *K pneumoniae* (2 isolates), *S. marcescens* (2 isolates), and *C. freundii* (1 strain), all of which belong to Enterobacteriaceae the most prevalent Gram negative bacteria

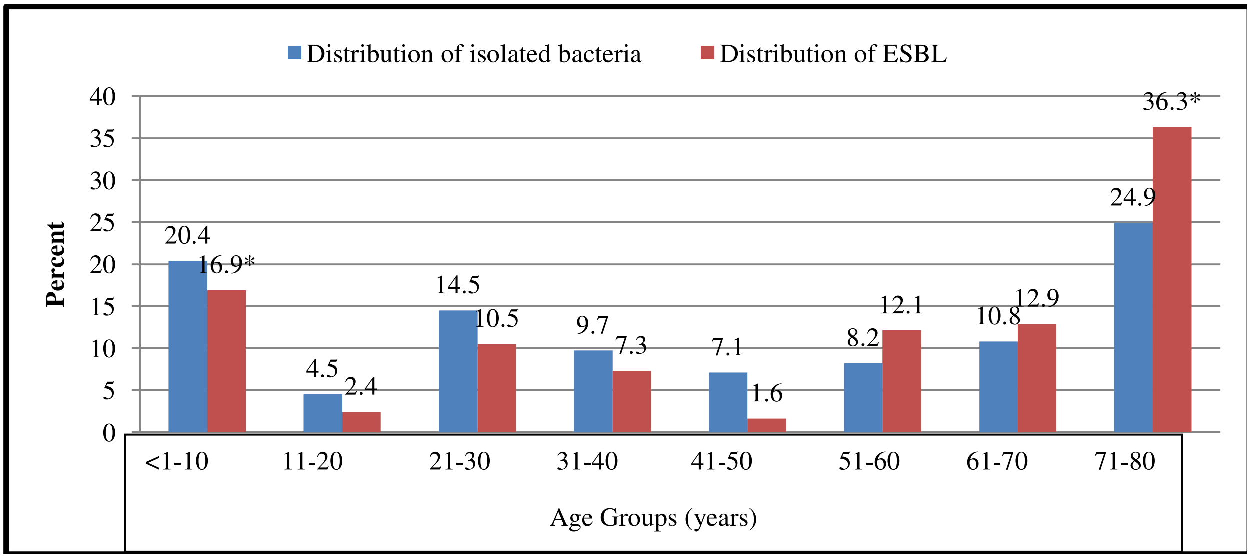

Figure 2. Histogram shows the distribution of recovered Gram negative bacteria and respective positive ESBL (%) in relation to age groups; *Significant p value ≤ 0.05

DISCUSSION

Urinary tract infection (UTI) is the second most common community acquired infection in clinical practice worldwide, ranked only after upper respiratory tract infections (Kurtaran *et al*., 2010; Khawcharoenporn *et al*., 2013; Sharma *et al*., 2013). Hence, it is an important cause of morbidity and mortality in healthcare delivery in both community and hospital patients, besides costing the global economy in excess of 6 billion US dollars annually (Kurtaran *et al*., 2010). Obviously, the microorganisms causing UTI and their resistance rates should be known, and the prudent management is to investigate the antibiotic susceptibility of agents isolated from urinary system infections at periodic intervals (Khawcharoenporn *et al*., 2013). Accordingly, the current study was designed to determine the prevalence of CA-UTI by Gram negative bacteria, in relation to studied risk factors and their antimicrobial susceptibility patterns, as well as prevalence of ESBL producers among isolated Gram negative bacteria. The most prevalent Gram negative bacteria were *E. coli, K. pneumoniae,* and *E. cloacae* and *E. aerogenes* with a frequency of 77 %, 16%, and 3% respectively.

Previous studies (Gupta *et al.,* 2011; Al-Jiffri *et al*., 2011; Pondei *et al*., 2012; Ahmad, 2012) have shown that the frequency of *E. coli* causing CA-UTI varied from 36 %,44% ,54%, to 66%.,versus 77 % in this study, which it is comparable to that 72 % or 73 % previously reported from Chicago, USA (Khawcharoenporn *et al*., 2013) and Iran (Irajian and Moghadas, 2010) . *K. pneumoniae* represented only an overall 16% of recovered Gram negative bacteria in this study, which confirms to those of 11.4%, 12.1%, 15% and 22.4%, as reported from Saudi Arabia (Al-Tawfiq and Anani, 2009), Nigeria (Tijjani *et al.,* 20012), Chicago –USA (Khawcharoenporn *et al,* 2013) and Kashmir (Ahmad, 2012) respectively. In contrast, other members of *Enterobacteriaceae : S. marcescens*, *C. freundii* and *C. koseri* ,*M. morganii*, *P. mirabilis*, *Providencia stuartii*, or *Pantoea* spp. *and non-Enterobacteriaceae P. aeruginosa* were encountered at a very low frequency (ranged from < 1% to 3%) which agrees with similar findings reported from Bosnia (Uzunovic-Kamberovic et al, 2006). These authors also reported, that most Gram negative bacteria were obtained from female patients, (77.2%) which is consistent with the finding of this study (74.3%) as well as others (Alzohairy and Khadri, 2011; Pondei *et al*., 2012).

In agreement with Moyo *et al*, (2010) *E. coli* isolates as well as other Gram negative bacteria, positive for ESBLs, were more commonly found in children as well as the elderly subjects, as compared to middle aged subjects. This vulnerability to UTI may be associated with less developed, waned immune system efficacy, and/or frequent use of antibiotics among those age categories. In contrast, Al-Otaibi and Bukhari, (2013) reported that, the non-ESBL *E. coli* was more commonly found in children. This difference is probably attributed to variation in age, number, and/or magnitude of sustained misuse of antibiotics *per se* region, especially among children, where it is unfortunately customarily practiced, even without a drug-prescription in Saudi Arabia as well other Middle East countries (Alam *et al.,* 2009).

According to Patil *et al, (2012)* urinary tract is the principle site of infection in diabetic patients. Changes in host defense mechanisms, the presence of diabetic cystopathy and of microvascular disease in the kidneys, as well as urine stagnation in bladder (due to sphinctercontrol neuritis) may play a role in the higher incidence of UTI in diabetic patients. Unlike *E. coli* and other recovered Gram negative bacteria, diabetic patients were much more vulnerable to UTI by *K. pneumoniae* isolates, than in those of non-diabetic ones. Yet in all cases, isolates exhibiting positive ESBLs were significantly (p=0.004) much more encountered among those isolates from diabetic patients; thereby emphasizing the risk effects of diabetes. These findings are consistent with those previously reported from India (Mahesh *et al.*, 2011; Meier *et al.*, 2011). Likewise, this same trend also holds true for complicated UTI in patients with chronic renal or cardiac diseases. With any of these risk factors, only the incidence of *K. pneumoniae* isolates that significantly correlated with these risk factors as compared to those patients without. These results seem to emphasize the superior efficient opportunistic nature of *K. pneumoniae* as compared to other recovered Gram negative bacteria in relation to the studied risk factors.

Furthermore, it is well recognized that pregnancy represents a risk factor in the occurrence of urinary tract infections as well as vulvovaginitis (Patil *et al., 2012)*. The findings of the present study also indicated that unlike the above mentioned risk factors, the risk of pregnancy exhibited different trends in both recoveries of Gram negative bacteria as well as prevalence of positive ESBLs production. Thus *E. coli* isolates and other enterobacteria were more significantly frequently recovered (p=0.004) from pregnant patients (87%) than those recovered from non-pregnant patients (76%),but the prevalence of positive ESBLs production was significantly much less (p=0.047) frequently encountered among isolates of the former group (28%) than those among isolates of the latter group (47%); presumably due to community's and/or physician's –culture regarding implemented curtailment of frequent use of antibiotics therapy in pregnant patients, and thereby less antibiotic selective pressure for ESBLs production (Rizvi *et al.*, 2011).

Moreover our susceptibility data indicated that with the exception of *E. coli* isolates (less<20% sensitive) all tested Gram negative bacteria exhibited complete resistance (100%) to ampicillin, but the inclusion of the β lactamase inhibitors (clavulanate or tazobactam) restored ampicillin activity (amoxicillin/clavulanate) in 37%, 37%, and 16 % in *E. coli*, *K. pneumoniae*, and other Gram negative bacteria respectively, suggesting the implication of Ambler type A (TEM,SHV, and/or CTX-M types) ESBLs (Numanovic *et al.*, 2013). This trend holds also true for piperacillin/tazobactam, and explained the observed remarkable activity of this combined drugs where susceptibility reached 65.5%, 53.5%, 50%, and 50% for *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and other Gram negative bacteria respectively, which confirms previous findings by Magliano *et al*, (2012). Additionally, the current study also revealed that most of Gram negative bacteria were far more resistant to gentamicin as compared to that of amikacin. The observed high activity of amikacin (~2-folds effective) against tested Gram negative bacteria isolates, as compared to gentamicin may be explained by the fact that the former antibiotic is stable to the enzymatic inactivation by Aminoglycoside 6'-N-Acetyltransferase AAC [6'] (Kitao *et al.,* 2009)]. These findings are compatible with the study of Khadri and Alzohairy, (2009) who found that the resistance rates for gentamicin and amikacin for *E. coli*, *K. pneumoniae*, and other GNB were (28%, 12 %,); (31%, 25%); and (34%, 24%) respectively. As expected, there are regional differences in the rate of resistance against aminoglycoside antibiotics among Gram negative bacteria reported worldwide. Bano *et al*, (2012*)* in Pakistan found that the gentamicin and amikacin resistance rates among uropathogenic *E. coli* were 77% and 44% respectively. While in India (Sharma *et al.*, 2013) slightly lower resistance rates of 33.3% were reported for both drugs. However, recently in Turkey, Yolbas *et al.*, (2013) claimed that only 3% of *E. coli* and 0.0% of *K. pneumoniae* isolates were resistant to amikacin, whereas its respective rates for gentamicin were 35% and 48%. In contrast Bano *et al*, (2012), from Pakistan, reported that as much as 54% of *K. pneumoniae* were resistant to amikacin as compared to 44% in *E. coli* isolates. These authors also reported that 77% of *E. coli* and 60% in *K. pneumoniae* isolates were resistant to gentamicin.

Because of high resistance rates of community urinary *E. coli* isolates to ampicillin and amoxicillin/clavulanate, quinolones are usually considered as an option, especially in the outpatient setting, due to availability of oral formulations. In this study, ciprofloxacin resistance rates among *E. coli* (44%), *K. pneumoniae* (49%), and other Gram negative bacteria (39%) isolates, were relatively higher than those previously found by Kashef *et al*, (2010) in Iran for *E. coli* (32%), *K. pneumoniae* (29%), and other Gram negative bacteria (13%). Similarly, in Bangladesh (Ahmed *et al.*, 2011) and Korea (Lee *et al.*, 2010), the ciprofloxacin resistance rates among the uropathogenic *E. coli* isolates were 39% and 32% respectively. However, in Italy, Magliano *et al,(2012)* found significantly lower frequency of ciprofloxacin resistance among CA-UTI caused by *E. coli* (23%) and *K. pneumoniae* (6%) reflecting its rational use in Italy as expected. An even lower ciprofloxacin resistance rate of 17 % was reported by Sanchez *et al, (2012)* in USA. According to these authors, the increase in provider use of fluoroquinolones may account for the rapid rise in antimicrobial resistance of *E. coli* to ciprofloxacin, as resistance to this agent has been shown to correlate with the level of use. Due to the propensity of *E. coli* to acquire resistance to this agent, use of ciprofloxacin for empirical treatment of UTI in outpatients should be used sparingly and only where local antimicrobial resistance rates remain low (Gupta et al., 2011; Sanchez *et al.*, 2012). This would support the previous conclusion

(Arslan *et al*., 2005) that prior exposure to fluoroquinolones appears to be an independent risk factor for ciprofloxacin resistant *E. coli* from CA-UTI. The findings of this study also showed that in general, resistance levels to antibiotics in Saudi Arabia are rising with time. Thus almost 2 decades ago, even with hospital Gram negative bacterial isolates (n=106) from ICUs, El-Kersh *et al*, (1995) reported that apart from *P. aeruginosa* , none of these isolates was resistant to ciprofloxacin or imipenem. Unlike ciprofloxacin (resistance rates ranged from 39% to 49%), the current study showed that meropenem still exhibited the highest antimicrobial activity against all tested Gram negative bacteria isolates, with only 1 %, 5 % and 15% resistance rates for *E. coli, K. pneumonia* and other Gram negative bacteria isolates respectively; suggesting porin loss and/or carbapenemase metallo β-lactamases (MBL) enzymatic inactivation. These isolates not only exhibited complete resistance to β-lactam antibiotics but also to all un-related antibiotic groups tested i.e. to all tested 14 drugs (pan-resistant isolates).

Globally, TMP/SMZ or a quinolone is the most commonly used drug for the treatment of UTI (Khawcharoenporn *et al*., 2013). The present findings indicated that as much as 53% of tested *E. coli* isolates are resistant to TMP/SMZ versus 39 % and 28% for *K. pneumoniae* and other Gram negative bacteria respectively. In a similar study conducted in Iran, Kashef *et al, (2010)* reported that the resistance rates of TMP/SMZ against CA-UTI caused by *E. coli*, *K. pneumoniae*, and other Gram negative bacteria isolates were 62%, 53 %, and 42%, respectively. Whereas, in Taiwan (Lin *et al*., 2008) the TMP/SMZ resistance rate in *E. coli* of CA-UTI was more than 50%. Such high TMP/SMZ level of resistance may be attributed to the fact that generally, this drug is frequently prescribed empirically to pregnant /non-pregnant women (high risk UTI), because of its high safety margin, in Saudi Arabia as well as in other countries. It has also been reported (Patricia, 2001) that women who had taken recently TMP/SMZ were >16 times as likely as women who had not taken recently antibiotics to be infected with an isolate resistant to this drug. Hence, the Infectious Diseases Society of America guidelines do not recommend fluoroquinolones as initial empirical treatment for uncomplicated UTI, except in communities with 10% to 20% resistance to TMP/SMZ mong uropathogens (Lin *et al*., 2008).

Finally, our results also indicated that 75% and 70% of *E. coli* and other Gram negative bacteria isolates were susceptible to nitrofurantoin respectively, compared to only 14% of sensitive *K. pneumoniae* isolates. In Russia (Stratchounskim and Rafalski, 2006) however, a significantly lower nitrofurantoin resistance rates of 1.2%, and 46.7% against *E. coli* (n=423), and *K. pneumoniae*(n=33) isolates were reported respectively. Whereas its resistance rates in Italy (27) were 5% and 65% respectively. Although, this drug was introduced into clinical use for more than half a century, it still effective against Gram negative bacteria primarily *E. coli*, as a single resistance mechanism is difficult to mount(due its multiple non-specific protein synthesis inhibition (McOsker and Fitzpatrick, 1994) and due to its rather narrow range, hence it is less frequently prescribed (Arredondo-García and Amábile-Cuevas, 2008). This highlight the nitrofurantoin –crucial role in the management of CA-UTI especially in cases of multidrug-resistant isolates, in which oral active antimicrobials are limited resources nowadays as recently stated by Munoz-Davila, (2014).

In conclusion, this study suggests that children with age of (≤1- 10 year), elderly with age of (71- 80 year), and diabetic patients were the major risk factors for the highest prevalence of Gram negative bacteria as well as ESBLs production. *E coli* was the predominant isolate, but *K pneumoniae* is significantly more associated with tested risk factors except pregnancy. The detection of pan–resistant-isolates among the community is alarming, which narrow therapeutic options, and increase mortality rate. The different antimicrobial resistance patterns of Gram negative bacteria, found in this study may be taken in consideration by local physicians to ensure appropriate empiric use, and hopefully help in the treatment of CA-UTI, and the curtailment of pan-resistant Gram negative bacteria isolates among Saudis population.

ACKNOWLEDGEMENTS

The authors extend their appreciation to the College of Applied Medical Sciences Research Center and the Deanship of Scientific Research at King Saud University for funding this research

REFERENCES

Ahmad S (2012). Pattern of urinary tract infection in Kashmir and antimicrobial susceptibility. *Bangladesh Med. Res. Counc. Bull.* 38(3): 79-83.

Ahmed MU, Khairuzzaman M, Begum A, Ahmed I (2011). Isolation and Antimicrobial Susceptibility Pattern of *Escherichia coli* Causing Urinary Tract Infection in Enam Medical College Hospital. *J. Enam. Med. Col.* 1(2): 60-62.

Alam MF, Cohen D, Butler C, Dunstan F, Roberts Z, Hillier S, Palmer S (2009). The additional costs of antibiotics and re-consultations for antibiotic-resistant *Escherichia coli* urinary tract infections managed in general practice. *Int. J. Antimicrob. Agents*, 33(3):255-257.

Al-Jiffri O, El-Sayed ZM and Al-Sharif FM (2011). Urinary Tract Infection with *Esherichia coli* and Antibacterial Activity of Some Plants Extracts, *Int. J. Microbiol. Res.* 2 (1): 01-07.

Al-Otaibi FE, Bukhari EE (2013). Clinical and laboratory profiles of urinary tract infections caused by extended-spectrum β-lactamase-producing *Escherichia coli* in a tertiary care center in central Saudi Arabia. *Saudi Med. J.* 34 (2): 171-176

Al-Tawfiq JA, Anani AA (2009). Antimicrobial Susceptibility Pattern of Bacterial Pathogens Causing Urinary Tract Infections in a Saudi Arabian Hospital. *Chemotherapy;*, 55:127–131.

Alzohairy M, Khadri H (2011). Frequency and Antibiotic Susceptibility Pattern of Uro-Pathogens Isolated from Community and Hospital-Acquired Infections in Saudi Arabia – A Prospective Case Study. *Br. J. Med. Med. Res.* 1(2): 45-56.

Arredondo-García JL, Amábile-Cuevas CF (2008). High resistance prevalence towards ampicillin, co-trimoxazole and ciprofloxacin, among uropathogenic *Escherichia coli* isolates in Mexico City. *J. Infect. Developing Countries*; 2(5): 350-353.

Arslan H, Azap OK, Ergönül O, Timurkaynak F (2005). Risk factors for ciprofloxacin resistance among *Escherichia coli* strains isolated from community-acquired urinary tract infections in Turkey. *J. Antimicrob. Chemother.* 56:914-918.

Bano K, Khan J, Begum RH, Munir S, Akbar N, Ansari JA, Anees M (2012). Patterns of antibiotic sensitivity of bacterial pathogens among urinary tract infections (UTI) patients in a Pakistani population. *Afr. J. Microbiol. Res*. 6(2): 414–420.

El-Kersh T, Tawfik A, Al-Shammary F, Al-Salah S, Kambal A, Shibl A (1995). Antimicrobial resistance and prevalence of extended spectrumβ-lactamase among clinical isolates of gram-negative bacteria in Riyadh. *J. Chemother.* 7(6):509-514.

Elsharkawy AA , Elkady LM, Mansour SA, Esmaeel NE (2013). Detection of extended-spectrum and plasmid-mediated AmpC β-lactamases in nosocomial *Klebsiella* isolates. *J. Microbiol. Infect. Dis.*3(1): 24-30

Fam N, Gamal D, El Said M, Aboul-Fadl L, El Dabei E, El Attar S, Sorur A, Fouad S and Klena J (2013). Detection of Plasmid-Mediated AmpC β-Lactamases in Clinically Significant Bacterial Isolates in a Research Institute Hospital in Egypt. *Life Sci. J.* 10(2) http://www.lifesciencesite.co.

Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, Moran GJ, Nicolle LE, Raz R, Schaeffer AJ (2011). International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. *Clin. Infect. Dis.* 52:e103–e120.

Irajian G, Moghadas AJ (2010). Frequency of extended-spectrum β-lactamase positive and multidrug resistance pattern in Gram-negative urinary isolates, Semnan, Iran. *Jundishapur J. Microbiol.* 3(3): 107-113.

Kashef N, Djavid GE, Shahbazi S (2010). Antimicrobial susceptibility patterns of community-acquired uropathogens in Tehran, Iran. *J. Infect. Dev. Ctries*. 4(4):202-206.

Khadri H, Alzohairy M (2009). High prevalence of multi-drug-resistance (MDR) and extended spectrum β-lactamases (ESBL) producing bacteria among community-acquired urinary tract infections (CAUTI). *J. Bacteriol. Res.* 1(9):105-110.

Khawcharoenporn T, Vasoo S, Singh K (2013). Urinary Tract Infections due to Multidrug-Resistant Enterobacteriaceae: Prevalence and Risk Factors in a Chicago . *Emergency Medicine International*; Volume 2013, Article ID 258517, 7 pages, http://dx.doi.org/10.1155/2013/258517

Kitao T, Miyoshi-Akiyama T, Kirikae T (2009). AAC(6')-Iaf, a Novel Aminoglycoside 6-N-Acetyltransferase from Multidrug-Resistant *Pseudomonas aeruginosa* Clinical Isolates. *Antimicrob. Agents Chemother.* 53(6): 2327-2334.

Kurtaran B, Candevir A, Tasova Y, Kibar F, Inal A S , Komur S, Aksu HSZ (2010). Antibiotic resistance in community-acquired urinary tract infections: Prevalence and risk factors. *Med. Sci. Monit.*, 16(5): 246-251

Lee DS, Lee CB, Lee SJ (2010). Prevalence and Risk Factors for Extended Spectrum β-Lactamase-Producing Uropathogens in Patients with Urinary Tract Infection. *Korean J. Urol.* 51:492-497.

Lin CY, Huang SH, Chen TC, Lu PL, Lin WR, Chen YH (2008). Risk factors of ciprofloxacin resistance in urinary *Escherichia coli* isolates. *J. Microbiol. Immunol. Infect.* 41: 325-331.

Magliano E, Grazioli V, Deflorio L, Leuci AI, Mattina R, Romano P, and Cocuzza CE (2012).Gender and Age-Dependent Etiology of Community-Acquired Urinary Tract Infections. *The Scientific World Journal*., Article ID 349597; doi:10.1100/2012/349597.

Mahesh E, Ramesh D, Indumathi VA, Khan MW, Kumar PS, Punith K (2010). Risk Factors for Community Acquired Urinary Tract Infection caused by ESBL producing Bacteria. *JIACM* 11(4): 271-276.

McOsker CC, Fitzpatrick PM (1994). Nitrofurantoin: mechanism of action and implications for resistance development in common uropathogens. *J. Antimicrob. Chemother.* 33: 23-30.

Meier S, Weber R, Zbinden R, Ruef C, Hasse B (2011). Extended-spectrum β-lactamase producing Gram-negative pathogens in community-acquired urinary tract infections: an increasing challenge for antimicrobial therapy. *Infection*. 39:333–340.

Moyo S, Aboud S, Kasubi M, Lyamuya E, Maselle S (2010). Antimicrobial resistance among producers and non-producers of extended spectrum β-lactamases in urinary isolates at a tertiary Hospital in Tanzania. *BMC Res. Notes*; 3: 348.

Munoz-Davila MJ (2014). Role of Old Antibiotics in the Era of Antibiotic Resistance. Highlighted Nitrofurantoin for the Treatment of Lower Urinary Tract Infections. *Antibiotics*, 3, 39-48; doi:10.3390/antibiotics30100

Numanovic F, Hukic M, Delibegovic Z., Tihic N, Pasic S, Gegic M (2013). Comparison of double disk synergy test, VITEK 2 and Check-MDR CT102 for detection of ESBL producing isolates. *Acta Medica Academica* 42(1):15-24

Ogutlu A , Guclu E, Karabay O, Utku A A, Tuna N and Yahyaoglu M (2014). Effects of Carbapenem consumption on the prevalence of *Acinetobacter* infection in intensive care unit patients. *Annals of Clin. Microbiol. and Antimicrobials* 13:7 http://www.ann-clinmicrob.com/content/13/1/7.

Otajevwo FD (2013). Urinary Tract Infection among Symptomatic Outpatients Visiting a Tertiary Hospital Based in Midwestern Nigeria. *Glo. J. Health Sci.* 5(2): 187-199

Patil N, Mali U, Ramtirthkar M, Bhave P, Mali S, Mane V (2012). Bacteriuria in diabetic patients. *World J. Sci. Technol.* 2(12):25-27.

Patricia AB (2001). Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin. Microbiol. Rev.* 14(4): 933-951.

Pondei K, Orutugu L, Pondei J (2012). Current microbial and culture sensitivity pattern of urinary tract infection in a private hospital setting in Bayelsa State, Nigeria. *Int. Res. J. Microbiol.* 3(12): 393-398

Rizvi M, Khan F, Shukla I, Malik A, Shaheen K (2011). Rising Prevalence of Antimicrobial Resistance in Urinary Tract Infections During Pregnancy: Necessity for Exploring Newer Treatment Options. *J. Lab. Physicians*. 3(2): 98–103.

Sanchez GV, Master RN, Karlowsky JA, Bordond JM (2012). In Vitro Antimicrobial Resistance of Urinary *Escherichia coli* Isolates among U.S. Outpatients from 2000 to 2010. *Antimicrob. Agents Chemother.*; 56(4): 2181-2183.

Sharma I, Paul D (2013). Prevalence of community acquired urinary tract infections (UTI) in Silchar Medical College, Assam, India and its antimicrobial susceptibility profile. *Int. J. Microbiol. Res. Rev.* 1 (1): 001-005.

Stratchounskim LS, Rafalski VV (2006). Antimicrobial susceptibility of pathogens isolated from adult patients with uncomplicated community-acquired urinary tract infections in the Russian Federation: two multicentre studies, UTIAP-1 and UTIAP-2. *Int. J. Antimicrob. Agents*. 28(1):S4-9.

Thomson KS, Cornish NE, Hong SG, Hemrick K, Herdt C, Moland ES (2007). Comparison of Phoenix and VITEK 2 Extended-Spectrum β-Lactamase Detection Tests for Analysis of *Escherichia coli* and *Klebsiella* Isolates with Well-Characterized β-Lactamases. *J. Clin. Microbiol.* 45 (8):2380–2384

Tijjani J, Arzai AH, Sadiq NM (2012). Antimicrobial Susceptibility Pattern of Extended Spectrum β-Lactamase Producers in Gram-Negative Urogential Isolates in KANO, NIGERIA. *Bayero J. Pure and Appl. Sci*. 5(1): 20 – 25.
Uzunovic-Kamberovic S, Saric D, Sestic S (2006). Community-acquired urinary tract infections by extended-spectrum β-lactamase-producing Enterobacteriaceae in Zenica-Doboj Canton, Bosnia and Herzegovina. *Medicinski glasnik*. 3(2): 46-52.
Vincent C, Boerlin P, Daignault D, Dozois CM, Dutil L, Galanakis C, Reid-Smith RJ, Tellier P, Tellis PA, Ziebell K, and Manges AR (2010). Food reservoir for *Escherichia coli* causing urinary tract infections. *Emerg. Infect. Dis.* 16(1): 88–95.
Yolbas I, Tekin R, Kelekci S, Tekin A, Okur MH, Ece A, Gunes A, Sen V (2013). Community-acquired urinary tract infections in children: pathogens, antibiotic susceptibility and seasonal changes. *Eur. Rev. Med. Pharmacol. Sci*. 17: 971-976.