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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

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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

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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), nitrofurantoin, or quinolones for lower uncomplicated UTI (cystitis) while fluoroquinolones, 3rd and 4th generations cephalosporins, 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 beta-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 outpatients 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 ≤1 year 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 10⁵ 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
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). Fisher’s exact test one way analysis variance (ANOVA), Kruskal-wallis 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 bacteriuria (≥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%), 3E. cloacae (43%), 2S.
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 ≤1 year 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 diabetic patients. 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%).

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

<table>
<thead>
<tr>
<th>Risk factors subjects (n)</th>
<th>E. coli n=206</th>
<th>K. pneumonia n=43</th>
<th>Other GNB n=20</th>
<th>Total Positive ESBL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic 67</td>
<td>44 (65.7)</td>
<td>27 (61.4)*</td>
<td></td>
<td>41 (61.2)*</td>
</tr>
<tr>
<td>Non diabetic 202</td>
<td>162 (80.2)*</td>
<td>64 (39.5)</td>
<td>10 (55.6)</td>
<td>83 (41.1)</td>
</tr>
<tr>
<td>Renal disease 45</td>
<td>29 (64.4)</td>
<td>16 (55.2)</td>
<td>8 (57.1)</td>
<td>25 (55.6)</td>
</tr>
<tr>
<td>Non renal disease 224</td>
<td>177 (79.0)*</td>
<td>75 (42.4)</td>
<td>8 (57.1)</td>
<td>99 (44.2)</td>
</tr>
<tr>
<td>Cardiac 18</td>
<td>13 (72.2)</td>
<td>7 (53.8)</td>
<td>2 (50)</td>
<td>10 (55.6)</td>
</tr>
<tr>
<td>Non cardiac 251</td>
<td>193 (76.9)</td>
<td>84 (43.5)</td>
<td>19 (7.6)</td>
<td>114 (45.4)</td>
</tr>
<tr>
<td>Pregnant 37</td>
<td>32 (86.5)</td>
<td>9 (28.1)</td>
<td>9 (24.3)</td>
<td>71 (44.1)</td>
</tr>
<tr>
<td>Non pregnant 232</td>
<td>174 (75.5)</td>
<td>82 (47.1)</td>
<td>10 (52.6)</td>
<td>115 (49.6)*</td>
</tr>
</tbody>
</table>

*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)

**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,
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 cefotaxin 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.

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

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

*Other GNB: Enterobacter cloacae and *E. aerogenes*, Serratia marcescens; Citrobacter freundii; *C. koseri*; Pseudomonas aeruginosa, Morganella morganii; Proteus mirabilis, Providencia stuartii; and Pantoea spp*
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 sphincter-
control 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 Maglino 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, Maglino 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 therapy for uncomplicated UTI, except in communities with 10% to 20% resistance to TMP/SMZ among 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 (Strachounskim and Rafański, 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-Garcia 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 Saudi population.

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REFERENCES


