Antibiotic susceptibility patterns of *Escherichia coli* isolates from patients with significant bacteriuria

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Abstract

The aim of this study was to the isolation and identification of *E. coli* bacteria from patients with significant bacteriuria and Antibiotic susceptibility patterns. During the period from period from March 2011 to May 2012, a total of 2000 urine samples were collected from patients with suspected UTI. Isolates were identified by traditional biochemical tests, and then confirmed by VITEK 2 system. 455 (22.8%) samples were recognized as significant bacteriuria. The study documented that *E. coli* is the most important uropathogen causing UTI and recovered from 207 (45.5%) patients. However, of the patients with significant bacteriuria, a total of 143 (31.4%) positive urine cultures were implicated in nosocomial infections. Additionally, 312 (68.6%) positive urine cultures were implicated in community-acquired infections. According to demographic data, it was observed that the number of patients with significant bacteriuria was higher in females, 309 (67.9%) compared to males, 146 (32.1%). The mean age of these patients was 39.1 years range from 2-90 years (standard deviation, 18.1years). Majority of patients with significant bacteriuria (269, 59.1%) were in the age group 20-50 years. Sensitivity of all isolates was tested against 23 Antibiotics. Results showed all isolates of *E. coli* were resistant 100% to ampicillin but sensitive100% to imipenem, the antibiotics resistance rate among the tested *E. coli* isolates ranged from 92.7%-74.9%, 69.6%-31.4% and 48.3%-10.2%, present to cephalosporins, fluoroquinolone and aminoglycosides respectively.

Introduction

Increased use of β-lactam antibiotics, particularly the third generation of cephalosporins, has been associated with the emergence of β-lactamases mediated bacterial resistance, which subsequently led to the development of ESBLs producing bacteria. ESBLs are enzymes that mediate resistance to extended spectrum e.g., third

generation cephalosporins as well as monobactams (CLSI, 2012), but not the cephamycins or carbapenems (Bush and Fisher, 2011), produced by the Gram-negative bacteria more commonly in *E. coli* and *K. pneumoniae* (Peirano and Pitout, 2010). A shift in the distribution of different ESBLs has recently occurred in different part of the world, with a dramatic increase of CTX-M enzymes over TEM and SHV variants (Coque *et al*., 2008). CTXM- ESBL-producing *Escherichia coli* have emerged as a significant and developing problem in many parts of the world, occurring in patients in the community as well as in those with recent hospital contact (Bonnet, 2004). Currently >100 different CTX-M enzymes that can be divided into six different groups based on their amino acid sequences: CTX-M-1,-2,-8,-9 and 25 (Smet *et al*., 2010), named after the enzyme first discovered for each lineage (Pagani *et al*., 2003). The diversity and increasing prevalence of CTX-M-type ESBLs pose a serious threat to the clinical use of third-generation cephalosporins for the treatment of severe infections (Livermore *et al*., 2007). Studies over the last 10 years have revealed that unlike some exceptions, the CTX-M enzymes have nearly displaced other ESBLs enzymes in *Enterobacteriaceae*, including TEM and SHV ESBL variants (Iroha *et al*., 2012). This study is carried out to evaluating the current occurrence and antibiotic susceptibility profiles of *E. coli* isolated from patients with significant bacteriuria.

Materials and methods

Collection and Handling of Samples

During the period from March 2011 to June 2011, a total of 2000 urine samples were taken (by standard mid-stream "clean catch" method) from patients with clinical suspected urinary tract infection (UTI) according to (Collee *et al.*, 1996).

Identification of Bacterial Isolates

Escherichia coli (207 isolates) and other bacterial isolates (248 isolates) were identified depending on the traditional morphological and biochemical tests according to the methods of MacFaddin (2000) as mentioned in Table (1). Selected isolates were further confirmed as *E. coli* by the VITEC 2 identification system (BioMerieux, Marcy L'E toile, France). This system was prepared in accordance with the manufacturer's instructions fixed on their strips.

Antibiotic Susceptibility Testing

Antimicrobial susceptibility testing of uropathogenic *E. coli* isolates (n= 207) performed identification to susceptibility testing by modified disc-diffusion method (Kirby-Bauer) (Bauer *et al.*, 1966).

Results

Patient Demographics and Etiological Agents

The present study received and examined 2000 urine samples from Teaching Hospital in Al-Diwaniya during the research period. Among these, 455 (22.8%) demonstrated as significant bacteriuria (presence of $\geq 10^5$cfu/ml) and/or significant bacteriuria with pyria (more than 10 polymorphonuclear pus cells/high power field). Present results also revealed that 552 (27.6%) samples show insignificant growth, 900 (45.0%) samples were no bacterial growth, and in 93 (4.6%) others the cultures were contaminated (1). Patients with renal stones and indwelling urinary catheters were excluded.

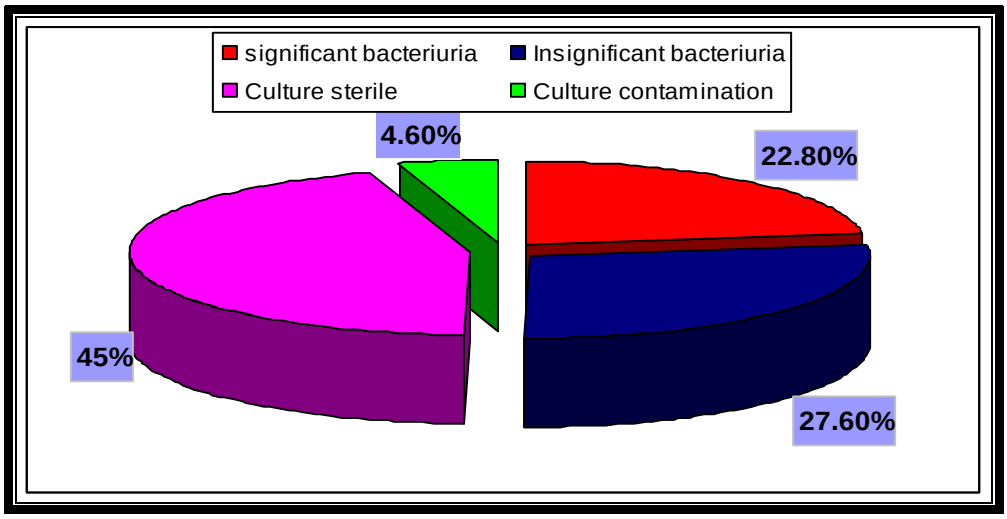

Figure (1): Occurrence of significant bacteriuria in patients suspected urinary tract infection.

For purposes of this study, nosocomial infections were defined as infections acquired at least 48 hr after hospital admission, whereas patients with community-acquired infections were those who had positive urine cultures at the time of or within 48 hr of hospitalization. However, of the patients with significant bacteriuria, a total of 143 (31.4%) positive urine cultures were implicated in nosocomial infections.

Additionally, 312 (68.6%) positive urine cultures were implicated in community-acquired infections (Figure 2). According to demographic data, it was observed that the number of patients with significant bacteriuria was higher in females, 309 (67.9%) compared to males, 146 (32.1%) (Figure 3). The female to male ratio in the present study was 2.1:1. However, significant difference between females and males was noticed in these patients (P<0.05).

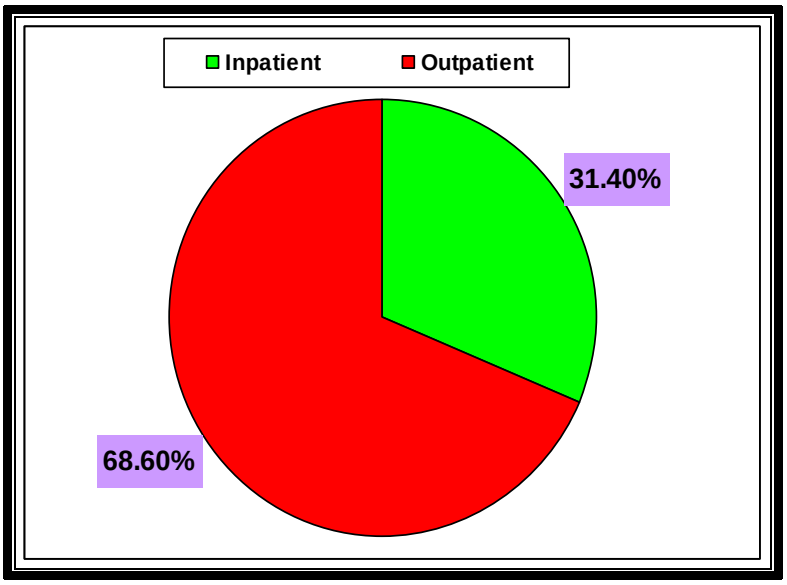

Figure (2): Occurrence nosocomial and community-acquired infections of patients suspected urinary tract infection.

The patient's age were categorized into three age groups: <20, 20-50, and >50 years. The mean age of these patients was 39.1 years range from 2-90 years (standard deviation, 0.3 years). Majority of patients with significant bacteriuria (269, 59.1%) were in the age group 20-50 years. This age group including; 200 females and 69 males, followed by 130 (28. 6%) in age group up to 50 years (77 females and 53 males),followed by the lowest incidence in age group lower than 20 years, where the number of recorded patients was 56 (12.3%), 39 females and 17 males (Figure 4). In this investigation, all urine samples were routinely cultured on MacConkey and blood agar plates. The bacterial isolates obtained as a pure and predominant growth from urine samples were only considered for the present study, and only one isolate per patient from UTI were included in the study.

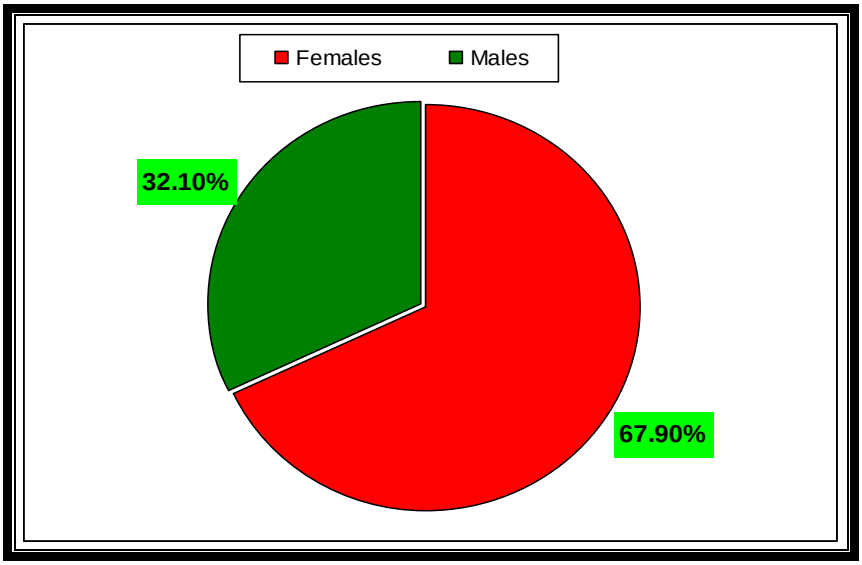

Figure (3): The percentage occurrence rate of urinary tract infection according to sex.

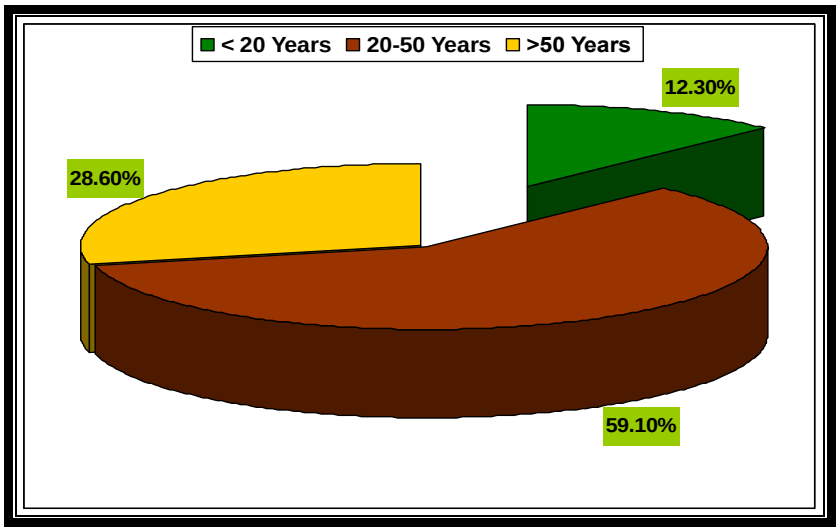

Figure (4): The percentage occurrence rate of urinary tract infection according to age.

Totally, 455 consecutive nonduplicate bacterial isolates were recovered from urine samples of patients with significant bacteriuria. The isolates were identified by their cultural characteristics, Gram staining, and reactions to standard biochemical tests. Primary tests were carried out on the isolated colonies of isolates that they behave as a typical *E. coli* on MacConkey agar, and biochemical tests. After confirmation of the presence of suspected *E. coli* isolates, the colony was subcultured on eosin methylene blue (EMB) agar, which is a differential and selective medium to differentiate between different *Enterobacteriaceae* in terms of the morphological characteristics and color on the agar. According to the color of *E. coli* on EMB, a colony was streaked on EMB to obtain pure cultures of *E. coli* colonies. From the pure culture, a

distinct *E. coli* colony was screened with additional biochemical tests, the phenotypic characteristics of suspected *E. coli* are listed in Table (1). VITEK 2 system was then carried out for the final identification of nine isolates had a phenotype consistent with production of a CTX-M ESBL.

Present study revealed that, out of the total of 455 positive urine culture recovered from patients with significant bacteriuria, 207 (45.5%) isolates were identified as *E. coli*, 71 (15.6%) *Klebsiella* spp, 64 (14.0%) *Proteus* spp, 51 (11.2%) *Enterobacter* spp, 19 (4.2 %) *Pseudomonas aeruginosa*,9 (2.0%) other Gram-negative bacteria, 29 (6.4%) *Staphylococcus* spp and 5 (1.1%) *Streptococcus* spp, thus *E. coli* proved to be the major etiology in patients with significant bacteriuria (Figure 5). These *E. coli* isolates screened for the antibiotic susceptibility profiles and then investigated for the presence of CTX-M-β-lactamases groups.

Table (1): Morphological and biochemical tests of 207 *E. coli* isolated from patients with significant bacteriuria (n= 455)

Test	Result
Gram-negative bacilli	100%
Growth on the EMB agar	Metallic sheen colonies
Indole	100%
Methyl red	100%
Vogas-Proskaur	0%
Citrate utilization	0%
Motility	80%
Acid from glucose	100%
TSI (A/A+ G**)**	100%

H_2S production	0%

EMB, eosin methylene blue; TSI, triple sugar iron; A, acid; G, gas

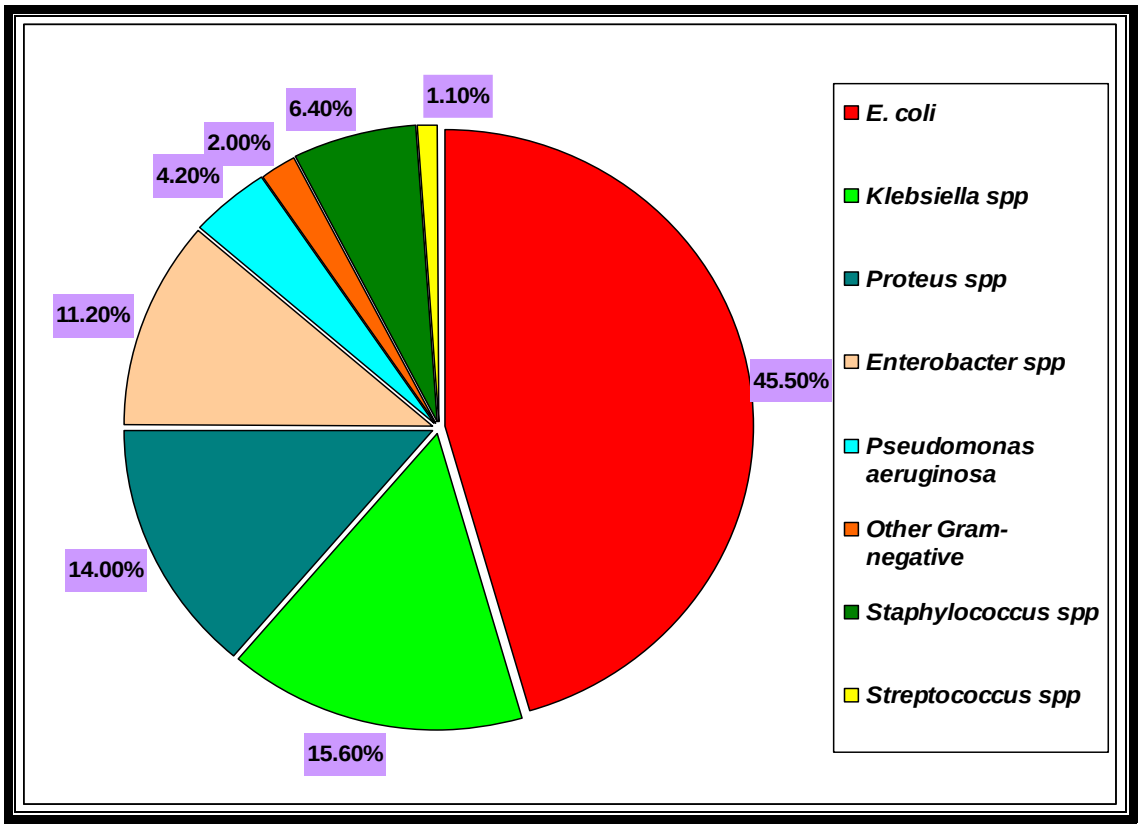

Figure (5): Growth pattern of different members of bacteria in urine culture of patients with significant bacteriuria.

Antibiotic Susceptibility of *E. coli* Isolates

All the 207 *E. coli* isolates obtained from patients with significant bacteriuria were tested for their antibiotic susceptibility against the selected 23 antibiotics. Figure (6) gives the resistance, intermediate resistance and susceptibility of the isolated *E. coli* to different antibiotics as represented by the diameter in mm. The highlight indicates cases considered to be resistant to the respective antibiotics. Aminopenicillin including ampicillin showed a resistance of 100% from all the isolates. The distribution of amoxicillin-clavulanic acid resistance among the isolated *E. coli* showed high frequency of resistance (92.7%), with an intermediate resistance of 0.5% and 6.8% were susceptible. Resistance to first generation cephalosporin (cefazolin) was found at rate of 92.3%, additionally 6.7% were intermediates, and 1.0% was

susceptible to this particular antibiotic. The resistance against third generation cephalosporins was as follows: ceftazidime (81.6%), cefotaxime (74.9%), and ceftriaxone (74.9%), while 8.7%, 13.0%, and 2.4% were intermediates resistance, respectively. However, the percentage of resistance to fourth generation cephalosporin (cefepime) was 74.9%, with an intermediate resistance of 9.2 and 15.9% were susceptible to this antibiotic. Beside this diverse resistance to cephalosporins, most of the isolates were resistant to cefoxitin (56.0%), with a percentage intermediate resistance of 35.3%. Of the *E. coli* isolates tested for monobactams (aztreonam), all isolates (100%) were nonsusceptible, including 87.0% resistant, and 13.0% intermediate resistant. The most effective β-lactam antibiotic was imipenem; all the isolates were found to be susceptible to this antibiotic. Interestingly, the isolates showed low susceptibility to meropenem, results revealed that 39.6% of the isolates exhibited resistance to meropenem and 9.7% indicated as intermediate resistant. The aminoglycosides resistance rate among the tested *E. coli* isolate ranged from 48.3%-10.2%, present study showed that amikacin was the most potent aminoglycoside its overall potency over the isolated *E. coli* was 10.2%, while tobramycin was 26.6% and finally gentamicin was 48.3%. The isolates exhibited high resistances to tetracycline (73.4%), trimethoprim (72.5%), and co-trimoxazole (71.0%), with an intermediate resistance of 6.8%, 8.2% and 2.4%, respectively. Chloramphenicol susceptibility data were obtainable for all isolates tested, of these, 14.0% were nonsusceptible, including 1.0% intermediate and 13.0% resistant. Regarding susceptibility to nitrofurantoin, most isolates displayed susceptibility to this antimicrobial agent (63.8%), while 10.1% and 26.1% exhibited resistant and intermediate resistant, respectively.

The isolates of *E. coli* showed diversity antibiograms with fluoroquinolone antibiotics tested (Figure 6). The most active fluoroquinolone was levofloxacin with resistance and intermediate resistance rates 31.4% and 15.9%, respectively. A relatively high rate of resistance in isolates was observed for lomefloxacin; 69.6% were resistant and 9.6% were intermediates resistant to this certain antibiotic. Norfloxacin had a percentage susceptibility of 45.9%, with a percentage resistance and intermediate resistance of 53.1% and 1.0%, respectively, whereas, 52.2% and 3.4% of the isolates were resistant and intermediate resistant to ciprofloxacin, respectively. However, present study revealed that all *E. coli* isolates were considered

to be multi-drug resistant (MDR), because they were resistant to at least three classes of antibiotics tested.

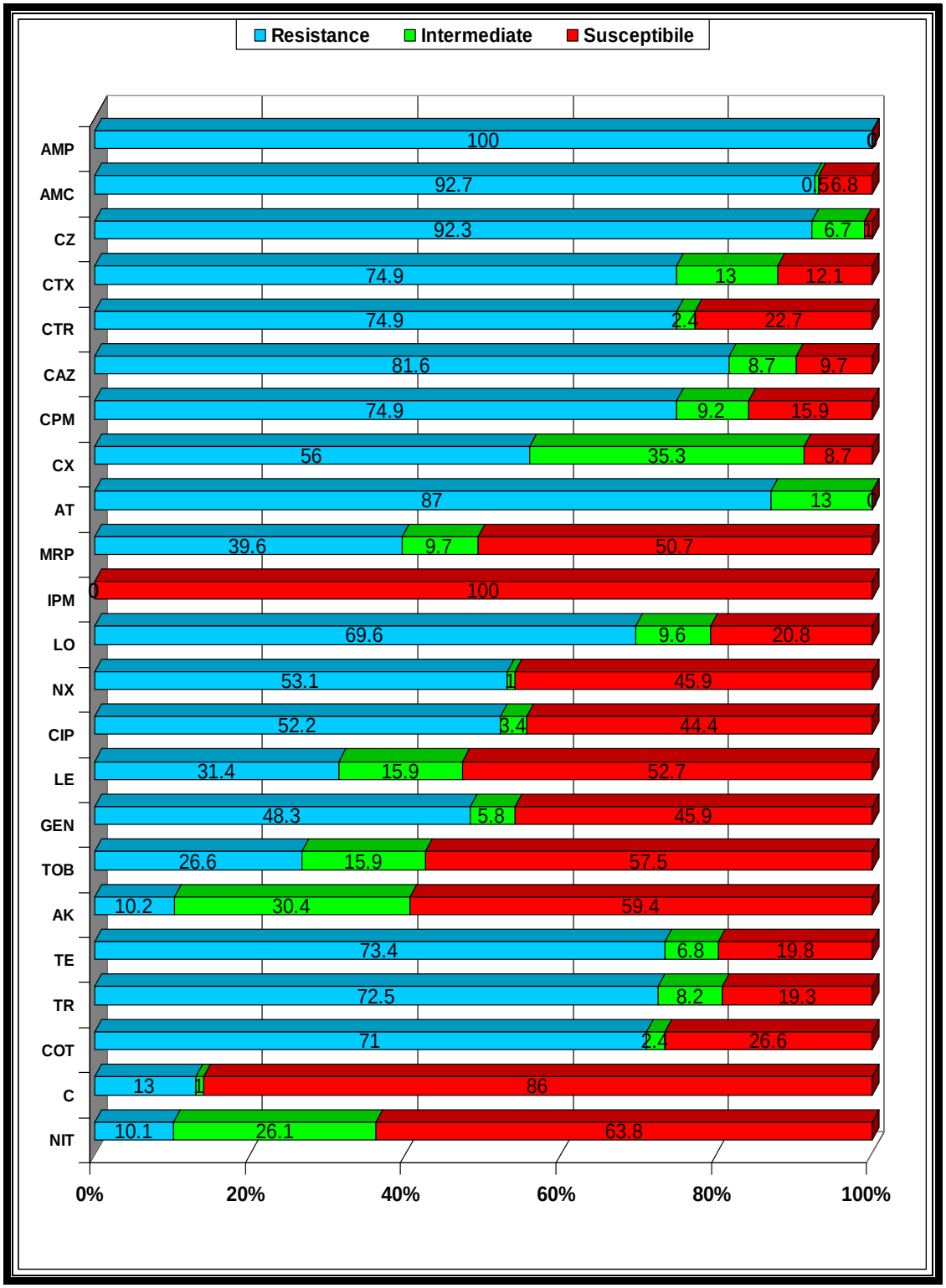

Figure (6): Susceptibility profile of *E. coli* isolates (n=207) for different antibiotics. AMP, ampicillin; AMC, amoxicillin-clavulanic acid; CZ, cefazolin; CTX, cefotaxime; CTR, ceftriaxone; CAZ, ceftazidime; CPM, cefepime; CX, cefoxitin; AT, aztreonam; MRP, meropenem; IPM, imipenem; LO, lomefloxacin; NX, norfloxacin; CIP, ciprofloxacin; LE, levofloxacin; GEN, gentamicin; TOB, tobramycin; AK,

amikacin; TE, tetracycline; TR, trimethoprim; COT, co-trimoxazole; C, chloramphenicol; NIT, nitrofurantoin.

Discussion

Urinary tract infection (UTI) is one of the common infectious diseases diagnosed in outpatients and constitutes the most common nosocomial infection in many hospitals, for about one third of all nosocomial infections (Daoud and Afif, 2011). UTI has been defined as the presence of significant numbers of pathogenic bacteria or organisms in the urinary system, depending on the presence or absence of symptoms (Grabe *et al*., 2012). UTIs are among the most prevailing infectious diseases with a substantial financial burden on Iraqi society (Hadi, 2008; Al-Sehlawi, 2012; Fayroz-Ali, 2012). There are only limited data from Al-Diwaniya. During the study period, a total of 2000 cases of suspected UTI were admitted in Teaching Hospital in Al-Diwaniya. Significant bacteriuria and/or significant bacteriuria with pyuria were observed in 22.8% of cases. In this study, laboratory confirmation of UTIs with significant bacteriuria ($\geq 10^5$cfu/ml on urine culture) and pyuria ($\geq$10 white blood cells on urinalysis) was agreed on as minimum necessary but not sufficient criteria for diagnosis of UTI in this population. However, this study rate is less than the rates observed by Hadi (2008) (51.4%) and Fayroz-Ali (2012)(76.3%) in Najaf hospitals wide study of UTI-associated with community-acquired and hospital-acquired infections.

Results have demonstrated that in general, majority of UTIs were community-acquired infections. The positive urine cultures obtained from outpatients samples were 68.6% and were 31.4% from inpatients samples. Present study found no reports in Al-Diwaniya for comparison. However, recent identification of UTI in inpatient and outpatient health care settings may provide a more accurate estimate of UTI infection incidence.

This study observed a higher proportion of UTI in females (67.9%) than in males (32.1%). The finding that females had higher prevalence of UTIs than males agrees with earlier studies (Mordi *et al.,* 2010; Ruiz *et al.,* 2011; Fayroz-Ali, 2012; Vardi *et al.,* 2012 ; Livermore *et al.*, 2012).

The reason may be due to the higher number of the females than the males in the present study populations, or may be because those males are less potent UTI, possibly their longer urethra and the presence of antimicrobial substances in prostatic fluid (Orhiosefe *et al.,* 2009). The high frequency of UTI in females, which might be

due to diversity of factors related with females, such as the close proximity of the female urethral meatus to the anus and they lack the bacteriostatic properties of prostatic secretions (Akinloye *et al.*,2006). Alterations in vaginal microflora that play a critical role in encouraging vaginal colonization with coliforms that may lead to UTI (Aiyegoro *et al.,* 2007). In agreement with present study, Todar (2002) reported that the UTIs are fourteen times more common in females than males by virtue of the abbreviated urethra. However, Ryan and Ray (2004) statement that UTI is more widespread in females, 40.0% of women have an episode in their life time. Sexually active females are also more predisposed to UTI than their male counterparts (Younis *et al*., 2009). The term "honeymoon cystitis" has been applied to this phenomenon of frequent UTI during early marriage. Microorganisms can reach the urinary tract by haematogenous or lymphatic spread, but there is abundant clinical and experimental evidence to show that the ascent of microorganisms from the urethra is the most common pathway that leads to a UTI, especially organisms of enteric origin (e.g. *E. coli* and other *Enterobacteriaceae*). This provides a logical explanation for the greater frequency of UTIs in women than in men (Grabe *et al.,* 2012). It is estimated that 40 to 50% of healthy adult women have experienced at least one UTI episode (Klemm *et al.,* 2007). In other reports, 50% of all women will experience at least 1 UTI in their lifetime and, of those, about 25% will have 1 or more recurrent infections (Dhakal *et al.,* 2008). Nevertheless, in male UTI most commonly occur in older men with prostatic disease, outlet obstruction, or urinary tract instrumentation. In later life, UTI is more common among men until the age of prostatic hypertrophy (above 40 years of age) (Schroeder *et al.,* 1990).

UTI is one of the most common diseases among all age groups encountered in medical practice today (Thulasi and Amsaveni, 2012). In this study, peak in the incidence of UTI was observed in the age groups 20-50 years (59.1%) followed by >50 years (28.6%). As such, there is not much information available from Al-Diwaniya on the dissemination of UTI within age groups. This information should therefore be construed as the first analysis performed on a diverse type of age groups related with UTI. Other study in Najaf has also reported similar findings rates (Fayroz-Ali, 2012).The high incidence of UTI in age group 20-50 years, probably because the high sexual activity within this age group. Orhiosefe *et al.*(2009) also reported this finding. Similar observations regarding the relative occurrence of the age groups have been documented in other developing as well as by the developed

countries (Getenet and Wondewesen, 2011). In Libya, Alhubgel *et al.* (2008) observed that the age group between 20 to 30 years was the most vulnerable (the highest exposed) age group to UTI recording about 1683 (49.9%) patients.

The most other age group associated with UTI in this investigation was age over 50 years. Among age groups, elderly patients are likely predisposed to conditions such as urinary tract obstruction, poor bladder emptying, and diabetes mellitus, etc. These factors favor colonization of bacteria and play an important role in UTI. Other studies have also reported similar findings (Ulleryd, 2003). However, within the group aged less than 20 years, the true incidence of UTI in children is difficult to estimate, particularly because young children with UTI may only have fever and no specific urinary tract symptoms or signs. Unrecognized UTI in infancy and childhood may have serious long-term effects and chronic pyelonephritis may occur in adults (Schlager, 2003). However, the infection occurs in all persons regardless of sex or age with particular impact on the young and the very elderly (Rubin *et al*., 1986).

One purpose of this study was to evaluate the dissemination of *E. coli* and to a certain the detection rate of this microorganism from the Gram- negative and Gram-positive pathogens in patients with significant bacteriuria, since the present study focused on detection of *E. coli* rather than other microorganisms. For many years, pathogens associated with uncomplicated UTI have remained constant, with *E. coli* was identified as

the etiological agent in about 75%–90% of infections (Lorenzo *et al*., 2010). However, present study documented that *E. coli* remain the most important uropathogen causing hospitals and community associated UTI in Iraq and was isolated from 45.5% of patients with significant bacteriuria in Al-Diwaniya hospital. Detection of *E. coli* as the predominant pathogen of community associated UTI has been extensively reported in many studies (Hryniewicz *et al*., 2001). The present finding was similar with that reported by other investigators in Iraq; Fayroz-Ali (2012) also reported *E. coli* as the most frequent organism (55.7%) isolated in urine samples suspected of UTI in Najaf. Hadi (2008) found that *E. coli* was the highest common bacteria (42%) isolated from patients with significant bacteriuria. Other recent study has documented that *E. coli* was important nosocomial pathogens representing the first leading causes of UTI in Najaf (Al-Yassery, 2011).In other study by Orhiosefe *et al.* (2009) who found that pure bacterial cultures were obtained with Gram-negative bacteria, being predominant *E. coli* was the highest isolate (45.7%).

Although, the decline in *E. coli* isolation (45.5%) rate in present setting remains unclear compared with most studies, but similar low rate isolation *E. coli* have also been reported by investigators from developed and developing countries (Mohammed *et al*., 2007). However, Abdulla *et al*. (2004) and Akram *et al*. (2007) reported that the urinary tract was the most common site of infection by *E. coli* strains. Moreover, *E. coli* accounts for more than 90% of the more than 7 million cases of cystitis and 250,000 of pyelonephritis estimated to occur in otherwise healthy individuals every year in the United States (Ryan and Ray, 2004). The ability of uropathogenic *E. coli* to cause UTI is related to general virulence factors such as α-hemolysin together with pili-mediated adherence to uroepithelial cells (P pili) (Wilson and Gidol, 2004).

This study also investigated the recent trends of incidence of other bacterial species that cause UTI in Al-Diwaniya province. As expected from UTI isolates, *Klebsiella* spp. was the second most common pathogen (15.6%) followed by *Proteus* spp. (14.0%), *Enterobacter* spp.(11.2%),*P. aeruginosa*(4.2%), *Staphylococcus* spp. (6.4%),other Gram-negative bacteria(2.0%), while the least common pathogen was *Streptococcus* spp.(1.1%). In other studies, the most common organism implicated in UTIs (80 to 85%) is *E. coli*, while *K. pneumoniae* is the cause in 5 to 10% (Foster, 2008). The reports detection rates of *Klebsiella* spp. in patients with significant bacteriuria in other parts of Iraq were 23.1% (Hadi, 2008), 22.5% (Al-Yassery, 2011) and 16.8% (Fayroz-Ali, 2012). However, present finding similar to other studies that reported by Nihar *et al*.(2008) who found that *E. coli* was the most common organism isolated from patients with significant bacteriuria (56%), followed by *Klebsiella* spp.(18%), *P. mirabilis* (17%) and *P. aeruginosa* (14%). Podschun and Ullmann (1998) reported that *K. pneumoniae* accounts for 6-17% of all nosocomial infections of urinary tracts. In the United States, *K. pneumoniae* comprises 3-7% of all nosocomial bacterial infection of the urinary tracts (Sahly and Podschun, 1997).

As expected from UTI isolates, *S. aureus* and *Streptococcus* spp. were the only Gram-positive isolates recovered in urine culture and represented as the fifth and eighth frequently isolates, respectively. Nevertheless, a study in Najaf found that *Streptococcus* spp. and *S. aureus* are the fourth and fifth frequently, occurring microorganism as causes of nosocomial UTI, accounting for 16.7% and 2.5%, respectively (Al-Yassery, 2011). Fayroz-Ali (2012) showed that *S. aureus* and *Streptococcus* spp. represented6.9% and 0.9% of all bacterial isolates and represented as the fourth and sixth frequently isolates, respectively. On the other hand, in one

study of Tunis, Larabi *et al*.(2003) reported that *Enterobacteriaceae* were the most frequently identified strains including *E. coli*, while Gram-positive strains are not a frequent care of UTI. However, the results of present study are variable (lower and higher) with the reports by others, this could be attributed to difference in geographical location and hygienic measures. The observed diversity of microorganisms in this study has serious implications as most clinicians treat patients without recourse to laboratory guidance. Such treatments are usually based on known etiological agents and susceptibilities (Orrett and Davis, 2006). This observed change in the occurrence of uropathogens may lead to a change in the antimicrobial susceptibility and ineffective treatment. Therefore, clinicians should rely on laboratory guidance before therapy as this will overcome the problem of mistreatment and reduce the emergence of resistant uropathogens.

Finally, the main risk factors associated with UTI were feminine sex and age 20-50 years. The present study revealed an occurrence of 22.8% of Gram-negative and Gram-positive bacteria among patients suspected with UTI in Al-Diwaniya province and the *E. coli* was the most obvious uropathogen among the patients.

Antibiotic Susceptibility of *E. coli* Isolates

Epidemiologic surveillance of antimicrobial resistance is indispensable for empirically treating infections, implementing resistance control measures, and preventing the spread of antimicrobial-resistant microorganisms in Al-Diwaniya province. *E. coli* is among the most important causes of nosocomial infections especially UTI (Fayroz-Ali, 2012). Unfortunately, extensive use of antibiotics is the cause of resistance phenomena, and treatment of these infections especially nosocomial infections faces a serious problem. It has been observed that antibiotic susceptibility of *E. coli* isolates is not constant and varies with time and environment. This therefore demands the need for periodic screening of *E. coli* isolates for their antibiotic susceptibility profiles in different communities and hospitals. Widespread occurrence of drug resistant *E. coli* in the community and hospitals has necessitated the need for regular monitoring of antibiotics susceptibility trends to provide the basis for developing rational prescription programs and assessing theirs effectiveness. The purpose of the present investigation is to test susceptibility of 207 uropathogenic *E. coli* isolates collected from urine of patients with significant bacteriuria to antibiotics of different classes. The isolates were obtained from Teaching Hospital in Al-

Diwaniya, the largest hospital in Al-Diwaniya, where extensive usage of antibiotics is currently very common.

The resistance level for aminopenicllins of the *E. coli* isolated in Al-Diwaniya is very high. All isolate exhibited resistance to ampicillin, which could be explained by the large-scale use of this antibiotic without a real need. Present study differs from the study of Al-Fatlawi (2012) who showed *E. coli* isolates to be having 84.6% resistance to ampicillinin Al-Diwaniya city. Other results from Iraq showed that *E. coli* isolates were 93.8% -100% resistant to ampicillin (Al- Al-Asady, 2009; Al-Hilli, 2010; Fayroz-Ali, 2012). TEM, SHV, OXA and CTX-M enzymes have been reported as the most frequent β-lactamase found in ampicillin-resistant *E. coli* in Iraq (Hadi, 2008; Al-Hilali, 2010; Fayroz-Ali, 2012). Present result is congruent to the results reported in Nigeria (Mobaleghi *et al.,* 2012), who found more than 90% resistance of their *E. coli* isolates to ampicillin. On the other hand, most countries reported resistance to aminopenicillins in 50% to 66.5% of *E. coli* with Austria and Estonia decreasing from already low trends (Gonsalves, 2011).

Amoxicillin-clavulanic acid antibiotic is the most common antimicrobial agent used in the community setting. However, incidence of amoxicillin-clavulanic acid resistant *E. coli* is very high in present study (92.7%),and other authors in Iraq have reported similar observations (Al-Muhannak, 2010; Al-Fatlawi, 2012). The low activity of amoxicillin-clavulanic acid was more likely explained by high rates of coproduction of ESBL and other plasmid mediated β-lactamase such as those belonging to Ambler classes A (e.g penicillins TEM-30 and SHV-10), C (e.g. AmpC), D (OXA enzymes) or carbapenemase (Bush and Jacoby, 2010). This is also likely to be due to the heavy selection pressure from the overuse of this amoxicillin/clavulanic acid combination and seem to be losing the battle.

Resistance frequencies were also noted for the cefazolin, 92.3% of the isolates were resistant to this antibiotic. Cefazolin is a classic antibiotic represents the first generation of cephalosporins and the bacterial resistance to cefazolin may arose from the multiuse in hospitals for different infections as parenteral antibiotic. This result is similar to previous studies in Iraq and other developing countries (Al-Muhannaak, 2010; Riaz *et al.,* 2011) perhaps are due to wide use of this drug because of their relatively cheap cost and easily administration.

During the past decade, the emergence of resistance to the third generation cephalosporins among the *Enterobacteriaceae* has become a serious problem

worldwide that now threatens the safe empirical use of these antibiotics in severe infections (Wilcox, 2009). The starting hypothesis of this thesis was that increasing resistance to the third generation cephalosporins within clinical isolates of *E. coli* would be attributed to the emergence and spread of ESBL producers, especially CTX-M, TEM and SHV ESBL variants, and they would frequently be MDR. Present findings demonstrated that *E. coli* isolates were highly resistant to ceftazidime (81.6%), cefotaxime (74.9%) and ceftriaxone (74.9%). This was consistent with earlier study in Al-Diwaniya reported by Al-Fatlawi (2012) who found that 73.7% of *E. coli* isolates were resistant to both cefotaxime and ceftriaxone. As reported previously in Najaf, Hadi, (2008) reported that 42.1%, 36.8% and 55.3% of *E. coli* isolated from patients with significant bacteriuria were resistant to cefotaxime, ceftazidime and ceftriaxone, respectively. Present study also correlates with other study done by Fayroz-Ali (2012) in Najaf where they found 71.4% resistance to cefotaxime and 71.4%, 68.8% resistant for ceftriaxone and ceftazidime, respectively. In India, Mukherjee *et al*. (2011) found that 81.2% and 85%of *E. coli* isolates were resistant to ceftazidime and cefotaxime respectively. However, it might be possible that this high level of resistance to third generation cephalosporins in current study was most probably due to acquisition of β-lactamase, which encode by *bla*-genes possibly during therapy. These antibiotics usually used for treating of urinary tract, respiratory tract and burn wound infections caused by *Enterobacteriaceae* (Naseer, 2008). The resistance to third generation cephalosporins was caused mainly by group of class A β-lactamases, which consisting of TEM, SHV and CTX-M β-lactamases that has extended hydrolytic spectrum activity on cephalosprins. However, it also may be attributed to AmpC cephalosporinases, which can result from the over expression of the chromosomally encoded AmpC enzyme in *E. coli* or by the acquisition of a plasmid-mediated AmpC β-lactamase enzyme (Woodford *et al.,* 2007). It's well established that the excessive consumption of third generation cephalosporins especially ceftazidime is not a suitable treatment plan because of its effects on acquisition of ESBL producing organisms (Livermore, 2008). Therefore, this kind of antibiotic therapy should be discouraged in the medical society. Pongpech *et al*. (2008) reported that, the persistent exposure of bacteria to a multitude of β-lactams has induced dynamic changes in terms of increasing production of β-lactamases and mutations in their restricted spectrum enzymes to become ESBLs. Eventually, most of the β-lactamases are integrated within plasmids and transposons that enable the rapid

transfer of these resistance genes between microbes and the association of insertion sequences with these β-lactamase genes are involved in their dissemination and expression of resistance (Bradford, 2001).

In this study, high rate of resistance was detected against cefepime (74.9%). However, in a previous study of *E. coli* isolates at the same province, 78.9% of isolates were found to be resistant to cefepime (Al-Fatlawi, 2012). In other reviewed study from Najaf, the resistance rate to cefepime was higher than this study (Fayroz-Ali, 2012). Cefepime resistance may be more frequent in isolates, which produce the CTX-M-type ESBLs (Yu *et al.,* 2002). Therefore, present study established that treatment using fourth generation cephalosporins is not recommended before the susceptibility testing is known.

Although, cefoxitin is not use in the treatment of bacterial infections in Al-Diwaniya province, the present research showed that 56.0% of uropathogenic *E. coli* isolates were cefoxitin resistant. Closely result reported that 42.0% of uropathogenic *E. coli* isolates were cefoxitin resistant in Al-Diwaniya (Al-Fatlawi, 2012). Other reports give high level of cefoxitin resistance in Najaf (Al-Muhannak, 2010; Al-Hilali, 2010), as well as, to reports give low level 2% and 37% of cefoxitin resistance in Belgium and Iran, respectively (Smet *et al*., 2010). The major causes of cefoxitin resistant in *E. coli* are encoding of plasmid mediated AmpC β-lactamase or other factors like; over expression of the chromosomal *ampC* genes, acquisition of plasmidic *ampC* genes, porin or permeability mutations, or a combination of these factors might cause the reduce susceptibility of *E. coli* to cefoxitin (Tan *et al*., 2009). However, cefoxitin was used as a marker for the production of AmpC β-lactamases in Najaf hospitals (Al-Sehlawi, 2012). In present study, the *E. coli* isolates resistant to cefoxitin may be considered probably AmpC producers.

Of all the *E. coli* isolates characterized in this study, 87.0% displayed resistance to aztreonam. The high rates of resistance might be taken as a marker for the production of ESBLs by these isolates. Generally, an isolate is suspected to be an ESBL producer, when it shows *in vitro* resistance to the third-generation cephalosporins and to aztreonam (Samaha-Kfoury and Araj, 2003). Present study carried out that the frequency of aztreonam resistance isolates was expected comparing with previous study conducted in Al-Diwaniya, Hilla and Najaf who identified high rates of isolates belonging to *E. coli* were aztreonam resistant (Al-Hilli 2010;Fayroz-Ali, 2012; Al-Fatlawi, 2012). However, clinical failure of cephalosporin and aztreonam therapy due

to ESBLs is a growing problem in Iraqi hospitals (Hadi, 2008; Al-Muhannak, 2010; Al-Sehlawi, 2012).

Although, imipenem resistance in *Enterobacteriaceae* has been rarely reported in past, resistance rates have recently increased. Nonetheless, imipenem remains the first choice of treatment for infections involving ESBL-producing *E. coli*. It has been estimated that worldwide rate of carbapenem resistance in *Enterobacteriaceae* is nearly 2% (Queenan and Bush, 2007). When national data are taken into account, rates of carbapenem resistance in *E. coli* are estimated to be 0% (Hadi, 2008; Al-Hilali, 2010; Al-Hili, 2010; Fayroz-Ali, 2012). However, present survey did not observe resistance to imipenem in any of the *E. coli* isolates tested. The high efficiency of this antibiotic may be due to rarely usage in Iraqi hospitals. The present study suggested that imipenem should be kept on reserve, and its use should be controlled. Interestingly, the isolates showed low susceptibility to meropenem, results revealed that 39.6% of the isolates exhibited resistance to meropenem. The finding of meropenem-resistant isolates in Al-Diwaniya hospitals may have important implications for the prevention and dissemination control of this drug-resistant *E. coli*.

Aminoglycosides continue to play an important role in antimicrobial therapy against Gram-negative pathogens, usually in combination with β-lactam agents. Resistance to the class can be widespread and has primarily been the result of aminoglycoside inactivation through the chemical processes of acetylation, phosphorylation, and /or adenylation, with varying effects depending upon the particular agent (Thomas *et al.*, 2008). According to present study the resistance rates to aminoglycosides ranged from 10.2% - 48.3%. Amikacin, however, remained active against 89.8% of *E. coli* isolates, indicating that this agent can still be used in the treatment of infections caused by uropathogenic *E. coli*. Similar results have been observed by Al-Fatlawi (2012) in Al-Diwaniya and Fayroz-Ali (2012) in Najaf. Several studies also showed that amikacin was more potent than gentamicin but if it is over used, it may also become resistant (Yasmin, 2012). In this study, the better activity of amikacin may be due to its less vulnerability to bacterial enzymes than other aminoglycosides. However, the high resistance to gentamicin (48.3%) in this survey is not surprising because of its extensive use, particularly in UTIs. This phenomenon was also observed in other studies in Egypt (Al-Agamy *et al.,* 2006), Saudi Arabia (Tawfik *et al.,* 2011), Pakistan (Hussain *et al.,* 2011), India (Shahid *et al.,* 2008) and Brazil (Kiffer *et al.,* 2006).

Nitrofurantoin is considered as one of the oldest urinary anti-infective drugs in use (Garau, 2008), surprisingly, resistance to this drug remains minimal (10.1%) in this investigation. Other study in Najaf showed that uropathogenic *E. coli* was highly susceptible to nitrofurantoin (Fayroz-Ali, 2012). In addition, the present result was very closely to Ho *et al.* (2011) in China, Marhova *et al.* (2009) in Bulgaria and Yasmin (2012) in Bangladesh who found that 6.6%, 3.5% and 2.9% of *E. coli* isolates was resistant to nitrofurantoin, respectively. The low rate of resistance may be related to the fact that nitrofurantoin has multiple mechanisms of action, requiring organisms to develop more than a single mutation in order to develop resistance. In addition, limited usage of nitrofurantoin for treating uncomplicated cystitis may also be a contributing factor to the lack of development of widespread resistance to this drug (Gupta, 2003).

The *E. coli* isolates exhibited high resistances to tetracycline (73.4%), trimethoprim (72.5%), and co-trimoxazole (71.0%). In agreement with the present study, Al-Fatlawi (2012), Hadi (2008) found that 69.8% and 60.5%, respectively of *E. coli*, isolates recovered from patients with significant bacteriuria in Najaf were resistant to trimethoprim. Smet *et al*.(2010) reported that 77.8% and 80% of *E. coli* isolates were resistant to tetracycline and trimethoprim. However, the major cause of trimethoprim resistance in Gram-negative bacteria is plasmid-borne dihydrofolate reductase (*dfr*) genes, which are commonly found as gene cassettes in class 1 integrons (Ashraf *et al.*, 2007). Heavy and widespread use of antibiotics in hospital does not only force the emergence of antibiotic resistance, but also promotes selection of drug-resistant organisms in the hospital environment (Beneiae *et al.*, 2001).

Chloramphenicol susceptibility data were obtainable for all isolates tested, of these, 13.0% were resistant. The low rate of resistance against chloramphenicol probably due to rarely use of this antibiotic to treatment of UTI and other infections caused by *E. coli* (Fayroz-Ali, 2012).

The isolates of *E. coli* showed diversity antibiograms with fluoroquinolone antibiotics tested (Figure 6). The most active fluoroquinolone was levofloxacin with resistance rate 31.4% and the less active was ciprofloxacin with resistance rate 52.2%. In Iraqi private clinics and hospitals, ciprofloxacin is the most frequently prescribed fluoroquinolone for UTIs because of its availability in oral formulations, which may account for the accumulation of multi-drug resistance among isolates. Quinolones have become the most frequently prescribed antimicrobials worldwide due to their

broad-spectrum antimicrobial activity (Yang *et al*., 2010). However, in the last few decades, an increase in quinolone resistance has been documented among human and veterinary isolates of *E. coli*. In Iraqi studies, Hadi (2008), Al-Janabi (2011), Al-Fatlawi (2012) and Fayroz-Ali (2012) found that 60.5%, 44%, 57.9% and 49% of *E. coli* isolates was resistant to ciprofloxacin, respectively. Many studies worldwide have also reported a sharp increase in ciprofloxacin resistant *E. coli* isolates from UTIs. For example, in China, from1998 to 2002, the prevalence of ciprofloxacin resistance has increased steadily from 46.6% to 59.4% (Kariuki *et al.*, 2007), and in Bangladesh the prevalence was 26% (Gupta *et al.*, 2001).

This research project also focuses on the measurements of MDR uropathogenic *E. coli* in Al-Diwaniya, these measures are necessary to prevent resistance organisms to become endemic in the hospitals. For purposes of this study, resistance to three as well as to more classes of antibiotics was considered MDR, XDR, or PDR. Detail analysis of antibiotic resistance profiles of isolates show that all of isolates were resistant to at least three classes of antibiotics tested. Present data support reports about often-difficult therapy for infections caused by uropathogenic *E. coli*. Frequently, these isolates are associated resistance to several classes of antibiotics. Although, MDR rates are high and therapeutic options are limited, some therapeutic options remain for *E. coli* in Al-Diwaniya such as amikacin and imipenem.

In finale, many factors may have contributed to such high rates of resistance including misuse of antibiotics by health care professionals or non-skilled practitioners, misuse of antibiotics by the general public and inadequate surveillance due to lack of information arising from routine antimicrobial susceptibility testing, like reports from other developing countries (Toukam *et al.*, 2010). Iraq is one of the developing countries where antibiotics are sold over the counter, an attitude that encourages self-medication. On the other hand, it is remarked that during period, a group of antibiotics become more used than others without susceptibility tests, which may lead to variability in their resistance.

Conclusion

الخلاصة

هدفت الدراسة إلى عزل وتشخيص بكتريا *E. coli* من المرضى المصابين بالتهاب المجاري البولية وإجراء فحص الحساسية الدوائية لها. جمعت 2000 عينة إدرار من المرضى المصابين بالتهاب المجاري البولية

المراجعين إلى مستشفى الديوانية التعليمي للفترة من آذار 2011 إلى مايس 2012, شخصت العزلات اعتمادا على الفحوصات البايوكيمياويه التقليدية وتقنية الفايتك 2- Vitek. أظهرت 455 (22.8 %) عينة إدرار بيله جرثوميه، وسجلت الدراسة إن بكتريا *E. coli* هي البكتريا الأكثر شيوعا 207 (45.5 %) المسببة لالتهاب المجاري البولية في الديوانية . كما وأظهرت النتائج أن ١٤٣ (٤٣,٣١ %) من العينات هي من إصابات المستشفيات و ٣١٢(٦٨,٦ %) من العينات هي من الإصابات المكتسبة من المجتمع, وكانت الإناث أعلى إصابة 309 (67.9 %) من الذكور 146 (32.1 %). كان معدل أعمار المرضى 39.1 سنة والمدى يتراوح بين ٢-٩٠ سنة بانحراف معياري 18.1 سنة, وكانت أعمار الغالبية العظمى من المرضى 269 (59.1 %) متركزة في الفئة العمرية ٢٠ ـ٥٠ سنة . اجري فحص الحساسية الدوائية للمضادات الحيوية للعزلات تجاه ٢٣ مضاد حيوي وقد أظهرت النتائج بان كل 100% عزلات بكتريا *E. coli* كانت مقاومة لمضاد ampicillin و حساسة 100% لمضاد الاميبينيم, فيما تراوحت نسب المقاومة لهذه البكتريا 74.9-92.7 % , 69.6- 31.4 % و 10.2-48.3 % تجاه مضادات السيفالوسبورينات , الفلوروكوينولونات و الامينوكلايكوسيدات على التوالي.

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