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Research Article

Prevalence and antimicrobial susceptibility patterns of uropathogenic *E. coli* among people in Zakho, Iraq

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ABSTRACT

Background: Antimicrobial resistance in uropathogenic *E. coli* is of major concern worldwide due to its increasing resistance to several commonly prescribed antibiotics. The aims of this study were to determine the prevalence and to evaluate the presence and antibiogram pattern of uropathogenic *E. coli* among people in Zakho city, Iraq.
Methods: 1120 urine samples were collected from people. Samples were screened for *E. coli* isolates and their susceptibility to different antibiotics was analyzed.
Results: Diagnoses tests showed that only 106 (9.4%) isolates were *E. coli*. Females (90, 85%) were more susceptible to urinary tract infections (UTIs) than males (16, 15%) ($p < 0.05$). The age 11-21 years old (23, 21.7%) was the most affected group ($p < 0.05$). All isolates were resistant to penicillin, ampicillin and aztreonam. While, all these isolates were sensitive to imipenem and meropenem.
Conclusions: The incidence of uropathogenic *E. coli* among people is comparable with reports from elsewhere. *E. coli* isolates are highly susceptible towards imipenem, and meropenem antibiotics, and highly resistant towards β-lactam and cephalosporins antibiotics. Education programs and improving the hygienic measures are necessary to prevent contaminations with *E. coli* and minimize the use of β-lactam and cephalosporin antibiotics.

Keywords: Uropathogens, *E. coli*, Antimicrobial resistance, Iraq

INTRODUCTION

Escherichia coli (*E. coli*) is a very diverse species of bacteria that forms a major part of the normal intestinal flora.[1] Extra intestinal Pathogenic *E. coli* (ExPEC) cause extra intestinal infections including urinary tract infections (UTIs), diverse intra-abdominal infections, pneumonia, surgical-site infections, meningitis, osteomyelitis, soft-tissue infections and bacteremia.[2,3] Uropathogenic *E. coli* (UPEC) strains are responsible for about 90% of all community-acquired UTIs and up to 50% of all nosocomial UTI.[4] The development of UTIs depends on anatomical factors, the integrity of host defense mechanisms, and the virulence of the infecting organisms.[1,5] Antibiotic resistance is a major threat to public health worldwide.[6,7] There are high prevalence of multi drug resistant *E. coli* in UTI infections against β-Lactam groups (penicillins, cephalosporins, carbapenems and Monobactams) because of their ability to produce Extended Spectrum β-Lactamases (ESBLs).[8,9] *E. coli* may acquire other antibiotic resistance traits from surroundings bacteria and conversely it can spread its resistance genes to different potential pathogens.[10] The aims of this study were to determine the prevalence of uropathogenic *E. coli* among people, of both genders aged nine months to 63 years, referring to Zakho emergency hospital and private laboratories in Zakho city/Kurdistan Region, Iraq. Also, to study their resistance against antimicrobials used to treat urinary tract infections (UTIs) to provide data to help direct empirical therapy.

METHODS

Sample collection

A total of 1120 urine samples were collected from people admitting to Zakho emergency hospital and private laboratories in Zakho city from September 2013 to February 2014. Urine samples were collected from people directly at their arrival to the emergency hospital and laboratories for different reasons. Samples were collected from both genders aged nine months to 63 years. Samples were transported to the laboratory within 30 minutes to one hour then characterized using different conventional bacteriological and biochemical methods. The study was conducted with the approval of ethics committee in the University of Zakho, School of Science.

Laboratory screening for E. coli isolates

Urine samples were cultured on MacConkey agar and incubated for overnight at 37°C. The lactose-fermenting colonies were selected and sub-cultured on MacConkey agar to obtain a pure culture. Suspected isolated colonies were diagnosed and characterized using microscopical (Gram stain, capsule stain and motility test) and biochemical tests including indole test, methyl red test (M.R), Voges-Proskaur test (V.P), citrate utilization test, urease test.[11]

Antibiotic sensitivity test

Table 1: Antibiotics used in this study class antimicrobial symbol concentration.

Class	Antimicrobial	Symbol	Con^{n} µg/ml
β-lactams			
Penicillins	Penicillin	P	30
Carbapenems	Imipenem	IPM	10
	Meropenem	MEM	10
Monobactams	Aztreonam	ATM	10
Aminopenicillin	Ampicillin	AM	25
Cephalosporins	Cefazolin (1G)	CZ	30
	Cefuroxime (2G)	CXM	30
	Ceftazidime (3G)	CAZ	30
	Cefotaxime (3G)	CTX	30
	Ceftriaxone (3G)	CRO	30
	Cefepime (4G)	FEP	30
β-lactamase Inhibitors			
Amoxicillin-clavulanic		AMC	20/10
Ampicillin-Sulbactam		SAM	25/5
Piperacillin-Tazobactam		TZP	20/10

All *E. coli* isolates were tested against 14 different antimicrobial drugs (Bioanalyse, Turkey) as listed in Table 1. Screening for antibiotic resistance was performed using disc diffusion assay according to Bauer, et al.[12] Bacterial suspensions were prepared in 1.0 ml of sterile saline solution. 0.5 ml of suspensions was spread on Mueller-Hinton agar plates and then incubated for 24 hours. The inhibition zones were measured in comparison to the Clinical Laboratory Standards institute.[13]

Statistical analysis

The relationships between categorical variables were examined using the Chi-square test with significance set at a p value of <0.05 using Minitab 15 software (Minitab Ltd., Coventry, UK).

RESULTS

Biochemical characterization of E. coli isolates

A total of 1120 urine samples were submitted for analysis and culture. Biochemical and morphological characterization of lactose fermenting isolates was carried out.[11,14] These biochemical tests were capable to give a clear cut differentiation between *E. coli* and other gram negative bacteria species. A total of 205 (18.3%) isolates showed positive growth on MacConkey agar. Lactose fermenting and lactose non fermenting colonies were 171 (83.4%) and 34 (16.6%) respectively. Among these 205 isolates, 106 (51.7%) were identified as *E. coli*.

The occurrence of E. coli isolates according to gender and age

The prevalence of *E. coli* uropathogens was analysed according to person's gender and age group. Among the 205 positive growth on MacConkey agar, 88 (43%) were from males and 117 (57%) from females. The prevalence of *E. coli* among people was significantly higher in females than males 90 (85%) and 16 (15%) respectively, (p <0.05). People were divided into 5 groups according to their age (Table 2). Our study showed that the prevalence of urinary pathogens was not consistent across all age groups. Overall, it is found that the percentage of *E. coli* isolates was high in people older than 11 years and younger than 44 years of age and lower percentage in people younger 11 years and older than 44 years. Statistical analysis showed that the incidence of infection with uropathogenic *E. coli* was significant (p <0.05) among age group of 11-21 years old (23, 21.7%).

Antibiotic susceptibility test

All *E. coli* isolates (106) were tested for their antibiotic susceptibility pattern against 14 antibiotics which include different β-lactam and cephalosporin groups. All isolates were 100% resistant to β-lactam antibiotics penicillin, ampicillin and aztreonam, whereas, all isolates were 100% susceptible to β- lactam antibiotics imipenem and meropenem (Table 3).

Table 2: Incidence of *E. coli* isolates among people according to gender and age.

Age groups (year)	No. of infected patients of both genders		Total and percentage of infection (%)
	Male	Female	
9 months-10	2	9	11 (10.4)
11-21	2	21	23 (21.7)*
22-33	7	37	44 (41.5)
34-44	3	15	18 (17.0)
45-63	2	8	10 (9.4)
Total	16	90	106 (100)

* P< 0.05

Table 3: Antimicrobial susceptibility among uropathogenic *E. coli*.

Antibiotics		No. of resistant isolates (%)	No. of sensitive isolates (%)
β-lactam	P	106 (100)	0 (0.0)
	AM	106 (100)	0 (0.0)
	ATM	106 (100)	0 (0.0)
	IPM	0 (0.0)	106 (100)
	MEM	0 (0.0)	106 (100)
Cephalosporins	CZ	55 (52)	51 (48.1)
	CXM	55 (52)	51 (48.1)
	CAZ	55 (52)	51 (48.1)
	CTX	55 (52)	51 (48.1)
	CRO	55 (52)	51 (48.1)
	FEP	55 (52)	51 (48.1)
Beta-lactamase inhibitors	AMC	18 (17)	88 (83)
	SAM	73 (68.9)	33 (31.1)
	TZP	44 (41.5)	62 (58.5)

This study revealed that only 55 (52%) *E. coli* isolates were resistant for all four cephalosporin generations including cefazolin, cefuroxime, ceftazidime, cefotaxime, ceftriaxone and cefepime. These results indicate that these isolates are ESBLs producers. *E. coli* isolates showed variable patterns in their susceptibility against the antibiotics containing β-lactamase inhibitors as it is shown in Table 3.

DISCUSSION

Urinary tract infections (UTIs) are the second most common infectious presentation in community medical practice. Worldwide, about 150 million people are diagnosed with UTI each year.[15] About 80% of UTIs are caused by *E. coli*. In this study, a total of 205 isolates showed positive growth on MacConkey agar. Lactose fermenting and lactose non-fermenting colonies were 171 (83.4%) and 34 (16.6%) respectively. Studies from different parts of Iraq and worldwide showed that lactose fermenters *E. coli* and *Klebsiella* were the most common pathogens associated with UTIs.[16-18] Non-lactose fermenting were found to be the least dominant uropathogens causing UTIs like *Pseudomonas*, *S. aureus*, *Proteus*, *Enterobacter*, *Acinetobacter* and *Enterococcus*.[19]

Biochemical and morphological tests detected 106 (51.7%) *E. coli* isolates among the 205 isolates. These results were in agreement with the results obtained from other studies conducted worldwide which approved that *E. coli* is the major pathogen that cause UTIs.[20,21] As *E. coli* is a major normal flora in the gut and most of the times poor hygiene will lead to cross contamination and then urinary tract infections.[1,22]

Various studies noted that uropathogenic *E. coli* is the predominant organism in females than males.[19,23] In the present study, the higher rate of *E. coli* was found in females (85%) compared to (15%) males. The higher incidence of urinary tract infections in females is due to unique anatomical features of the female genitourinary tract, which include a shorter urethra and the more proximal location of the urethral meatus to the anus makes it easy for bacteria to ascend in the urinary tract.[24] Fecal-perineal-urethral contamination is the most probable explanation for infections caused by *E. coli* strains causing UTI in women.[25] About 50% of women will experience at least one UTI in their lifetime, and of those about 25% will suffer from one or more recurrent (or relapsing) infections.[26] UPEC isolates can also be transmitted via sexual activities.[27]

E. coli was common among people aged 11-44 years. This result comparable to a study conducted by Kiffer, et al they found that the higher percentage of *E. coli* isolates in people of age group 13-60 and lower percentage in people younger 13 years or older than 60 years.[28] Another study found that the lowest percentage of *E. coli* was among age group less than 10 years and high within the age group 26-36 years.[29] In our current study a high percentage of Uropathogenic *E. coli* in people were found in ages (11-21) years (p<0.05). This may be due to a poor hygiene condition or other reasons. However, more study is needed to explore this result.

Antimicrobial resistance in uropathogenic *E. coli* is of major concern worldwide due to its increasing resistance to several commonly prescribed antibiotics.[30] In our study, *E. coli* isolates were various in their susceptibility to different antibiotics belonging to different groups. All isolates were 100% resistant to penicillin, ampicillin and aztreonam, whereas, all isolates were 100% susceptible to imipenem and meropenem. This results was comparable to different locally and worldwide studies.[31] This high resistance may be due to the spontaneous and uncontrollable use of these antibiotics.[32] The carbapenems (imipenem and meropenem) are known to be stable against ESBL enzymes and effective in the treatment of infections caused by ESBL-producing bacteria.[33] The administration routes of these antibiotics

either intravenous or intramuscular make this group of limited usage by most patients.[34]

55 (52%) *E. coli* isolates were resistant for all four cephalosporin generations. *E. coli* and other genera of gram negative bacteria possess a naturally occurring, chromosomally mediated β lactamase and plasmid mediated β-lactamase.[35,36] The plasmid-mediated resistance had spread all over the world and into many different members of the Enterobacteriaceae family.[37] In our study, *E. coli* isolates susceptibility patterns against the β-lactamase inhibitors was variable. These results were found to be in agreement with results obtained by Kumar et al.[38] The addition of clavulanate significantly expands amoxicillin's spectrum to include penicillinase-producing *E. coli, Klebsiella spp., S. aureus, Neisseria gonorrhoeae, and Pseudomonas mirabilis*.[39] The efficacy of β-lactam-β-lactamase inhibitor combinations may be reduced for organisms producing multiple ESBLs.[40]

CONCLUSION

The incidence and causative uropathogenic *E. coli* are comparable with reports from elsewhere. Females were more susceptible to UTI than males. The age group of 11-21 year was with higher percentage of *E. coli*. Uropathogenic *E. coli* isolates are highly susceptible towards imipenem, and meropenem antibiotics, and highly resistant towards β-lactam and cephalosporins antibiotics. For this reason, the prevalence of these pathogens and their susceptibility to antibiotics must be considered in the choice of treatment for UTI. Additionally, education programs and improving the hygienic measures are necessary to prevent contaminations and cross-infections with *E. coli* and minimize the use of β-lactam and cephalosporin antibiotics in order to minimize the emergence of ESBLs producing bacteria.

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