EXCELLENT PUBLISHERS

International Journal of Current Research in Biosciences and Plant Biology

ISSN: 2349-8080 Volume 2 Number 6 (June-2015) pp. 189-197

www.ijcrbp.com

Original Research Article

Antibiotic Resistance Pattern of *Escherichia coli* Isolated from Urinary Tract Infections

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A b s t r a c t

Forty seven of Gram-negative-lactose sugar fermenting bacteria grown on MacConkey agar was isolated from UTI collected from Al-Sadar Medical City and AL-Zahraa Hospital Education in Al-Najaf province, during 15-7-2013 to 20-10-2013. The bacterial isolates were identified according to cultural characteristics and biochemical activities. The results have revealed that 40(85%) isolated bacteria were identified as *E. coli*. The susceptibility of *E. coli* isolates were tested with 10 types of antibiotics, using disc diffusion method. The results revealed that *E. coli* isolates were highly resistant to most common antibiotics. Hence, all isolates 40 (100%) exhibited resistance to ampicillin, while 36 (90%) of isolates were resistant to piparacillin, while these isolates showed different sensitivity to different types of antibiotics. The present study showed that 68% of the isolates were sensitive to sulfamethoxazole – trimethoprim; 60% and 62.5% of the isolates were sensitive to amikacin and gentamicin respectively; 18 (45%) of isolates were found to be sensitive to nitrofurantoin. Furthermore, all isolates 40 (100%) showed sensitive to imipenem and this antibiotic was highly active amongst the antibiotics tested against *E. coli* isolates.

K e y w o r d s

Antibiotic resistance

Escherichia coli

Imepenem

Urinary tract infection

Introduction

Escherichia coli is the most common member of the genus *Escherichia* belong to Enterobacteriaceae family. The normal habitat of *E. coli* is the intestinal tracts of humans and animals. Some strains of *E. coli* are capable of causing disease like urinary tract infection (UTI), wounds, the lungs, and blood. UTIs are the most common diseases caused by bacteria and occur at any time in the life of an individual. About 95% of UTI are caused by uropathogenic *E. coli* (UPEC), fecal bacteria that colonize in the urethra and spread up the urinary tract to the bladder as well as to the kidneys or the prostate in males (Harvey, 2010).

Many studies have reported that the resistant of antibiotics have been examined phenotypically in

clinical isolates. Resistance to antibiotics is highly prevalent in bacterial isolates in worldwide, particularly in developing countries (Iruka et al., 2000). *E. coli* isolates are producing extended-spectrum β-lactamase (ESBL). The spread of ESBL-producing bacteria has become a dangerous factor and causing infection about 50% in non-hospitalized patients. Bacteria that produce β-lactamases are capable of resistant to the penicillins, first-, second-, and third-generation cephalosporins, by hydrolysis of these antibiotics. Infections of *E. coli* that are able to produce ESBL are difficult to treat (Kallen et al., 2006).

The study attempts to reach the aim of detecting the resistance of bacteria to β-lactamas by the following objectives: (1) isolation and identification of *E. coli* from UTI; (2) Phenotypic detection of antibiotics resistance of bacterial isolates; (3) Detecting the effective antibiotics and non-effective antibiotics for UTI.

History and classification

The genus *Escherichia* had been discovered before 102 million years ago, and which is found in mammals, birds and reptiles, and also found in soil and water. In 1885, it was discovered by a German pediatrician, Theodor Escherichia in the feces of healthy individuals and called it *Bacterium coli* because it is found in the colon and their classifications were based on shape and motility (Daegelen, 2009). *Bacterium coli* was reclassified as *Bacillus coli* by Migula in 1895 and later classified in the newly created genus *Escherichia*, the genus belongs to a group of bacteria informally known as "coliforms". *Escherichia* belongs to Enterobacteriaceae family. Then this genus was divided into five species (*E. albertii, E. coli, E. fergusonii, E. hermannii* and *E. vulneris*) (Lederber, 2004).

General characteristics

E. coli is a gram negative bacteria, facultative anaerobic and non-spore forming. They are typically rod-shaped, about 2.0 micrometers (μm) long and 0.25-1.0 μm in diameter, with a volume of 0.6–0.7 μm. It has peritrichous flagella. It can produce lactate, ethanol, acetate and dioxide. Optimal growth of *E. coli* occurs at 37°C and can multiply at temperatures up to 49°C (Kubitschek, 1990). On MacConkey agar *E. coli* isolates produce bright pink colonies, while producing opaque, cherry red colonies and non-hemolytic on blood agar, so growth on eosin methylene blue agar produce black colonies with a greenish-black metallic sheen (Madigan and Martinko, 2006). Also *E. coli* presented the result of indole-positive (red ring) and methyl red (bright red), but gave negative results for Vogasproskaur (VP) (without change in colour) and citrate (green colour) (does not change in colour) (Fotadar et al., 2005).

Pathogenicity

E. coli is found in large intestine of human and can cause severe infections in humans and animals. It also can cause many diseases like urinary tract infections, respiratory tract infections and bloody diarrhoea in humans (Rama et al., 2005). Uropathogenic *E. coli* (UPEC) cause 70–95% of UTI. UTI can be caused by ascending infections to urethra. This infection can be found in both adult male and female, and some infants (Cohn and Schaeffer, 2004). While Enterohemorrhagic strain of the *E. coli* (EHEC) can lead to bloody diarrhea and kidney failure when one gets infected by contaminated ground beef, unpasteurized milk or contaminated water (Darnton et al., 2007).

The enteroaggregative *E coli* (EOEC) is found only in human, so we can determine the source of contamination of the stools (from human or from other animals) by examining which strain of *E. coli* is present in it (Darnton et al., 2007). *E. coli* is commonly used as an indicator in the field of water purification. *E. coli* index can indicate how much human feces contamination is in water. Because significant larger amount of *E. coli* in human feces is found than other bacterial organisms, with a short life cycle (Zinnah et al., 2007).

Antibiotics resistance

Resistance of Gram-negative bacteria to beta-lactam antibiotics has been increasing, and become a particular problem in recent decades, because strains of bacteria were produced extended-spectrum beta-lactamases and became more common. Most strain of *E. coli* are producing Extended-spectrum beta-lactamase (ESBL *E. coli*) which are highly resistant to an array of antibiotics, and infections by these strains are difficult to treat (Nicolle, 2005).

Antibiotics were used to treat *E. coli* infections include many type of cephalosporins, carbapenems, aztreonam, trimethoprim-sulfamethoxazole, ciprofloxacin, nitrofurantoin and the aminoglycosides (Paterson and Bonomo, 2005).

Antibiotic resistance is due to overuse of antibiotics in humans and use of antibiotics as growth promoters in animal feeds. A study published in the journal, *Science* in August 2007 found the rate of adaptive mutations in *E. coli* is in the order of 10^{-5} per genome per generation, which is 1,000 times as high as previous estimates (Perfeito et al., 2007).

E. coli bacteria often carry multiple drug-resistance plasmids, and under stress, readily transfer those plasmids to other species, where many species of bacteria exist in close proximity to each other. This mixing of species allows *E. coli* strains that are piliated to accept and transfer plasmids from and to other bacteria (Salyers et al., 2004).

Materials and methods

The materials

Apparatuses: The apparatuses were used in this research include: autoclave, incubator, sensitive balance, distillator, compound microscope, refrigerator, loop and micropipettes.

Reagents

Kovacs reagent: It has been prepared by dissolving 5 g of P-dimethylaminebenzylaldehyde in 75 ml of amyl-alcohol and then 35 ml of concentrated HCl was added, it was used to detect indole production (Baron and Finegold, 1991).

Methyl Red reagent: It has been prepared according to MacFaddin (2000) by dissolving 0.1 g of methyl red powder in 300 ml of (95%) ethanol, then the volume was completed to 500 ml with distilled water.

Voges–Proskaur reagent: It has been prepared according to MacFaddin (2000) by mixing two solutions: Solution A: prepared by dissolving 5 g of alpha-naphthol in 100 ml of ethyl alcohol (absolute). Solution B: prepared by dissolving 40 g of KOH in 100 ml distilled water, it has been used to detect the partial glucose hydrolysis.

Solutions

Normal saline solution: It has been prepared by dissolving 0.85g of NaCl in 90 ml of distilled water, then the volume was completed to 100 ml and autoclaved at 121°C for 15min; it was used for many tests (MacFaddin, 2000).

Antibiotic discs

Ten antibiotics discs were used to detect susceptibility pattern of bacterial isolates to common antibiotics, they were chosen according to the Performance standards for Antimicrobial Susceptibility Testing. All antibiotic discs of these were from Bioanalyse Company (Table 1).

Culture media

Blood agar medium: It has been prepared according to Difico Manual (1953) by dissolving 40 g of Blood Agar base (Himedia, India) per one litter of distilled water and autoclaved at 121°C for 15 min, 8% human blood was added after cooling the medium to 50-55°C, it is used for primary cultivation and to detect the type of hemolysis.

Table 1. Antibiotic discs used in the present study.

Class of antibiotic	Antibiotic disc	Symbol	Disc potency (µg)
Penicillin	Ampicillin	Am	10
	Piperacillin	PI	100
	Amoxicillin-clavulanic acid	AMC	30
	Ampicillin	Am	10
Cephalosporins	Cefixime	CFM	5
Carbapenems	Imipenem	IPM	10
Nitrofurans	Nitrofurantoin	NIT	30
Folatepathway inhibitor	Trimethoprim-sulfamethoxazole	TMP-SMZ	25
Aminoglycosides	Gentamicin	GEN	10
	Amakicin	AK	10
Quinolones	Ciprofloxacin	CIP	5

MacConkey agar medium (Himedia, India): It has been used for the isolation and differentiation between Gram negative and Gram positive bacteria, and differentiation between lactose fermenting and non-lactose fermenting bacteria.

Muller–Hinton agar (Himedia, India): It has been used for testing the susceptibility of bacterial isolates to antibiotics (Cruikshank et al., 1975).

Pepton Water medium (Himedia, India): It has been used to detect the ability of bacteria to produce indole (MacFaddin, 2000).

MR-VP medium (Himedia, India): It has been used to detect the partial and complete hydrolysis of glucose (MacFaddin, 2000).

Simmon Citrate medium (Himedia, India): It has been used for determining the ability of bacteria to utilize citrate as the sole of carbon (MacFaddin, 2000).

The methods

Specimen collection: Forty seven of Gram–negative bacteria that fermented lactose were collected from Al-Sadar Medical City and Zahraa Hospital teaching in Al- Najaf province, during the period from 15-7-2013 to 20-10-2013 and represented by 34 specimens from females and 13 from males.

Cultivation and identification of clinical isolates

The samples were cultured on MacConkey and blood agar, incubated at 37°C for 18-24 h. The bacterial isolates were identified according to MacFaddin (2000).

Typical characteristics

After the incubation period, the typical characters of *E. coli* were used for the identification of bacterial isolates. *E. coli* isolates were distinguished by the production of opaque, grayish-white and non-hemolysis on blood agar while on MacConkey agar pink to rose-red was produced. Colonies may be surrounded by a zone of precipitated bile (Forbes et al., 2007).

Biochemical tests

The following biochemical tests were performed for distinguishing *E. coli* isolate from other related isolates (MacFaddin, 2000).

Indole test

Peptone water was inoculated with young culture and incubated at 37°C for 24h, 3-5 drops of Kovacs reagent (0.5 ml) was added, forming a red colour ring in the alcohol layer, indicating a positive result

Methyl Red test

MR-VP was inoculated with a single colony of young culture and incubated at 37°C for 24 h, 5 drops of Methyl red reagent were added, mixed, and the result was read immediately. The development of a bright red colour indicates positive test.

Voges–Proskaur test

MR-VP was inoculated with a single colony of young culture and incubated at 37°C for 24 h, 3ml of reagent A (5% alpha naphthol) and 1 ml of reagent B (40% KOH) were added, a positive reaction was indicated by the development of a pink color in 2-5 min.

Citrate utilization test

Simmon citrate medium was inoculated with a single colony of young culture and incubated in 37°C for 24h, a blue color appearance indicated a positive result.

Antimicrobial susceptibility test

Disc diffusion method was performed to determine the susceptibility pattern of E coli isolates to common antibiotics on Mueller–Hinton agar, with the inoculums equal to 108*1.5 MacFarland turbidity according to CLSI (Clinical Laboratory Standards Institue, 2011), the plates were incubated at 37°C for 18-24 h and the inhibition zone diameters around the antibiotics discs were measured.

Results and discussion

Specimen collection

Forty seven of lactose fermenting bacteria had been collected from urinary tract infections representing 34 (72%) from females and 13 (28%) from males (Table 2).

Bacterial identification

Identification of bacterial isolates based on cultural and morphological characteristics: The characters of

the bacterial colonies grown on MacConkey agar were studied; the lactose was fermented (pink color) pink to rose-red (may be surrounded by a zone of precipitated bile), while on blood agar, smooth, round, grayish-white non-hemolytic colonies appeared (Forbes et al., 2007).

Table 2. Distribution of bacterial isolates according to patients setting.

Hospital	AL- SADAR				AL-ZAHRAA				Total
Patients with	Negative		Positive		Negative		Positive		
UTI	Female	Male	Male	Female	Female	Male	Male	Female	47
	13	10	1	3	17	-	-	3	
Total	23		4		17		3		47

Biochemical tests

All Gram-negative isolates that grown on MacConkey agar undergo biochemical tests in order to distinguish *E. coli* isolates from other members of related lactose fermented bacteria, all biochemical tests have been carried out according to MacFaddin (2000).

Forty (85%) isolates have given positive results for Indole test, while 7 (15%) isolates gave negative results. The breakdown of tryptophan for nutritional leads to the release of the indole that can be detected through the use of Kovacs' reagent, which reacts with indole and produces a red color on the surface of peptone water. *E. coli* gave positive result (Macfaddin, 2000).

The results have been indicated that 40(85%) isolates were positive for methyl red test, *E. coli* gave positive result ,while 7(15%) isolates give negative result, glucose fermentation with acid accumulation in MR-VP medium, causing decreased of pH, leading to the formation of red colour when methyl red reagent was added (Bharti et al., 2008). Citrate utilization test were negative in 40 (85%) isolates, the *E. coli* gave negative results, and 7 (15%) isolates give positive result changing the color (green) of Simmon citrate slants to blue as results of using citrate as a carbon source. The result revealed that 40 (85%) of isolates were *E. coli*.

Clinical isolates of *E. coli*

The *E. coli* clinical isolates have been accounted to be 40 (100%). The isolates obtained from UTI infection were represented by 10 (25%) isolates from male, and 30 (75%) isolates from female. The finding may be true, because *E. coli* is one of the most important opportunistic pathogens which is commonly predominant in hospital environment (Johnson and Russo, 2005).

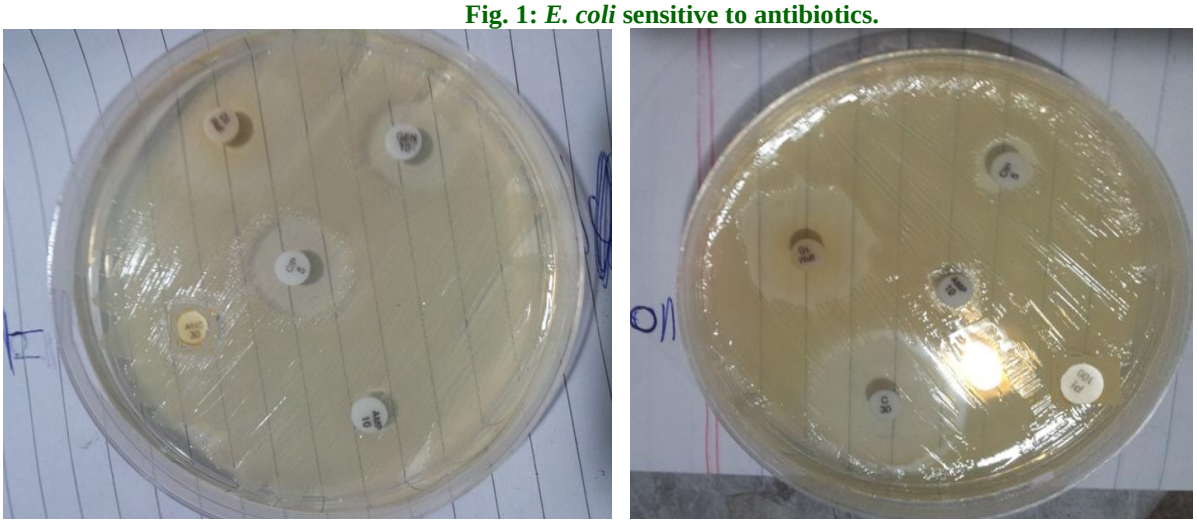
Urinary tract infection (UTI) is most common infectious presentation in hospital acquired and community acquired infections since long time. Approximately (60%) of women and (19%) of men will experience to symptomatic urinary tract infection during their lifetime (Tada et al., 2012). Among both outpatients and inpatients, *E. coli* is the primary urinary tract pathogen, accounting for 75% to 90% of both side - hospital acquired and - community acquired UTI (Mokady et al., 2005).

Antibiotic susceptibility test

The susceptibility of *E. coli* isolates to common antibiotics was tested to determine the resistance pattern of isolates to different antibiotics, penicillin, cephalosporins, carbapenems, nitrofurans, folate pathway inhibitor, aminoglycosides and quinolones. The diameters of inhibition zones around the antibiotic disks were measured and compared with the standard manual of CLSI (2011). The study found *E. coli* was highly resistant to penicillin group antiobiotics; all *E.coli* isolates (100%) showed resistance to ampicillin and 36 (90%) of isolates resistant piperacillin while 4 (10%) of isolates were sensitive to it. Present study results differed from the other studies with regard to the antibiotic resistance pattern of E. coli. The study of Anton et al. (2010) found 80% of *E. coli* was resistant to piperacillin.

E. coli resistance to penicillin has been attributed to the production of enzymes which inactivate penicillin group. These enzymes are known: penicillinase (3-lactamase), which hydrolyzes the CO-N bond in the 3-lactam ring of the penicillin molecule, and amidase, which hydrolyzes the CO-NH bond between the side chain and the 6-amino group in the penicillanic acid residue (Hooton and Samadpour, 2005) (Figs. 1A, 3 and Appendix-1).

Fig. 1: *E. coli* sensitive to antibiotics.



(A) **(B)**

The present study results showed that 32 (80%) of *E.coli* isolates were resistant to amoxicillin-clavulanic acid while 8 (20%) isolates were sensitive to it. The study of Mimica et al. (2010) and Ortega et al. (2012) reported 90% and 25% resistance in *E. coli* to amoxicillin-clavulanic acid respectively. Beta-lactamases are enzymes produced by *E. coli* that provide resistance to beta-lactam antibiotics. These antibiotics have four-atom ring known as a beta-lactam, the lactamase enzyme breaks the β-lactam ring and became inactive against microbes (Hooton and Samadpour, 2005).

E. coli isolates of 27 (68%) were sensitive to trimethoprim sulfamethoxazole (TMP-SMZ), while 13 (32%) isolates were found to be resistant to it. Brown et al. (2002) found 15.8% of *E .coli* was resistant to TMP-SMZ. This antibiotic belongs to folate pathway inhibitors. TMP-SMZ is still considered the first-line drug of choice, TMP-SMZ has a possible role as a second- or third-line treatment for patients who have UTI (Arslan et al., 2005).

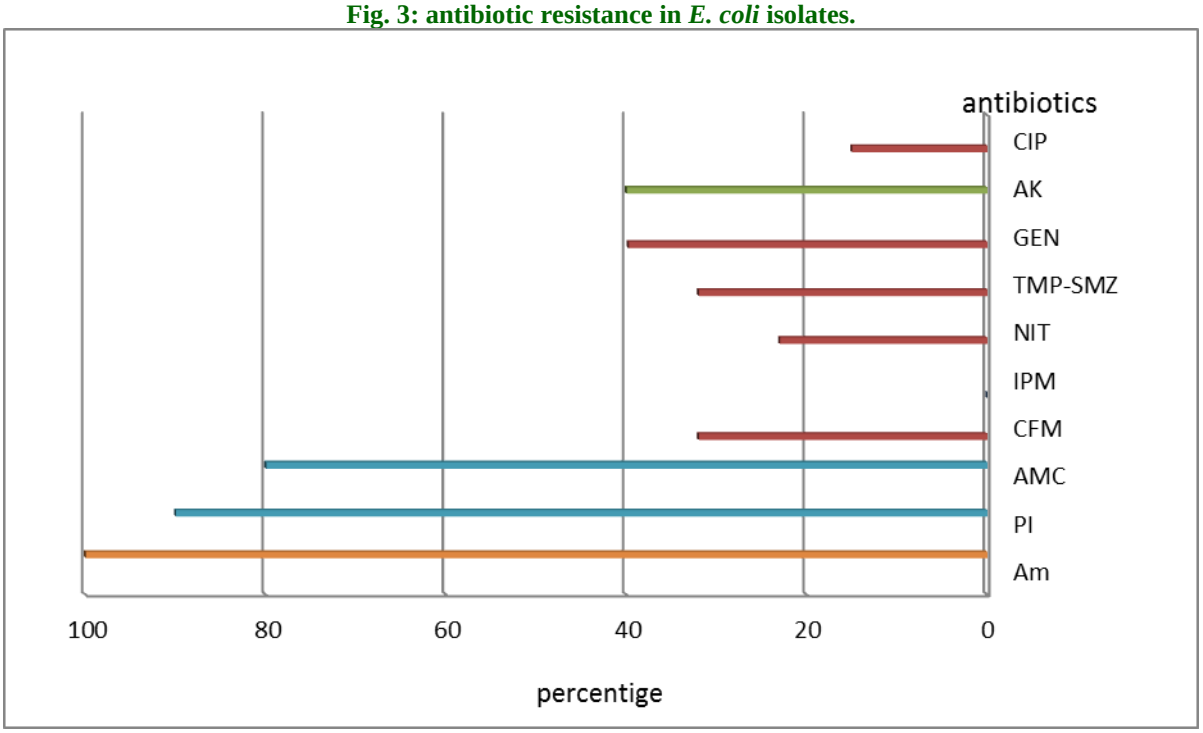
E. coli was found to be sensitive to aminoglycoside in the present study which recorded 24 (60%) and 25 (62.5%) of isolates were sensitive to amikacin and gentamicin respectively; whereas, 10 (25%) of isolates were intermediately resistant to gentamicin; also 16 (40%) and 5 (12.5%) of *E. coli* isolates were resistant to amikacin and gentamicin respectively. Sukumaran et al. (2012) reported the sensitivity of *E. coli* to amikacin (1, 33%) and gentamicin (2, 66%). The bacteria have three mechanisms to aminoglycoside resistance: decreased cell permeability, alterations at the ribosomal binding sites, and production of aminoglycoside modifying enzymes (Mingeot et al., 1999) (Fig. 1B, Fig. 3 and Appendix-1).

Fig. 2: *E. coli* isolates resistant to antibiotics.

The present study results showed 18 (45%) of isolates were sensitive to nitrofurantoin while 13 (32.5%) of isolates were intermediately resistant to it and 9 (22.5%) of isolates resistant to nitrofurantoin belonging to nitrofurans. However, Iqbal et al. (2002) observed 8% resistance in *E. coli* isolates to nitrofurantoin. The random administration of antibiotics has led to the development of multi-drugs resistant organisms, causing serious opportunistic infections (Shukla et al., 1998).

The antibiotic cefixime belong to cephalosporine group and the result show that 18 (45%) of isolates were resistant to cefixime; while 8 (20%) were intermediately resistant and 14 (35%) of isolates were sensitive to cefixime. The study conducted by Iqbal et al. (2002) recorded 39% resistance in *E. coli* isolates to cefixime.

Fig. 3: antibiotic resistance in *E. coli* isolates.



The resistance pattern of *E. coli* isolates to quinolones were represented by 34 (85%) of isolates sensitive to ciprofloxacin and 6 (15%) of isolates with resistance to it. The study of Arslan et al. (2005) found 14.7% of *E. coli* isolates were resistant to it. Antibiotic resistance is a major clinical problem in treating infections. The resistance to the antimicrobials has increased year to years and vary from place to place (Fig. 2, Fig. 3 and Appendix-1).

The results revealed that imipenem was the best choice antibiotic for the treatment of *E.coli* infection, since the effect of imipenem was observed in all bacterial isolates (100%) (Appendix-1). The result of the present findings is in agreement with the study of Tada et al. (2012) who reported that all *E. coli* isolates were sensitive to imipenem 100%. It is a broad spectrum antibiotic with activity against many aerobic and anaerobic Gram-positive and Gram-negative organisms, and it use for the treatment of complicated skin, tissue, respiratory tract, urinary tract and urogenital infections (Veronique, 2012) (Fig. 3).

Conclusions and recommendations

E. coli is the one of the predominant agents of UTI. Imipenem, in the present study has been observed to be highly active against *E. coli* isolates, while penicillin group doesn't. The UTIs are caused by *E. coli* and distributed 10 times more among women than men. Carbapenems antibiotics including imipenem can be used as best choice in treatment of serious *E. coli* infections.

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Appendix-1.

Results of antibiotics susceptibility test with a wide-range of antibiotics against *E. coli* isolates.

Antibiotics	B- lactam														
	Carbapenems			Cephalosporins			Pencillins								
	IPM			CFM			AMC			PI			Am		
UTI	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Women	10	-	-	9	6	15	5	-	30	3	-	29	-	-	30
Men	30	-	-	5	2	3	3	-	2	1	-	7	-	-	10
Total	40	-	-	14	8	18	8	-	32	4	-	36	-	-	40

Antibiotics	Aminoglycosides						Folate pathway inhibitor			Quinolones			Nitrofurans		
	AK			CN			TMP-SMZ			CIP			NIT		
UTI	R	S	I	S	I	R	I	R	S	S	I	R	S	I	R
Women	14	16	-	3	8	19	-	10	20	26	-	4	14	8	4
Men	2	8	-	2	2	6	-	3	7	8	-	2	4	5	5
Total	16	24	-	5	10	25	-	13	27	34	-	6	18	13	9

R: Resistant S:Sensitive I: Intermediate.