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Bacteriological Investigation of *E. coli* in Urinary Tract Infection in South Port Said City

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Authors' contributions

This work was carried out in collaboration between all authors. Author MB designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors ASH and MMZ reviewed the experimental design and all drafts of the manuscript. Author AMA managed the analyses of the study. Author MB identified the plants. Author AMA performed the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

UTI is characterized by the evidence of uropathogens and pyuria and is accompanied by various clinical manifestations depending on the area of involvement.
Aim: The aim of this study was to isolate the bacterial infection to urinary tract infection in men and women at different ages and to investigate the susceptibility of bacteria implicated in urinary tract infection to different antimicrobial drugs (antibiotics). Also to determine minimum inhibitor concentration for resistant bacteria to antibiotic and molecular characterization of *E. coli* associated.
Materials and Methods: One hundred urine specimens were received from patients admitted during January till December 2015. *E. coli* isolates were confirmed by typical colonial morphology, and identified by differential tests as well as by the growth on characteristic agar, Susceptibility testing was carried out by disk diffusion method. Among the 100 isolates, 72 bacterial strain was isolated from the urine specimens of infected Patient admitted at Port-said area out-patient clinics,

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using agar medium and macCkonkey agar, This organism was characterized by biochemical tests and showed similarity with *E. coli.* The genomic level confirmation done with 16S rDNA primer by submitting the genomic sequence to Gene Bank under acc.No-GU046545 after comparing, showed 98% sequence similarity with *E. coli*. Antibiotic susceptibility test revealed that imipenem, Amikacin, Nitrofurantoin and gentamicin are the lowest resistant rate with percent of 79.2, 76.4, 75 and 61% respectively and ampicillin showed the highest resistant rate with 89%. Pattern on antibiotic susceptibility test showed high resistant rate to some antibiotic that made it difficult for pregnant patients, although its frequency was low. The most prevalent bacterial pathogen in Port-saed city was *E. coli* and the most sensitive antibiotic against it is imipenem.

Keywords: Antibiotic sensitivity; molecular characterization; minimum inhibitor concentration.

1. INTRODUCTION

Urinary tract infection (UTI) is one of the most important causes of morbidity in the general population, and is the second most common cause of hospital visits [1]. The pathogenic causing UTIs are almost always predictable [2].

In recent studies microbial species that cause UTIs are classified by their target sites, such as urine infection (bacteriuria), bladder infection (cystitis) and kidney infection (pyelonephritis), which can be either asymptomatic or associated with symptoms [3]. Prevalence of infection differs with age, sex and certain predisposing factors [4]. The distribution of these bacteria is different in different parts of the world and studying the microbial factors that cause this infection in different geographical regions, indicates their dispersion [4]. Urinary Tract Infections involve the infection of kidneys, ureters, bladder, and/or urethra by pathogenic organisms invasion of the urinary tract, which ultimately leads to an inflammatory response of the urothelium. Prevalence of infections may differ with age, sex and certain predisposing factors. The incidence of infection is greater in females than in males with two exceptions, infections found in infants and catheter-related infections. Women tend to become infected by UTIs more often because their urethra is shorter and closer to the anus than men and hence, the pathogenic bacteria have easier access to the bladder [4]. In this context, *E. coli* is the most prevalent organism and is solely responsible for the majority of these infections. An accurate and prompt diagnosis is important in shortening the disease course and for preventing the ascent of the infection to the upper urinary tract [5].

Many studies reported that *E. coli* and *K. pneumonia* are the common pathogen that causing UTIs in various region of the world [6]. UTIs are ranked among the most common infectious diseases found in either the community or health care setting [7]. Its role in urinary tract infection is widely documented; in fact *E. coli* is incriminated in almost 60 to 80% of this infection [8,9].

With *E. coli* being the primary etiologic agent among both outpatient and inpatient accounting for 75 to 90% of urinary tract infection isolate [10]. Therefore, constant monitoring of drug resistance is acquired because only limited data describing multiple resistance among UTIs isolate is available.

E. coli like other bacteria show resistance to antibacterial through intrinsic or acquired resistance mechanism [5]. The evolution of bacterial resistance henceforth constitutes a major risk of public health, because, amongst the several pathogenic species, certain strains are sensitive to only a few antibiotics. The consequences are numerous: increased morbidity and mortality, increase in health care costs related to prolonged hospitalization and hence the need for more costly and often more toxic antibiotics. Some strains are resistant to all the antibiotics usually available on the market. The control of the appearance and the extended resistance of pathogens to antibiotics has become imperative for medical laboratories so as to establish a useful database and initiate an epidemiologic surveillance for resistance. Tunisia and Morocco, have noticed an increase in the resistance of *E. coli* to antibiotics [11,12]. Antibacterial agents as trimethoprime/ Sulpamethoxazole, Ciprofloxacine, Cephalosporine, Penicillin without penicillinase inhibitors, Nitrofurantoin, fosfomycin are generally used in the treatment of community acquired UTIs [9]. Identification of bacterial isolates is an essential task of clinical microbiology laboratories. In clinical laboratories, the present means of identification of

bacteria relies on phenotypic tests. Traditional phenotypic identification is difficult and time consuming [4]. In the late 1906 - 1910, genotypic identification emerged as an alternative or complement to the established phenotypic methods. Typically, genotypic identification of bacteria involves the use of conserved sequences within phylogenetically informative genetic targets, such as the small-subunit (16S) rRNA gene. Sequence analysis of the 16S ribosomal RNA (rRNA) gene has been widely used to identify bacterial species and diagnose microbial infections. However, these methods are yet to replace standard bacterial culture due to their prohibitive costs, complexity, and the need for highly-trained personnel [14]. The present study aims at analyzing the infectious epidemiology of UTIs in a general university hospital located in South Port Said city, In addition, it examines the susceptibility profiles of *E. coli* between January and December of 2015, studying Minimum inhibitory concentration of antibiotic and molecular characterization of *E. coli* associated.

2. Methodology

2.1 Sample Collection

Study period: This is across–sectional study carried out in outpatient clinic of south port-said city. Two hundred and fifty urine specimens were received from patients during January till December 2015. Two hundred and fifty Urine sample were obtained as clean catch voided or catheterized samples from all patients who were subjected for assessment for UTI.

2.2 Isolation and Identification of Bacterial Isolate

All specimens were cultured routinely in Microbiology Laboratory on Blood nutrient, MacConkey's and EMB agar and were incubated at 37℃ for 24 hr. All *E. coli* isolates were confirmed by typical colonial morphology, type of hemolysis, Gramstain, IMVC test, motility, microscopic examination, growth characteristics in agar and biochemical tests including indole, methyl red, Voges–Proskauer and citrate (IMViC), triple sugar iron,urease, and nitrate reduction [15]. A specimen was considered positive for UTIs if an organism were cultured at a concentration of at least 10^5 single bacteria colonies per ml of urine and >5 pus cells per high power field were observed on microscopic examination [11].

2.3 Antibiotic Susceptibility Testing

Susceptibility testing was carried out by disk diffusion methods recommended by CLSI, All *E. coli* isolates were tested for resistance against gentamicin, ampicillin, ciprofloxacin, tobramycin, trimethoprim-sulphamethoxazole, Imipenem, norofloxacine, amikacin, nitrofurantoin andamoxicillin, antimicrobial susceptibility and resistance was determined by isolate growth zone diameter.

E test are used for determination of Minimum inhibitor concentration (MIC): The lowest concentration which can inhibit growth of bacteria.

2.4 Molecular Characterization of Bacterial Isolate by 16SrDNA *Gene*

The DNA was extracted from the bacterial isolate by the method of Sambrook et al. (2001).

The polymerase chain reaction was carried out by following method of Sambrook and Russel (2007). forward primer 5' TAGGGAAGTAATGACGG 3' Reverse primer5' CCTCTATCCTCTTTCCAACC3' was used in PCR reaction.

2.5 Automated Sequencing

The sequencing of the genomic DNA amplicon coding for strain TS1 was carried out at Biotechnology center, Suez Canal University by 16S rRNA gene sequence analysis, PCR gene fragment of 16S rRNA was amplified from the purified genomic DNA using the universal primer. Aliquots of the amplification products were analyzed by agarose gel electrophoresis using 1.0% agarose containing 0.5 µg of ethidium bromide per ml. The results of Blast n for 16S rRNA DNA sequences were retrieved and aligned with the sequences of bacterial isolates using ClustalW embedded in MEGA 6 (Molecular Evolutionary Genetics Analysis) software. The multiple sequence alignment and 16S rRNA phylogenetic tree was constructed. Phylogenetic analysis was conducted based upon 16S rRNA gene data using Maximum Likelihood analyses (ML). Alignment gaps were treated as missing data. ML analysis was conducted using a heuristic search with tree bisection- reconnection (TBR) branch swapping and 100 random addition sequence replicates. Statistical support for the internal branches was estimated by bootstrap analysis based upon 1000

replications. Nucleotide sequence was compared to those in the Gene Bank database with Basic Local Alignment Search Too (BLAST algorithms to identify known closely related sequences. The tree was generated by the neighboring algorithm (SaitouandNei1987) join edtoitby implementation with phydit. The assemblage of 16SrDNAgene sequences in each library was analyzed by rarefaction analysis.

3. RESULTS

In this study, 100 (40%) patients out of 250 were showed to be urine culture positive (their colony count was equal or more than 10^4). The frequency of isolated microorganisms and their relation to sex is given in Table 3. The most common isolated uropathogens in Gram-negative bacilli and Gram-positive cocci were *E. coli* (72%), *E. coli* were isolated in high frequencies in females (63.8%) and (36.2%) in male. Also *E. coli* present in all ages from five months to 90 years with high frequency than other bacteria as shown in Table 3.

3.1 Susceptibility of *E. coli* against Different Antibiotic

The results revealed that isolates of *E. coli* were highly sensitive to imipenem 79.2%, amikacin 76.4%, Nitofurantoin with 75% followed by gentamicin and Ciprofloxacin with percentage of 61 and 52 respectively, but were highly resistant to Ampicillin with 89%, trimethoprim + sulphameth-oxazole with 70%, Amoxicillin (65%) and followed by Tobramycin with percentage of 43% as shown in Table 1.

3.2 Minimum Inhibitor Concentration (MIC)

(MIC) of five antibiotic drugs (Ampicillin, Amikacin, Gentamicin, Clindamicin and Imipenem) of the 10 clinical resistant bacterial strains was determined and these results were illustrated (Table 2). MIC of Clindamicin was the highest (400 µg/ml) for *E. coli* Isolate no 35. and the lowest (250 µg/ml) for *E. coli* Isolate no 22, 33 and 42. The maximum MIC of Ampicilin was obtained at concentration (4 µg/ml) for *E. coli* isolate no 40 and 42 and the minimum was obtained at (0.19 µg/ml) for *E. coli* 30. MIC for Amikacin was the highest (6 µg/ml) for *E. coli* 42 and the lowest (0.5 µg/ml) for *E. coli* isolate no 30. The maximum MIC of Gentamicin was obtained at concentration (2 µg/ml) for *E. coli* isolate no 22 and 42 and the lowest was obtained at (0.25 µg/ml) for *E. coli* isolate no 40.

MIC of Imipenem was the highest (1 µg/ml) for *E. coli* isolate no 30 and the lowest (0.19 µg/ml) for *E. coli* isolate no 5.

3.3 Molecular Characterization of Bacterial Isolated from Urine

The PCR product that was run in agarose gel electrophoreses showed 870 bp for strain. The DNA sequence of the strain having Gene Bank ACC.NO. GUO46545 when compared to those in Gene bank databases with BLAST were found to have 98% similarities with *E. coli*. This strain is named as *E. coli* (Gene bank ACC.No. GU046543)

3.4 Molecular Characterization of the Strain

The genomic DNA was amplified with16S rDNA primer, 16S F:5′-GAGTTTGATCCTGGCTTAG-3′16S R:5′-GGTTACCTTGTTACGACTT-3′.

DNA sequence revealed that it is high purified sequence, The amplified PCR product when run in agarose gel electrophoresis, strain TS1 was found having a molecular weight approximately of 870 bp when compared with the DNA marker (Fig. 1).

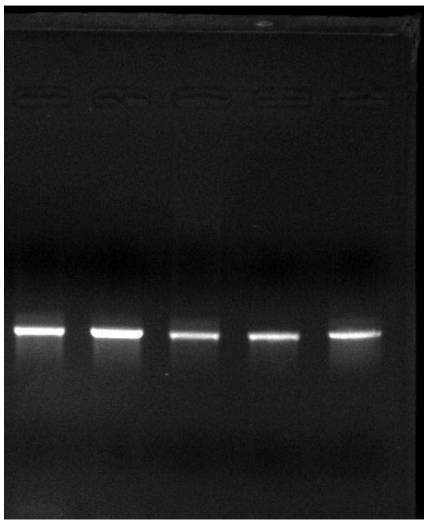

Fig. 1 . Agarose gel analysis. An unknown amount of a 5.5 kb DNA fragment (U) was run alongside known quantities (as indicated in ng) of the same DNA fragment. The unknown sample contained 75–100 ng DNA, as estimated by visual comparison with the standards M: 1 kb DNA ladder

Table 1. Resistant and susceptibility of gram negative bacterial isolates to various antibiotics, indicating sex and age of each individual infected with *E. coli* in the 72 sample, were Symbol S are Sensitive, Symbol R are Resistant and I are intermediate

Sex	Age	Tested antibiotics / Bacterial isolates	Gentamicin	Ciprofloxacin	Ampicillin	Amoxicillin	Amikacin	Nitrofurantoin	Trimethoprim-sulph	Norofloxacin	Tobramicin	Impenem
Female	45	*E. coli (Ec.1)*	R	R	R	R	R	R	S	R	R	R
Female	10	*E. coli (Ec.2)*	S	S	R	R	S	S	R	S	S	S
Female	43	*E. coli (Ec.3)*	S	S	R	R	S	S	R	S	S	S
Male	46	*E. coli (Ec.4)*	S	R	R	R	S	S	R	S	S	S
Female	20	*E. coli (Ec.5)*	R	S	R	R	R	R	R	S	S	R
Female	33	*E. coli (Ec.6)*	S	R	R	R	S	S	R	S	R	S
Female	36	*E. coli (Ec.7)*	R	S	R	R	S	S	R	S	S	S
Female	5	*E. coli (Ec.8)*	S	R	R	R	S	S	R	R	S	S
Male	35	*E. coli (Ec.9)*	S	S	R	R	S	S	R	S	S	S
Female	55	*E. coli (Ec.10)*	S	R	S	S	S	S	S	S	S	S
Male	60	*E. coli (Ec.11)*	I	R	R	R	S	S	R	R	S	S
Female	61	*E. coli (Ec.12)*	S	S	R	R	S	R	R	S	S	S
Male	66	*E. coli (Ec.13)*	R	I	R	R	S	I	R	S	R	S
Female	75	*E. coli (Ec.14)*	S	R	R	R	S	S	R	S	S	S
Female	33	*E. coli (Ec.15)*	S	S	R	R	S	S	R	S	S	S
Female	85	*E. coli (Ec.16)*	S	R	R	R	S	R	R	S	S	S
Male	18	*E. coli (Ec.17)*	S	S	R	R	S	S	S	S	S	S
Female	33	*E. coli (Ec.18)*	S	R	R	R	S	S	R	S	S	S
Female	45	*E. coli (Ec.19)*	S	S	R	R	S	S	R	S	R	S
Female	55	*E. coli (Ec.20)*	S	I	R	R	R	R	R	S	S	R
Male	13	*E. coli (Ec.21)*	R	R	R	R	S	S	S	S	R	S
Female	56	*E. coli (Ec.22)*	R	R	R	S	R	R	R	R	R	R
Male	68	*E. coli (Ec.23)*	S	R	R	S	I	S	R	S	R	S
Male	33	*E. coli (Ec.24)*	S	R	S	S	S	S	S	S	R	S
Female	25	*E. coli (Ec.25)*	I	I	R	S	S	R	R	S	R	S
Female	33	*E. coli (Ec.26)*	S	I	R	R	S	S	R	S	I	S
Female	40	*E. coli (Ec.27)*	S	S	R	R	S	R	R	S	I	S

Sex	Age	Tested antibiotics / Bacterial isolates	Gentamicin	Ciprofloxacin	Ampicillin	Amoxicillin	Amikacin	Nitrofurantoin	Trimethoprim-sulph	Norofloxacin	Tobramicin	Impenem
Male	57	*E. coli (Ec.28)*	I	S	R	R	S	S	R	S	S	S
Female	66	*E. coli (Ec.29)*	S	I	R	R	S	S	I	R	S	S
Female	30	*E. coli (Ec.30)*	S	S	R	R	R	R	R	I	R	R
Male	45	*E. coli (Ec.31)*	S	I	I	I	S	S	R	S	R	S
Female	12	*E. coli (Ec.32)*	I	S	R	I	S	S	R	S	S	S
Female	55	*E. coli (Ec.33)*	S	I	R	R	R	R	R	R	S	R
Female	31	*E. coli (Ec.34)*	S	S	R	S	S	S	R	S	R	S
Male	44	*E. coli (Ec.35)*	S	I	R	R	R	S	S	R	I	R
Female	31	*E. coli (Ec.36)*	S	S	R	R	S	S	S	S	R	S
Male	62	*E. coli (Ec.37)*	I	I	R	R	S	S	R	S	S	S
Male	48	*E. coli (Ec.38)*	S	S	R	I	S	S	S	R	S	R
Male	34	*E. coli (Ec.39)*	S	S	I	R	S	S	R	S	R	S
Female	50	*E. coli (Ec.40)*	I	I	R	R	R	R	R	S	S	I
Female	66	*E. coli (Ec.41)*	S	S	R	R	S	S	R	S	R	S
Male	80	*E. coli (Ec.42)*	S	S	R	R	R	R	S	S	S	R
Female	6	*E. coli (Ec.43)*	I	S	R	R	S	S	R	S	S	S
Female	21	*E. coli (Ec.44)*	S	R	I	R	S	S	R	R	R	S
Male	22	*E. coli (Ec.45)*	S	S	R	R	I	S	R	R	S	S
Male	57	*E. coli (Ec.46)*	I	S	R	S	S	R	S	S	S	S
Female	8	*E. coli (Ec.47)*	S	S	R	R	S	S	R	S	S	S
Female	69	*E. coli (Ec.48)*	S	R	R	I	S	I	S	I	R	R
Female	33	*E. coli (Ec.49)*	R	S	I	S	S	R	R	R	R	S
Female	67	*E. coli (Ec.50)*	S	S	R	S	R	S	S	S	R	S
Female	45	*E. coli (Ec.51)*	R	S	R	R	S	S	R	R	S	S
Female	56	*E. coli (Ec.52)*	R	R	R	S	S	S	R	S	R	S
Male	55	*E. coli (Ec.53)*	R	R	R	S	R	S	R	S	S	S
Female	53	*E. coli (Ec.54)*	S	S	R	R	S	S	R	S	R	R
Female	44	*E. coli (Ec.55)*	R	S	R	R	R	S	R	S	R	S
Male	65	*E. coli (Ec.56)*	S	S	R	S	R	S	S	R	S	S
Female	60	*E. coli (Ec.57)*	I	S	R	S	S	R	R	S	R	S

Sex	Age	Tested antibiotics / Bacterial isolates	Gentamicin	Ciprofloxacin	Ampicillin	Amoxicillin	Amikacin	Nitrofurantoin	Trimethoprim-sulph	Norofloxacin	Tobramicin	Impenem
Female	44	*E. coli (Ec.58)*	S	S	R	R	S	S	R	S	S	S
Male	56	*E. coli (Ec.59)*	R	I	R	R	S	S	S	R	R	S
Female	34	*E. coli (Ec.60)*	S	S	R	S	R	S	R	S	S	R
Male	56	*E. coli (Ec.61)*	I	S	R	R	S	S	R	R	R	S
Female	8	*E. coli (Ec.62)*	S	S	R	S	R	S	R	S	S	R
Male	6	*E. coli (Ec.63)*	S	R	R	S	S	S	S	S	R	S
Female	7	*E. coli (Ec.64)*	I	R	R	R	R	R	R	S	R	S
Female	55	*E. coli (Ec.65)*	R	R	R	R	S	S	S	S	S	S
Male	48	*E. coli (Ec.66)*	R	I	S	S	S	S	S	R	R	R
Female	56	*E. coli (Ec.67)*	S	I	R	S	R	S	R	S	S	S
Female	56	*E. coli (Ec.69)*	R	S	R	S	R	S	S	R	R	S
Male	34	*E. coli (Ec.70)*	S	S	R	S	S	S	S	S	S	S
Male	35	*E. coli (Ec.71)*	R	S	R	R	S	R	R	S	R	S
Female	55	*E. coli (Ec.72)*	S	S	S	S	S	R	R	S	R	R

Table 2. All bacterial species isolated from different ages and different sexes in 100 urine sample which are positive for bacteria

Patient age (years)	No of BI	BI in different sexes		Bacterial isolates
		M	F	
0 – 10	15	7	8	*E. coli, Klebsiella pneumoniae, Pseudomonas areuginosa, Proteus mirabilis and Enterococcus spp.*
10-20	6	4	2	*E. coli, Klebsiella pneumoniae and Proteus vulgaris.*
20-30	5	3	2	*E. coli.*
30-40	15	5	10	*E. coli, Klebsiella pneumoniae, Staphylococcus aureus and Enterococcus spp.*
40-50	20	8	12	*E. coli, Klebsiella pneumoniae, Pseudomonas areuginosa, Proteus mirabilis and Enterococcus spp.*
50-60	18	6	12	*E. coli, Klebsiella pneumoniae, Pseudomonas areuginosa, Proteus vulgaris, Staphylococcus aureus and Enterococcus spp.*
60-70	14	5	9	*E. coli, Klebsiella pneumoniae, Pseudomonas areuginosa and Proteus mirabilis.*
70-80	5	3	2	*E. coli, Klebsiella pneumoniae and Staphylococcus.*
80-90	2	1	1	*Klebsiella pneumoniae and Proteus mirabilis.*

3.5 The Phylogenetic Tree Showed Resemblance with *E. coli*

PCR amplified 16 SrRNA gene using 27F-1429R Primer Pairs were sequenced by Sanger sequencing method. Sequences were used for phylogenetic tree construction of UTI isolates. This sequences were used for construction of phylogenetic tree as illustrated in Fig. 2.

The evolutionary history was inferred by using the maximum likelihood method based on kimura 2-parameter model (kimura 1980). The tree with the highest log likelihood is shown. The percentage of trees in which the associated taxa cluster together is showed next to branches. Initial tree for the heuristic search were obtained automatically by applying neighbor- join and BioNj algorithms to matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch length measured in the number of substitution per site.

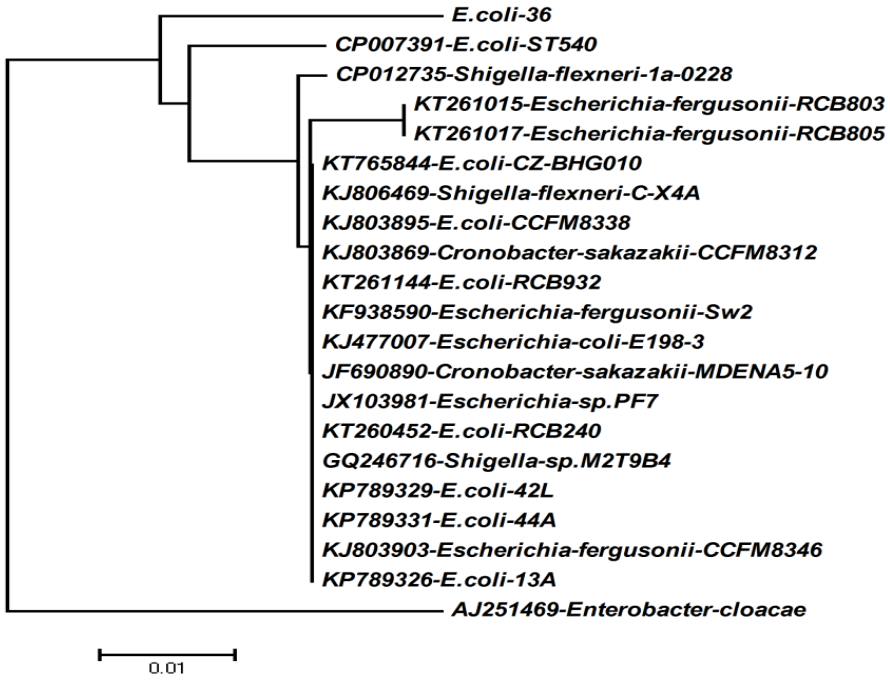

Fig. 2. phylogenic tree phylogenetic analysis the 16s rDNA sequences of the amplified products revealed that the strains TW1 had unique sequences which matched with *E. coli* present by phylogenetic tree

Table 3. Minimum inhibition concentration of the most resistant bacteria to some antibiotics

No of isolate	Concentration of antibiotic(µg/ml)				
	AM	AK	Cn	CL	IMP
E. coli(1)	0.2	1.5	1.0	300	0.45
E. coli(5)	1.0	1.5	0.75	300	0.19
E. coli(20)	0.4	1.0	1.0	300	0.35
E. coli(22)	0.25	1.0	2.0	250	0.45
E. coli(30)	0.19	0.5	0.75	350	1.0
E. coli(33)	2	2.5	1.0	250	0.5
E. coli(35)	2.5	3.0	0.75	400	0.05
E. coli(40)	4	3	0.25	350	0.45
E. coli(42)	4	6	2.0	250	0.2

4. DISCUSSION

In the present study a total of 250 urine samples were collected from patients who were examined for UTIs in South Port said hospitals or attending South earth Port said outpatients clinics, then cultured on CLED agar, blood agar and MacConkey agar media. The results of this study corroborated that 40% of samples (100 out of 250) gave positive bacterial growth when cultivated in CLED agar media. A positive bacterial growth was based on the presence of ≥ 10^4 CFU per ml in urine culture. Significant bacteriuria occurs when there are 10^5 colony forming unit per ml (CFU/ml) in a properly collected urine [16].

This study was consistent with other studies which showed that *E. coli* was the most frequent cause of UTIs at all ages [17].

Yuksel et al. [18] reported that the most causative agents was *E. coli* (87%of cases), Das et al. [19] reported that the most common pathogen isolated were *E. coli* (59.4 percent). Lerner, [20] demonstrated that *E. coli* was the most common organism present (80%) in UTI and this agree with this study. The results of the present investigation also revealed that *E. coli* were present in high frequencies in females (63.8%) than in males (36.2%), UTI affects all age groups, but women are more susceptible than men, due to short urethra, absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with faucal flora [21]. Women with recurrent UTI have an increased susceptibility to Vaginal colonization and uropathogens, which is due to a greater propensity for uropathogenics coliforms to adhere to uroepithelial cells [22]. In the present investigation the antibiotics used were Impenem, norfloxacin, amikacin, Amoxicillin, Tobramicin, nitrofurantion, Ciprofloxacin, gentamicin, Ampicillin and trimethoprim-sulphamethoxazole. Impenem and amikacin were highly active towards *E. coli but* were highly resistant to ampicillin, amoxicillin, Gentamicin and trimethoprim-sulphamethoxazole. The results of the present study showed that the susceptibility rate of urinary isolates was highest for Imipenem (79.2%), followed by amikacin (76.4%), nitrofurantion (75%), gentamicin (61) and ciprofloxacine (52%). Mean while, the resistant rate of urinary isolates was highest for Ampicillin (89%) and trimethoprim-sulphamethoxazole (70%). The increasing frequency of trimethoprim-sulfamethoxazole (TMP-SMX) resistance is worrisome, since this agent is frequently prescribed for uncomplicated UTIs in many developed and developing countries [23]. The highest percentages of resistance of *Escherichia coli* causing urinary tract infections were found for Ampicillin (89%), Trimethoprimsulphamethoxazole (70%), amoxicillin (65%), whereas the highest percentages of sensitivity were seen for imipenem (79.2%), Amikacin (76.4%) and nitrofurantoin (75%). These results correlates with a study done in Comilla Medical College, Bangladesh [24]. Khotaii et al. [25] reported resistance rates of 87.5% to ampicillin, 67.5% to trimethoprim—sulfamethoxazole. In other study, meropenem and imipenem were found to be 98% and 100% sensitive, respectively, against highly resistant gram negative bacilli [29]. A study done in King Fahd Hospital, Saudi Arabia showed that meropenem was 95.8% sensitive followed by amikacin (93.7%) and imipenem (91.71%) against extended spectrum β lactamase producing *E. coli* [30]. This significantly higher bacterial resistance to antibiotics in our region may be due to a higher rate of antibiotic usage, even in the absence of a prescription. Reducing the number of prescriptions of a particular antibiotic can lead to a decrease in resistance rates [26,27]. Another study conducted in India showed that meropenem was highly sensitive against Gram negative bacilli [28]. MIC of Ampicillin are the highest For *E. coli* No 40, 42 as 4 mg/ml were as the lowest for *E. coli* No 1 as 0.2 mg/ml this correlate with study on USA describe the MIC for Ampicillin, Ciprofloxacin and nitrofurantoin for *E. coli* as 4 mg/ml,0.03 ml/mland 16 ml gm/ml [31]. In conclusion, the PCR method provides a valuable tool for cheap and accurate diagnosis of Gram-negative bacteria in urinary tract infections, and can also be applicable for other infections.

5. CONCLUSION

The most prevalent microorganism which cause urinary tract infection in South Port Said city are *E. coli*, and the most sensitive antibiotic against it are imipenem, and the most resistant against *E. coli* are ampicillin. the minimum MIC for Ampicillin are 0.19 µg/ml and the maximum MIC for *Ampicillin* are 4 µg/ml. The 16SrDNA sequence of the amplified product revealed that the strain TW1 had aunique sequence which matched with *E. coli* present by phylogenetic tree.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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