Isolating and Identification Of Bacteria Causing Urinary Tract Infections From Hospital Patients And Nursing Staff

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KEYWORDS

UTI; gyrA; E. coli; K. pneumonia; P. aeruginosa

ABSTRACT

The current study aimed to isolate and identification the bacteria that cause urinary tract infections from recumbent patients and nursing staff, using traditional and molecular methods. 700 Clinical samples were collected from Azadi Teaching Hospital and Kirkuk Teaching Hospital in Kirkuk city for the period from October 2023 to March 2024 from (500) recumbent patients and 200 nursing staff. 347 (49.6%) of the total samples showed positive results for bacterial growth when cultured on ideal culture media, including mannitol, blood, and MacConkey agars. 353(50.4%) of total samples were appear as negative results for bacterial growth. The findings showed the distribution of bacterial isolates obtained from urine samples of recumbent patients and nursing staff at Azadi Teaching Hospital. Escherichia coli (44.6% and 41.67%) was the most prevalent bacterial species, followed by (13.08% and 13.89%) K. pneumoniae, (11.54% and 11.11%) P. aeruginosa. while, the distribution of bacterial isolates obtained from urine samples of recumbent patients and nursing staff at Kirkuk Teaching Hospital. Escherichia coli (46.9% and 51.85%) was the predominant bacterial species, followed by (16.6% and 14.81%) K. pneumoniae and (9% and 11.11%) P. aeruginosa. for molecular study, 7(50.0%) out of a total of 14 E. coli isolates possessed the gyrA gene. On the other hand, 12(100.0%) isolates out of a total of 14 isolates of K. pneumoniae possess the gyrA gene, while 11(91.7%) isolate out of a total of 12 isolates of P. aeruginosa possesses the gyrA gene. It is concluded that the most common cause of urinary tract infections is Escherichia coli followed by K. pneumoniae and P. aeruginosa, and that one of the most important causes of antibiotic resistance is the presence of the gyrA gene, which causes bacterial resistance to fluoroquinolone.

1. Introduction

Urinary tract infections (UTIs) can be classified as either complicated or uncomplicated. Simple UTIs are treatable with medication and can happen to otherwise healthy people. Patients with immunosuppression, renal calculi, pregnancy, indwelling catheters, and other conditions that compromise urinary tract function due to anatomical or neurological abnormalities are at a higher risk of developing severe UTIs [1-2]. People who have urinary tract infections (UTIs) may endure severe and debilitating symptoms, such as dysuria, increased urge to urinate, and persistent discomfort (stranguria) [3]. Gram positive and gram negative bacteria are among the various types of bacteria that cause urinary tract infections. Escherichia coli is a gram-negative bacterium that is responsible for over 75% of UTIs globally [4]. E. coli has identified virulence factors that contribute to the bacteria's persistence and incitement of inflammation in the urinary system. These variables include the bacterial capsule's pili or K antigen, fimbriae, the synthesis of haemolysin and colicin, the capacity to absorb iron, and more [4-5]. It is more common for penitents to have isolates of Klebsiella, Staphylococci, Enterobacter, Proteus, Pseudomonas, and Enterococci species to exhibit UTI symptoms [6-7]. Urinary tract infections are frequently caused by coagulase-negative Staphylococci, according to several publications. Infections caused by Staphylococci saprophyticus typically affect young women who are sexually active [8]. Due to widespread antibiotic abuse in clinical settings, there has been a discernible rise in the frequency of antimicrobial resistance among UTI-causing bacteria globally [7]. Every year, hundreds of thousands of deaths are attributed to antibiotic-resistant microorganisms. Increasingly, the problem is caused by bacteria that are resistant to common antibiotics, even prescription drugs used as a last option [9]. Global public health is affected by the alarming discovery that resistance genes can spread swiftly over the world, necessitating international cooperation. The World Health Organization (WHO) designated this phenomenon as a major global health issue in 2014 in response to the notable global increase in the population of multi-drug resistant strains [9–11]. The current study aimed to isolate and identification the bacteria that cause urinary tract infections from recumbent patients and

nursing staff, using traditional and molecular methods.

2. Methodology

Specimen Collection

700 Clinical samples were collected from Azadi Teaching Hospital and Kirkuk Teaching Hospital in Kirkuk city for the period from October 2023 to March 2024 from (500) recumbent patients and 200 nursing staff. The method of collecting samples included the following: Urine samples were taken from patients with UTI ranging in age from (20-50 years) of both sexes, after which they were transferred directly to the laboratory to be implanted on the culture media.

Bacterial Identification

Bacteria were diagnosed based on the following aspects:

Morphological diagnosis and media characteristics

Based on the culturing features of the *E. coli* colonies developing on blood agar, EMB, and MacConkey agar, were diagnosed after incubation for 24 hours at 37 ^{0}C.

microscopic examination

By using a microscope to examine the morphological characteristics of germ cells—specifically, how they contacted the gram stain, which indicates the shape and arrangement of the germ cells—bacterial colonies were found.

Biochemical reaction and motility test

Numerous biochemical tests, such as, methyl red, citrate, urease, voges-proskauer, catalase, oxidase, KIA, and indole test, were carried out in order to identify and diagnose bacteria.

Identification of bacteria isolates via VITEK2

For microbiological identification, VITEK 2, the most recent colorimetric technology generation, is the gold standard. Procedure: The subsequent steps were carried out in compliance with the instructions supplied by the manufacturer, Biomerieux.

Biofilm formation by Congo red test

Dissolve 37 g/L of brain heart infusion broth (BHI) and 50 g/L of sucrose in 500 ml of distilled water to make Congo red agar. Put in 10 grams of agar-agar. After that, the volume was adjusted to 900 ml of distilled water and the medium was autoclaved to achieve sterilization. 0.8 g of congo red stain and 100 ml of distilled water were combined to create the dye, which was then autoclaved to guarantee sterility. After autoclaving the medium and dye and allowing it to cool to 50 oC, the dye was applied to the agar. The medium was poured into Pyrex plates. Using this medium, the biofilm growth of the isolates was found [12].

Genetic Study

Template Preparation by boiling method

The boiling procedure described by [13] was used to create the DNA template. To sum up, two milliliters of distilled water were used to completely suspend fifteen distinct colonies of bacteria that grew over night, and the mixture was then brought to a boil in a water bath for ten minutes. The supernatant from centrifugation served as the PCR's template DNA.

PCR Amplification

The following steps comprise the PCR amplification process for the genetic level detection of K. pneumoniae, P. aeruginosa, and Escherichia coli local isolates table (1): A total of 25 µl (12.5 µl of Master Mix 2x, 5 µl template DNA, 1 µl primers for each forward and reverse primer, and 5.5 µl

nuclease-free water) was the final amount of the PCR mixture using uniplex PCR Eppendorf tubes. The multiplex PCR quantity was different. The mixture was put in a thermocycler polymerase chain reaction after being quickly stirred with a vortex.

PCR procedure

Detecting the *gyrA* sequences have been performed through the conventional PCR with 50µl of the PCR Mastermix. All primers were used at concentration of 30 pmol/µl. The primers that have been utilized in the presented research have been listed in table (1).

Table (1): Uropathogenic E. coli virulence genes PCR assay primers

Primer	Primer sequence	Length (bp)	TA (°C)
gyrA-F	TACACCGGTCAACATTGAGG	648	63.7
gyrA-R	TTAATGATTGCCGCCGTCGG		63.7

Agarose Gel Electrophoresis of DNA

In order to distinguish the bundle size of the PCR interaction on the agarose gel, electrophoresis has been used to detect DNA fragments after the procedure of extraction or to identify the result of the PCR interaction in the presence of standard DNA.

3. Result and Discussion

4.1. Sample distribution

The study included 500 recumbent patients (293 admitted to Azadi Teaching Hospital and 207 admitted to Kirkuk Teaching Hospital); the study further included 200 nurse staff (100 individuals work in Azadi Teaching Hospital and 100 in Kirkuk Teaching Hospital). Urine examination and urine culture were done for all the enrolled subjects, and bacterial species were isolated and clinical features of UTI were also noted.

In the current study, the urine specimens were collected from volunteers with clinical features of UTI. The findings were found that 347(49.6%) of total samples were appear as positive results for bacterial growth that cultured optimal cultured media such as blood, mannitol, and MacConkey agars. 353(50.4%) of total samples were appear as negative results for bacterial growth, table (2).

Table (2): Distribution of study group according to results

	No. (%) **+ve culture**	No. (%) **-ve culture**	Total No.(%)	P value
Volunteers	347(49.6%)	353(50.4%)	700 (100.0%)	0.172

Distribution of bacterial isolates from the urine of recumbent patients to Azadi Teaching Hospital

The table 3 presents the distribution of bacterial isolates obtained from urine samples of recumbent patients at Azadi Teaching Hospital. *Escherichia coli* was the most prevalent bacterial species, accounting for **58** isolates (44.6%), followed by *Klebsiella pneumoniae* with **17** isolates (13.08%), *Pseudomonas aeruginosa* with **15** isolates (11.54%)Proteus mirabilis with **11** isolates (8.46%), and Enterococcus faecalis with 8 isolates (6.15%). Other identified bacterial species included Staphylococcus aureus, Staphylococcus saprophyticus, Proteus vulgaris, Enterobacter cloacae, and Staphylococcus epidermidis, each comprising smaller proportions of the isolates.

The table displays the distribution of bacterial isolates obtained from urine samples of recumbent patients at Kirkuk Teaching Hospital. Escherichia coli was the predominant bacterial

species, constituting 62 isolates (46.9%), followed by Klebsiella pneumoniae with 22 isolates (16.6%), Pseudomonas aeruginosa with 12 isolates (9%), and Proteus mirabilis with 9 isolates (6.82%). Other identified bacterial species included Enterobacter cloacae, Enterococcus faecalis, and Proteus vulgaris, each comprising smaller proportions of the isolates.

Table (3): Distribution of bacterial isolates from the urine of recumbent patients to Azadi and Kirkuk Teaching Hospitals

Type of bacteria	**Azadi Teaching Hospital**		**Kirkuk Teaching Hospital**	
	Gram negative			
	No.	**%**	**No.**	**%**
E. coli	58	44.62	62	46.97
P. aeruginosa	15	11.54	12	9.09
P. mirabilis	11	8.46	9	6.82
P. vulgaris	5	3.85	4	3.03
K. pneumonia	17	13.08	22	16.67
E. faecalis	8	6.15	4	3.03
E. cloacae	3	2.31	6	4.55
Gram positive				
S. aureus	6	4.62	7	5.30
S. saprophyticus	6	4.62	3	2.27
S. epidermidis	1	0.77	3	2.27
Total	130	100	132	100

Distribution of bacterial isolates from the urine of nursing staff of Azadi and Kirkuk Teaching Hospitals

The table 4 illustrates the distribution of bacterial isolates obtained from urine samples of nursing staff at Azadi Teaching Hospital. Escherichia coli was the most prevalent bacterial species, constituting 15 isolates (41.67%), followed by Klebsiella pneumoniae with 5 isolates (13.89%), Pseudomonas aeruginosa with 4 isolates (11.11%), and Proteus mirabilis with 3isolates (8.33%). Other identified bacterial species included Enterobacter cloacae, Staphylococcus aureus, Staphylococcus epidermidis, and Staphylococcus saprophyticus, each comprising smaller proportions of the isolates. The table presents the distribution of bacterial isolates obtained from urine samples of nursing staff at Kirkuk Teaching Hospital. Escherichia coli was the most prevalent bacterial species, accounting for 14 isolates (51.85%), followed by Klebsiella pneumoniae with 4 isolates (14.81%), Pseudomonas aeruginosa with 3 isolates (11.11%). Other identified bacterial species included Enterobacter cloacae, Enterococcus faecalis, Proteus mirabilis, Staphylococcus aureus, Staphylococcus epidermidis and Staphylococcus saprophyticus, each comprising smaller proportions of the isolates.

Table 4: Distribution of bacterial isolates from the urine of nursing staff of Azadi and Kirkuk Teaching Hospitals

Type of bacteria	Azadi Teaching Hospital		Kirkuk Teaching Hospital	
	Gram negative			
	No.	%	No.	%
E. coli	15	41.67	14	51.85
P. aeruginosa	4	11.11	3	11.11
P. mirabilis	3	8.33	1	3.7

K. pneumonia	5	13.89	4	14.81
E. faecalis	-	-	1	3.7
E. cloacae	2	5.56	1	3.7
Gram positive				
S. aureus	3	8.33	1	3.7
S. saprophyticus	2	5.56	1	3.7
S. epidermidis	2	5.56	1	3.7
Total	36	100	27	100

Biofilm formation

Table () shows the number of isolates whose ability to produce biofilm was examined and the number of bacterial species studied. The number of positive isolates tested reached 143(50.4%) out of a total of 284 isolates examined. The number of positive E. coli isolates for the test was 109(76.2%), the number of *K. pneumonia* isolates was 18(12.6%), while the number of *P. aeruginosa* isolates was 15(10.5%), and *S. aureus* was 1(0.7%) out of a total of 143 which biofilm-producing isolate.

Table (): number and percentage of biofilm formation for isolated bacterial strains

Isolated bacteria	No. of isolated bacteria	Biofilm positive
E. coli	162(57.0%)	109(76.2%)
K. pneumonia	56(19.7%)	18(12.6%)
P. aeruginosa	46(16.2)	15(10.5%)
S. aureus	20(7.1%)	1(0.7%)
Total	284(100.0%)	143(50.4%)

The current study's findings, which were obtained using Congo red agar, showed that 76.2% of the total bacteria examined were E. coli. These outcomes fell short of those of the study by Al-Chalabi [14], which showed that 90% of E. coli had the capacity to form biofilms. It is thought that using CRA to identify E. coli biofilm development is beneficial since these bacteria do not ferment other sugars that could be required to produce certain metabolites, which mix with CRI to give the colonies a black color that indicates the production of slime [15]. Of the 44 bacterial isolates that were determined to be capable of forming biofilm, 26 isolates produced biofilm at a rate of 46.2%, 26 isolates produced biofilm at a rate of highly biofilm, and 46.2% produced biofilm at a moderate rate. These results are consistent with previous research [16]. Out of the 143 isolates that tested positive for K. pneumonia in the current investigation, 18 isolates (12.6%) showed positive results for the biofilm formation test. In the Chu et al. study [17], of the 45 clinical strains, 28 (62.2%) were biofilm-producing strains, while the remaining 17 (37.8%) were classified as biofilm-negative. This is more than the current study's findings, and the discrepancy between the two investigations could be explained by the quantity of K. pneumonia isolates that were found in each. This work's findings conflicted with those of Rewatkar and Adher's investigation [18], which found that out of 30 nosocomial infection P. aeruginosa isolates, 27 (90%) and 3 (10%) of them formed biofilms, respectively. The CRA test is a major approach for determining biofilm satisfaction, however it is not precise enough. As a result, other methods that are recommended must be used [19].

Genetic study

In the current study, isolates were selected for genetic study on the basis of their biofilm production. 109 isolates of *E. coli*, 18 isolates of *K. pneumoniae*, and 15 isolates of *P. aeruginosa* were studied.

gyrA gene

In the current study, 7(50.0%) out of a total of 14 E. coli isolates possessed the *gyrA* gene (fig: 1). On the other hand, 12(100.0%) isolates out of a total of 14 isolates of *K. pneumoniae* possess the *gyrA* gene (fig: 2), while 11(91.7%) isolate out of a total of 12 isolates of *P. aeruginosa* possesses the *gyrA* gene, as shown in figure (3).

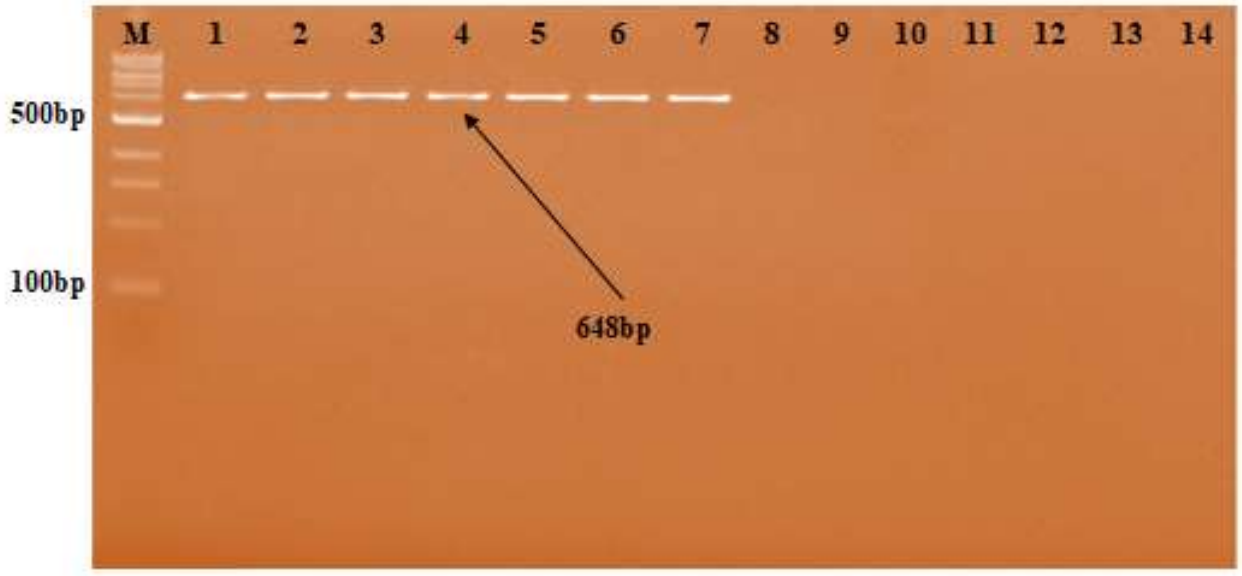

Figure (1): Agarose gel electrophoresis of *E. coli* (1.5% agarose,7v/cm^2 for 60 min) for *gyrA* gene (648 bp amplicon): M: represent M100bp DNA Ladder, lanes 1-15 represent *E. coli* isolates.

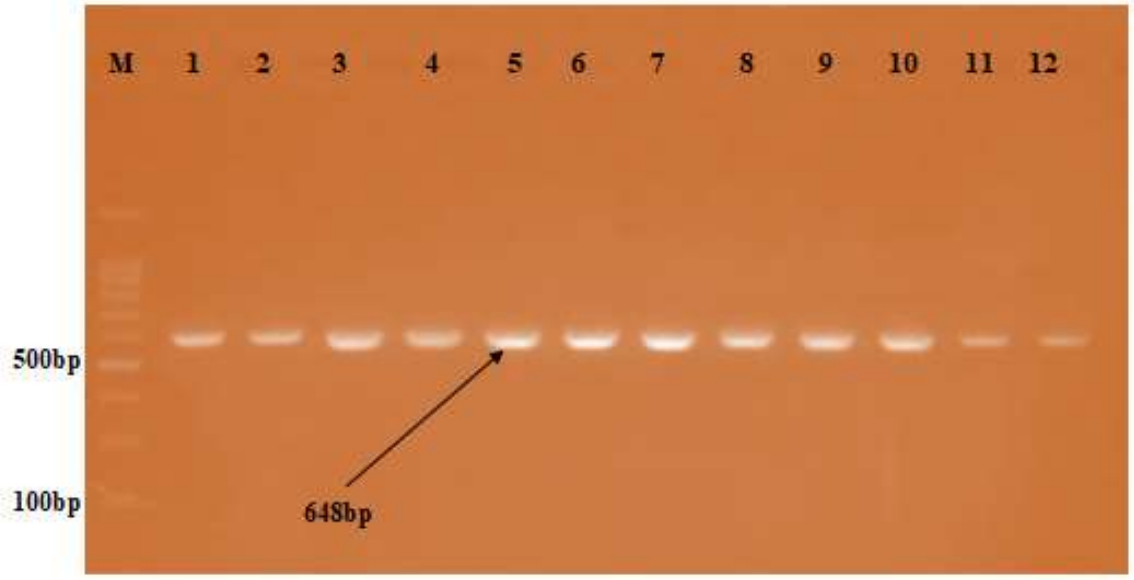

Figure (2): Agarose gel electrophoresis of *K. pneumoniae* (1.5% agarose,7v/cm^2 for 60 min) for *gyrA* gene (648bp amplicon): M: represent M100bp DNA Ladder, lanes 1-14 represent *K. pneumoniae* isolates.

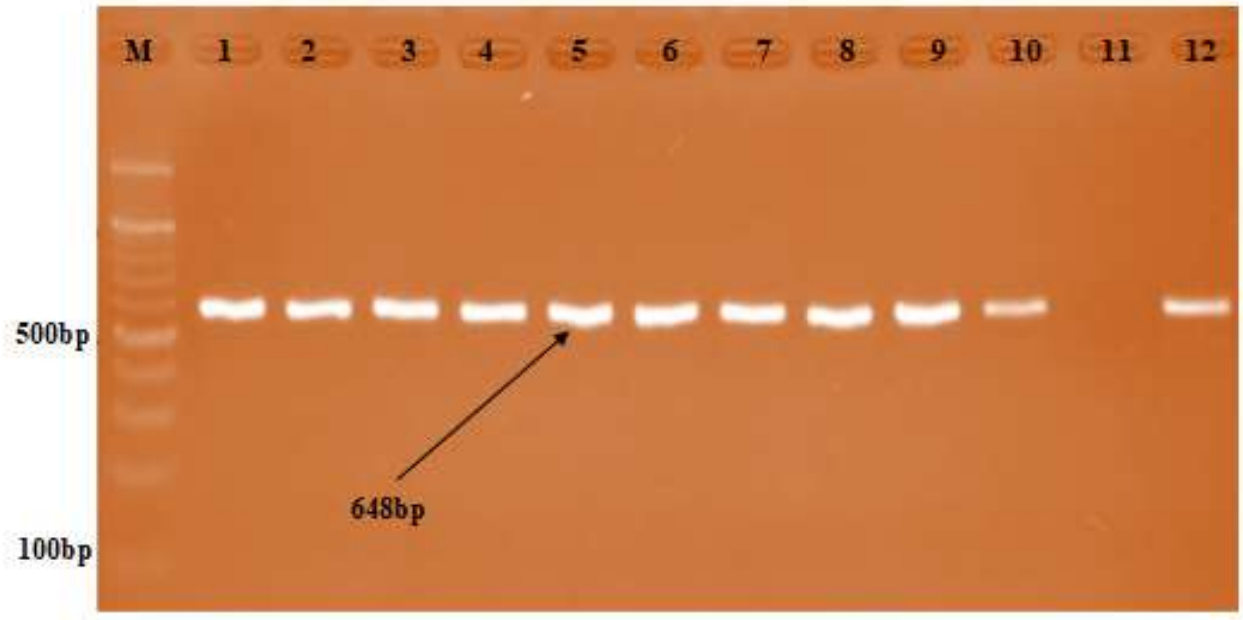

Figure (3): Agarose gel electrophoresis of *P. aeruginosa* (1.5% agarose,7v/cm^2 for 60 min) for *gyrA* gene (648bp amplicon): M: represent M100bp DNA Ladder, lanes 1-12 represent *P. aeruginosa* isolates.

The current study's findings concurred with the research done by Al-Zuhairy and Al-Dahmoshi [20], where Three genes—gyrB, gyrA, and gyrC—have had their fluoroquinolone resistance mutations examined using PCR in the E. Coli strains recovered from the UTI patient. 32 (57.1%) and 33 (58.9%) of the isolates, respectively, had mutated gyrA and gyrB genes, according to the results of the PCR investigation. The enlarged spectrum of fluoroquinolones, like ciprofloxacin, has considerable action against Gram-negative bacteria, especially E. coli. Fluoroquinolones are a commonly used and ingested class of pharmaceutical drugs. Fluoroquinolones' primary target in E. coli is DNA gyrase. The two subunits that make up this enzyme are gyr B and gyr A, which are encoded by gyr B and gyr A, respectively. Clinical strains resistant to fluoroquinolones are more likely to have mutations in either of the two genes responsible for quinolone resistance or in a portion of the quinolone resistance-determining region (QRDR) of gyr A [21]. Mutations that target the parC and QRDR of DNA gyrase and topoisomerase IV are the main source of fluoroquinolone resistance [22]. According to Varughese et al. [23], the examined Klebsiella isolates have gyrA and gyrB genes. Additionally, 88.9% of the isolates contained an extra copy of gyrA [24]. Quinolone-related phenotypic resistance is very prevalent. Mutations in the gyrase and topoisomerase IV genes compromise the interaction of quinolones with enzymes, making quinolones hazardous and capable of destroying bacterial chromosomes, which in turn causes bacterial resistance to quinolones [25]. The elevated degree of resistance to the quinolone group and its derivatives is caused by a gene mutation in the gyrA gene, which determines quinolone resistance [26]. The findings of Safika et al.'s study [27], which showed that 100% of Klebsiella pneumoniae isolates had the gyrA gene, were similar to those of the current investigation

4. Conclusion and future scope

It is concluded from the current study that the most common cause of urinary tract infections is Escherichia coli followed by K. pneumoniae and P. aeruginosa, and that one of the most important causes of antibiotic resistance is the presence of the gyrA gene, which causes bacterial resistance to fluoroquinolone.

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