Original Article

Molecular Detection And Antimicrobial Resistance Of Escherichia Coli Isolated From Uti Patients

Safa Hasan Radhi[1], Hassan Hadi Al-Kazzaz[2], Noor Flayyih Hasan[3], Reyam Abdul Khuder Mohammed[4]

1 Department of Biology, College of Science, Al-Qasim Green University, Iraq.
2 Al-Zahraa university for women, iraq.karbala, Hassan.alkazzaz@alzahraa.edu.iq
3 Health and Medical Technical College, Al-Zahraa University For Women, Iraq.
4 B.V.S, M.Sc. Oral Microbiology, Al-Mustaqbal University College, Hilla, Iraq.
DOI: 10.47750/pnr.2023.14.S02.97

Abstract

The purpose of this research was for molecular identification and antibiotic resistance of Escherichia coli isolated from UTI patients. More than 50 urine samples from patients with urinary tract infections were gathered from Hilla's hospitals and medical clinics. PCR was used to confirm the presence of 16srRNA, acrA, and acrB genes in the bacterial isolates, which were identified using standard methods. Out of 50 clinical samples, 23 isolates of E. coli were detected and identified using conventional techniques. From these 23 samples, 22 isolates were verified by PCR. The results of antibiotics sensitivity test showed that all isolates were MDR were higher resistant to the carbenicilline 23 (100 percent), Erythromycin 22 (95.7 percent), Cefotaxime 17 (73.9 percent), Novobiocin 16 (69.5 percent),Tetracycline 14 (60.9 percent) ,Ciprofloxacin 12 (52.2 percent), Gentamicin 8 (34.8 percent) and Nitrofurantoin 1 (4.3 percent). (4.3 percent).

All isolates of E. coli isolated from urinary tract infections showed multiple resistance for antibiotics), multidrug resistance (MDR) was the highest antibiotic resistance carbenicillin with a percentage of (100%) and less resistance to the antibiotic nitrofurantoin with a percentage of (4.3%).

Keywords: Molecular, E. coli, UTI

Introduction:

Escherichia coli is a member of the gram-negative Enterobacteriaceae family. It is rod-shaped, motile or some time non-motile, facultatively anaerobic, and ferments lactose producing ramenose and sorbetol. The optimal temperature for the development of the -glucoronidase enzyme (63-63°C) (Jawetz et al., 2016; Wanger et al., 2017) positive for catalase and negative for oxidase. Indole producer but not citrate consuming, positive for methyl red and Vogase-Proskauer negative (Hemraj et al., 2013).

It is naturally found in the intestines of people and animals, but it is also an opportunistic bacterium that causes a variety of illnesses such as diarrhea, bacteremia, blood poisoning, meningitis, and sepsis. It is one of the most prevalent forms of bacteria that causes these disorders. Urinary tract infections are frequent, accounting for about (90 percent) of urinary tract infections worldwide, and are especially prevalent in youth (Hadi et al., 2014; Shuwaikh and Jassim, 2016) .

This bacteria's pathogenicity is due to the incidence of many virulence factors, including siderophores, colisin, cytotoxic necrotizing factor, and surface structures such as capsule, flagella, as well as LPS,

which confer antigenic qualities on bacteria by producing the antigens O, H, as well as antigen K. Additionally, it has cilia (fimbreae or pilli) that aid in attachment to host tissues, allowing it to build biofilms (Zowawi et al., 2015; Terlizz et al., 2017).

E. coli bacteria are characterized by multidrug resistance (MDR) (Laird, 2016). It is characterized by a high degree of antibiotic resistance as a result of the existence of resistance enzymes such as -lactamases that confer resistance to beta-lactam antibiotics.

Resistance to aminoglycosides and antagonists is conferred by -lactams and enzymes. quinolones Additionally, these bacteria possess additional mechanisms that confer antibiotic resistance, such as altering the permeability of the cell membrane, inhibiting protein synthesis, altering the target site, and the acquisition of pumps by bacteria efflux, conferring antibiotic-resistant bacteria such as macrolides, rifamicin, and novobiocin (Kapoor et al., 2017).

The goal of this study was for molecular detection and antimicrobial resistance of Escherichia coli isolated from UTI patients

Materials and methods:

Fifty clinical samples of urine from patients with UTIs were obtained from multiple hospitals and medical clinics in Hilla city. PCR-based gene identification was used to identify bacterial isolates and confirm their diagnosis (Table 1) .

Table 1. Genes used in this study

Name of gene	Sequence	bp	Reference
16SrRNA	F CGAGTGGCGGACGGGTG AGT R TCGACATCGTTTACGGCGTGGA	727	(Maleki et al., 2017)
acrA	F CTCTCAGGCAGCTTAGC CCTAA R TGCAGAGGTTCAGTTTTG ACTGTT	107	
acrB	F GGTCGATTCCGTTCTCCG TTA R CTACCTGGAAGTAAACG TCATTGGT	105	

Two milliliters of template DNA, two milliliters of particular primer, ten milliliters of 2x Taq master mix, and eleven milliliters of PCR grade water were used to make the PCR mixture used in the experiment. When it came to doing PCR, a heat cycler was used.

The program of 16srRNA include, Initial denaturation took 5 minutes at 94°C, followed by 30 sec. at 60°C annealing, 1 minute at 72°C extension, followed by a final 5 minutes extension at 72°C. This was followed by 35 cycles of denaturation, extension, and annealing at 72°C.

For arcA and arcB program same of above gene only the differences in annealing at 52°C for 30 sec.

Electrophoresis agarose gel was used to examine DNA samples, ladder of 100bp was used.

The antibiotic susceptibility test done by using the followings antibiotics Erythromycin, Cefotaxime, Novobiocin, Tetracycline, Ciprofloxacin, Gentamicin and Nitrofurantoin. The method of this test was depending on Kirby baur technique.

Results and discussion:

Out of 50 clinical samples, 23 isolates of E. coli were diagnosed and identified by conventional methods. From these 23 isolates, 22 isolates were confirmed by PCR (Fig. 1,2,3).

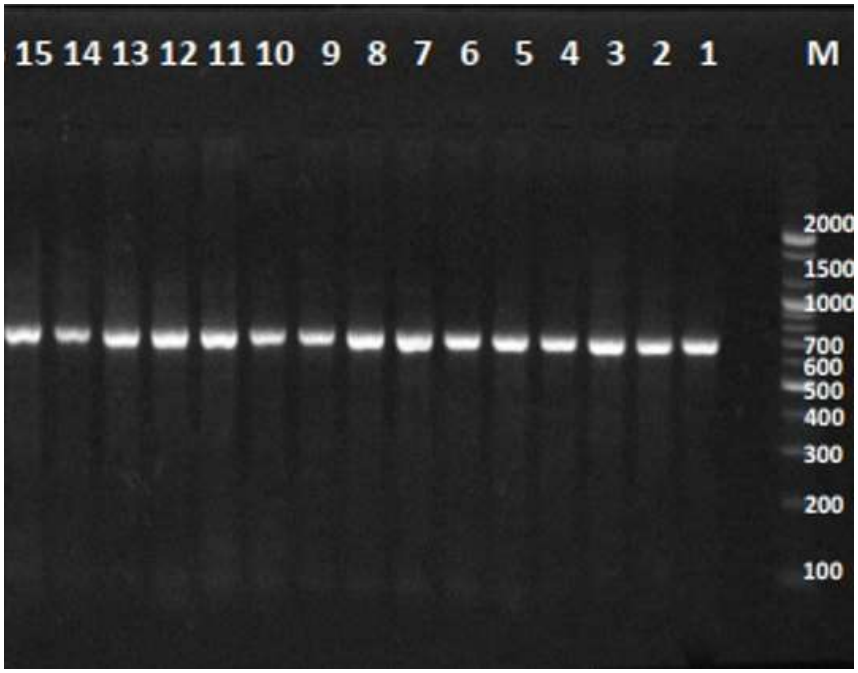

Figure 1. PCR of E. coli 16srRNA gene at 700bp. Lane M represents 100 bp DNA marker.

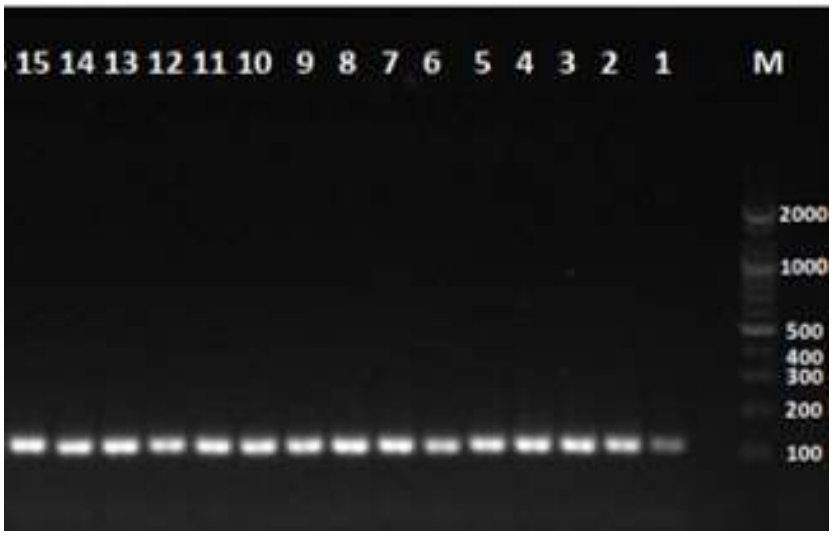

Figure 2. PCR of E. coli acrA gene at 107bp. Lane M represents 100 bp DNA marker.

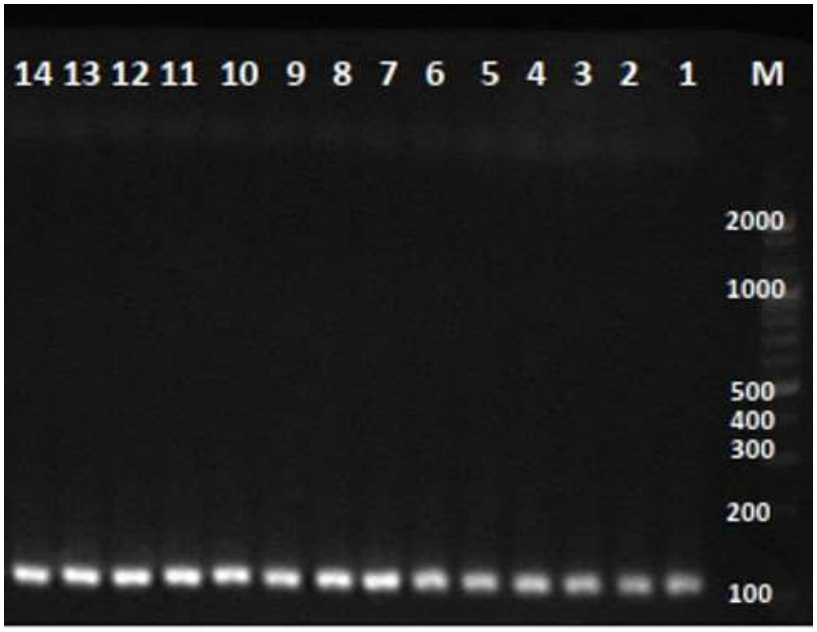

Figure 3. PCR of E. coli acrB gene at 105bp. Lane M represents 100 bp DNA marker.

The results of this study are in agreement with the results of the local study reached by Jaaz and Himim (2016), as it reached the percentage of bacterial isolates possessing (100%) 16SrRNA gene, as the current study also agrees with the study reached by the researcher Maleki et al (2017) in Iran and the study reached by the researcher Lai et. 2016 in Malaysia, as the molecular diagnosis of all bacterial isolates was done using the diagnostic gene, Also, the results of the current study agree with the results of the study conducted by the researcher. Jenkins et al ,(2018).who showed that all isolates of E. coli had the diagnostic gene 16SrRNA.

This method is considered one of the fastest and most effective methods for diagnosing bacteria pathogens, this gene contains conserved regions that overlap with regions the 9 variable regions are used to determine sex and gender identity bacterial because the diagnostic gene 16SrRNA is present in all bacterial species, as it is little heterogeneous or the random change in the genetic sequence is very small over time. Promote a rule of this gene to be Very sufficient for educational and marine purposes (Suardana, 2014; Srinivasan et al., 2015).

The current study is consistent with the clearness of local and global studies that mentioned that E. coli bacteria was one of the main causes of urinary tract infections, and among the local studies, that carried out by Shuwaikh and Jassim (2016) in hospitals in the city of Baghdad and its surrounding areas, and the local study carried out by Al-Busalah (2014) in the city of Al-Qadisiyah, the percentage of bacteria isolation was(41.6%) and (56%) of urine samples subsequently.

All MDR isolates were found to be highly resistant to antibiotics, according to the findings of an antibiotic sensitivity test to carbenicilline 100 %, Erythromycin 22 (95.7 %), Cefotaxime 17 (73.9%), Novobiocin 16 (69.5%),Tetracycline 14 (60.9 %) ,Ciprofloxacin 12 (52.2%), Gentamicin 8 (34.8%) and Nitrofurantoin 1 (4.3%) (Figure 4).

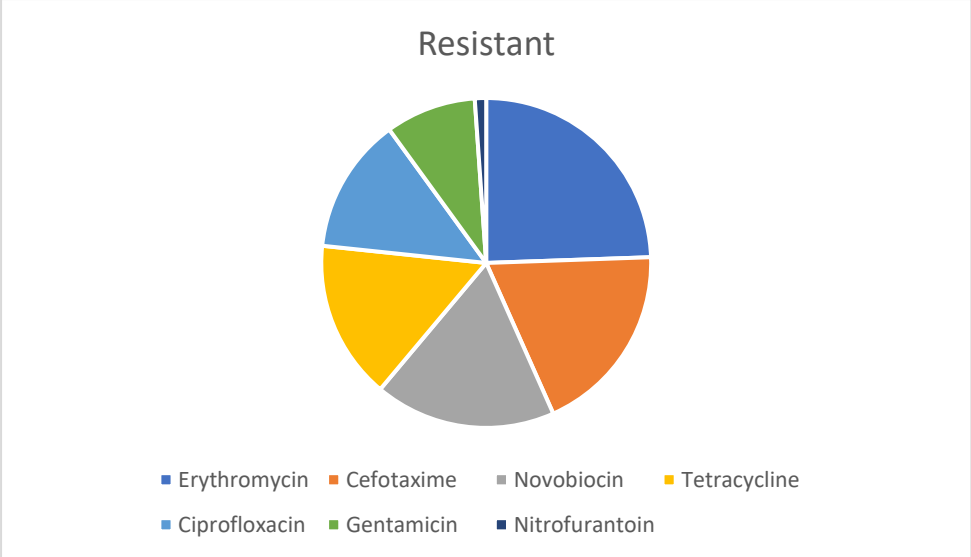

Figure 4. antibiotic sensitivity test of E. coli

The results of this study for the antibiotic carbenicillin agree with previous studies, including the results The local author Ibrahim et al, (2014) in which the percentage of bacterial isolates reached Antibiotic resistance, (011%) carbenicillin, as this study showed that many enzymes β-lactamases that are controlled by plasmid-borne genes in Gram-negative bacteria can degrade both carbenicillin antibiotics ampicillin, cephalothin, and other antibiotics of the same group Also, the results of the current study are consistent with the findings of the researcher. (Hinthon et al. 2017) In which the percentage of bacterial isolates resistant to this antibiotic reached (%92.2)

While the results of this study did not agree with the findings of the two authors, Kafilzadeh and Farsimadan ,2016 as the percentage of resistance of bacterial isolates to this antibiotic was (42.8%), and they indicated that the bacteria Several mechanisms have been used in the resistance to this antibiotic, and they also indicated that the reasons for the high resistance to antibiotics are due to the excessive use of antibiotics, mutations that encode the enzymes, as well as the Transfer of resistance mediated by plasmid.

It agrees with the study carried out by the researcher Harran (2018), who found that E. coli bacteria were highly resistant to antibiotics belonging to the . group β-lactams, as the percentage of bacteria resistance to these two antibiotics was (78.6%) and(%76.6) straight. It also agrees with the study reached by the researcher. . Suresh et al. (2016) in India, which amounted to in which the percentage of bacterial isolates resistant to these two antibiotics is (98%) and (100%), respectively, and they showed that the main reason for this high resistance of bacteria is that they have efficient effluent pumps that deliver antibiotics outside the cell and demonstrating its harmful effect on the cell, and that this study came close to what was reached by the researcher Hegazy(2018) et al. in Egypt, whose study revealed that 74.4 percent of E.coli isolates were resistant to cefotaxime, but the findings of this study contradicted the researcher's study.

The results of the current study agree with the dependent antibiotic ciprofloxacin For a group of quinolones with the results reached by the researcher Tajbakhsh et al. (2016) Iran, as the rate of bacterial resistance to this antibiotic was (56.25%).

Conclusion:

All isolates of E. coli isolated from urinary tract infections showed multiple resistance for antibiotics), multidrug resistance (MDR) was the highest antibiotic resistance carbenicilline with a percentage of (100%) and less resistance to the antibiotic nitrofurantoin with a percentage of (4%).

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