JMGCB
ISSN:3032-1085
https://doi.org/10.61796/jmgcb.v1i4.394

DETECTION OF OQXAB EFFLUX PUMPS GENE IN ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIA ISOLATED FROM PREGNANT WOMEN SUFFERED FROM URINARY TRACT INFECTIONS AND DIABETES MELLITUS IN BASRAH

Amna Ali Nasir
Department of Biology, College of Science, University of Basrah, Iraq
amnanali68@gmail.com

Adnan A.Al-Mousawi
Department of Biology, College of Science, University of Basrah, Iraq
adnan.hamad@uobasrah.edu.iq

Zainab R. Abdul-Hussein
Department of Pathological analysis, College of Science, University of Basrah, Iraq
zainb.abdulhussien@uobasrah.edu.iq

Received: Feb 22, 2024; Accepted: March 29, 2024; Published: Apr 18, 2024;

Abstract: Considerably, pregnant women with diabetes mellitus are highly susceptible to urinary tract infections. The aim of the study is investigate the incidence of OqxAB efflux pumps gene among Escherichia coli and Klebsiella pneumonia isolates from urine of pregnant women suffered from urinary tract infections and Diabetes mellitus. E.coli and K.pneumonia were identified using conventional methods and confirmed by specific 16S rDNA primers. Antibiotic susceptibility test of E. coli and K. pneumonia isolates against several antibiotics were examined using Vitek®-2 compact system .The production of efflux pump by E. coli and K. pneumonia isolates were investigated phenotypically using ethidium bromide-agar cartwheel method (EtBr CW). Moreover, OqxAB Efflux Pumps genes were tested. The results showed that among 100 urine samples, 40 urine samples showed positive bacterial growth where 21(51%) E coli isolates, 8(20%) K. pneumonia isolates. In terms of antibiotic sensitivity, all E.coli and K.pneumonia isolates were resistant to ampicillin, piperacillin and ticarcillin, respectively. The prevalence of OqxAB efflux pump genes frequency ranges from 72% in E. coli and 50% in K. pneumonia.

Keywords: OqxAB Efflux pumps, E. coli, UTI, UTI, Diabetes Mellitus, Antibiotic sensitivity

INTRODUCION

Urinary Tract Infections (UTIs) is highly considered as one of the leading infections that associated with pregnancy globally. These types of infection generally are hard to overcome because of the therapeutic challenges that consequently might affect mother and new born together (Onyango et al., 2018). Interestingly, pregnant women with diabetes are more susceptible to UTIs due to decrease of cytokines in urine and decreasing of neutrophil response and decrease of leukocyte concentration (Al-Bidhani, 2018). Moreover, It was found that the invasion of microbes to the epithelial cells lining the urinary tract with diabetes is easier in comparision to

non pregnancy due to the lack of local cytokines secretion (Hoepelman et al., 2003). Generally, the frequency of UTIs in diabetic pregnant women is enhanced to 27.6% (Onoh et al.,2013; Emiru et al., 2013; Naher et al., 2018). Interestingly, Around 80-90% of all bacterial infections are caused by uropathogenic E.coli, Uropathogen E. coli (UPEC) where it has emerged as the predominant bacteria that has recorded in 80% of community acquired and 40% of nosocomial infections (Mahdi et al., 2020; Albadery et al., 2023). Globally, resistance of bacteria against antibiotics triggered an alarming health issue that threat the life of individuals terms of mortality, the financial cost, and quality of the services. Moreover, health authorities expect that antimicrobial activity will get worse with an estimation that by 2050, it will cause 10 million fatalities each year (Praveenkumarreddy et al., 2020; Bengsston-Palme et al., 2018). Among various antimicrobial mechanisms, efflux pump is revealed as one of the main pathways that exploited effectively by bacteria that inhibit the actions of antibiotics (Ali and Al-Dahmoshi,2021; Moosavian et al.,2021).Generally, efflux pumps are proteins of membrane, play a pivotal role in expelling harmful substances from bacterial cells into the external environment, constituting a ubiquitous feature across all bacterial species. The mechanism governing efflux pumps is intricately regulated by genes residing within mobile genetic elements like plasmids or bacterial chromosomes (Webber and Piddock, 2003). What is worth mentioning is the fact that efflux pumps can be quite specific, in cases when they are involved in the transport of only one certain compound, or they can be of a broader spectrum, facilitating for the export substances with various structures (Piddock, 2006). Resistance-Nodulation Division (RND) family of efflux pumps occupies a central position in the family of efflux pump families, which act as antibiotic resistance promoters in Gram-negative bacteria. In addition, the RND family have two recognizable members, OqxAB, which are the most important microorganisms in this mechanism. The OqxAB pump, a significant player in the array of efflux pumps, is characterized by two principal domains: The enzyme complex includes two components: OqxA, found in the periplasmic part of the bacterial cell, and OqxB, a transmembrane protein. Surprisingly, there are at least two copies of genes encoding OqxAB - on the bacterial chromosome and plasmids (Piekarska et al., 2015). This is similar to what happens in MFS family efflux systems that include CmlA, Tet/BE, and Flo efflux systems, or OqxAB-TolC transporter that belongs to the RND family efflux pumps. These genes can encode for more than one substrate of different chemical make-ups and may be pumped out through the single efflux pump. These are called multidrug resistance efflux pumps, and thus confer the characteristic of multidrug resistance to antibiotics (Blair et al., 2014).

MATERIALS AND METHODS

One hundred urine samples were collected from pregnant women with both urinary tract infections and diabetes mellitus by transfer mid-stream urine sample in sterile containers. Samples of urine were cultured on MacConkey agar and Blood agar plates and after 18-24 h. the bacterial colonies were pre-identified phenotypically based on morphological diagnostic, microscopy, biochemical assays. VITEK®2 Compact equipment was utilized for the antibiotic susceptibility test.

2.1 profiling of bacterial isolates

2.1.1 Detection of *E.coli* using specific primer

E.coli. isolates were identified according to Tonu*et al*., (2011) and Gamal *et al*., (2017). Genomic DNA of *E. coli* was amplified using Specific 16SrDNA primers as the following primers: Forward primer: 5 ′GAC CTC GGT TTA CTT CAC AGA 3′, and Reverse primer: 5 ′CAC ACG CTG ACG CTG ACC 3 ′.a 585 bp result, which was used to confirm the presence of *E. coli*. The total volume of chemicals used in PCR amplification is 25, with each of the following volumes: DNA: 1 µI, Forward and Reverse primers: 1 µI each, respectively, GoTaq Green Master Mix: 12.5 µI, and Nuclease-free water: 9.5 µI. Table 1 illustrate the total volume of PCR tube reaction.

Table (1): Program used in PCR amplification

Steps	Temperature °C	Time	No. of cycles
Initial denaturation	94	4min	1

Denaturation	94	90 sec	30
Annealing	62	90 sec	
Extension	72	2min	
Final extension	72	7min	1

2.1.2 Detection of *K. pneumonia* isolates using specific primer

According to Osman *et al*. (2014), *K. pneumonia* were identified using specific 16SrDNA primer as the following : Forward primer (5′ATTTGAAGTTGCAAACGAT3′) and the reverse primer (5′TTCACTCTGAATTTTCTTGTGTTC3′). The total volume of chemicals used in PCR amplification is 20, with each of the following volumes. DNA : 2 µI, Reverse and Forward primers : 1 µI,1 µI, respectively, Go Taq Green Master Mix : 5 µI, and Nuclease-free water : 11 µI. Table 2 included an explanation of the PCR program.

Table (2): Program used in PCR amplification

Steps	Temperature °C	Time	No .of cycles
Initial denaturation	95	5min	1
Denaturation	94	30 sec	35
Annealing	58	90 sec	
Extension	72	90 sec	
Final extension	72	10min	1

2.2 Phenotypic Detection of efflux pumps

2.2.1 Efflux pumps activity

Efflux pump activity of *E.coli and K. pneumonia* isolates were investigated using cartwheel EtBr-agar (EtBrCW) method according to (Khalaf *et al*., 2021). The bacterial isolates were grown for 24 hours in a shaker with nutritional broth, and next day they were adjusted to 0.5 of a McFarland standard. The Nutrient Agar plates were divided into a cartwheel shape using radical lines. The bacteria were then cultivated for 16 hours at 37°C after being swabbed onto nutrient agar plates containing higher concentrations of EtBr using a sterilized cotton swab. Using a UV transilluminator, the culture on the nutrient agar plates was inspected. The isolates displaying increased fluorescence.

2.2.2 Detection of Efflux pumps genes

2.2.2.1 Detection of OqxA gene

OqxA gene was detected in bacterial isolates through the use of specific primer (Moosavian *et al*.,2021).Polymerase chain reaction(PCR)was utilized toConfirm the existence of OqxA gene in *E. coli* and *K. pneumonia* using primers pairs from the: Forward primer:(5′CTCGGCGCGATGATGCT3′), reverse primer: (5′CCACTCTTCACGGGAGACGA3′) to give 392 bp product.In PCR amplification, the reagents and their volumes are 25 total volume of reagents included : DNA:5µI, Reverse primer:2 µI, Forward primer:2 µI, Go Taq Green Master Mix:12 µIand Nuclease- free water:4 µI. The program of PCR was described in table (3).

Table(3) : Program used in PCR amplification

Steps	Temperature °C	Time	No. of cycles

Initial denaturation	94	5 min	1
Denaturation	94	45sec	34
Annealing	52.3	45sec	
Extension	68	1 min	
Final extension	72	10min	1

2.2.2.2 Amplification of OqxB gene

OqxBgene was detected in bacterial isolates through the use of specific primer (Moosavian *et al*.,2021) .Polymerase chain reaction (PCR)was utilized to Confirm the existence of OqxB gene in *E. coli* and *K. pneumonia* using primers as the following: Forward primer:(5′TTCTCCCCCGGCGGGAAGTAC3′), reverse primer: (5′CTCGGCCATTTTGGCGCGTA3′) to give 512 bp product. In PCR amplification, the reagents and their are 25 total volume of reagents included : DNA:5µI, Reverse primer:2 µI, Forward primer:2 µI, Go Taq Green Master Mix:12 µIand Nuclease- free water:4 µI. The program of PCR was described in table (4).

Table(4) :Program used in PCR amplification

Steps	Temperature °C	Time	No. of cycles
Initial denaturation	94	5 min	1
Denaturation	94	45sec	32
Annealing	64	45sec	
Extension	72	1 min	
Final extension	72	5min	1

RESULT

Following a general urine examination and urine sample collection, biochemical testing revealed that among 100 pregnant women with UTIs and DM, 41 (41%) urine samples showed positive culture of bacteria . Gram negative bacteria accounted for 40 (98%) of all isolates. and Gram positive bacteria accounted for only 1(2%) isolate of all isolates.

3.1 Pre-identification of bacterial isolates

Based on the outcomes of the present investigation microscopically, biochemical tests and cultural,21 isolates (51 %) belonged to *E. coli* and 8 isolates (20%) belonged to *K. pneumonia*.

3.2 Antibiotic susceptibility testing

Antibiotic susceptibility tested monstrated that the majority of bacterial isolates were resistant to most antibiotics, especially ampicillin, ticarcillin, piperacllin, in particular. The high resistance rate for *E. coli* (n=18) were found towards ampicillin (100%), piperacllin (100%), ticarcillin (100%) and to trimethoprim/ sulfamethoxazole (100%) and moderate resistance rate were piperacillin/tazonactam (52%), ticarcillin/ clavulanic acid (61%), aztronam (56%). Antibiotic susceptibility testing revealed that the majority of the bacterial isolates were extremely resistant to most antibiotics, especially ampicillin, ticarcillin and piperacllin, in particular. The high resistance rate for K.pneumonia (n=8) were found to be against ampicillin (100%). piperacllin (100%), ticarcillin (100%) and to aztronam (63%) and moderate resistance rate were ticarcilin/ClavulanicAcid(50%), trimethoprim/sulfamethoxazole(50%).

3.3 Genetic profiling of bacterial isolates

3.3.1 Detection of *E.coli* using specific primer

The DNA Extracted from (n=21) *E.coli* isolateswas subjected to PCR for characterized in (585bp) by comparison with standard molecular DNA ladder (3000bp), as seen in figure (1).

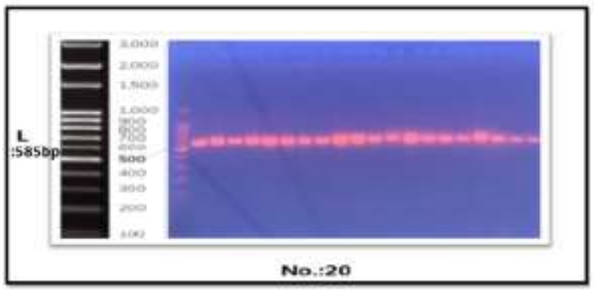
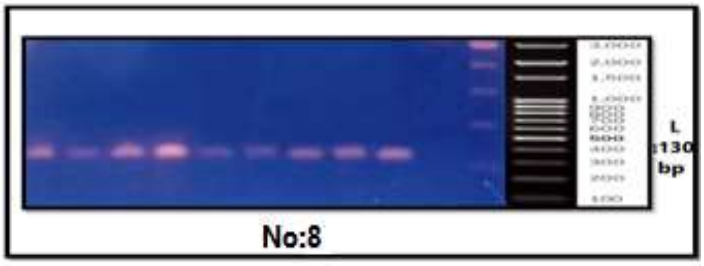


Figure (1): Detection of *E.coli* isolates using specific primers. Lane L:(3000 bp DNA ladder), Lane:(No.:20) specific primer band of *E. coli* isolates. using 1.5% agarose gel, 70V, 45minutes.

3.3.2 Detection of *K.pneumonia* using specific primer

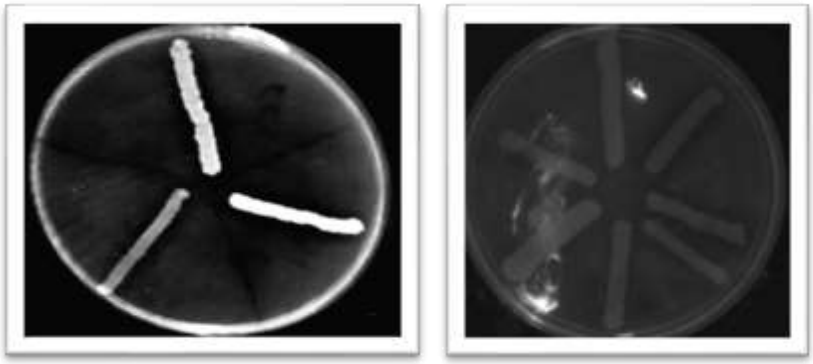
The DNA Extracted from (n=8)*K. pneumonia* isolates was subjected to PCR for characterized in (130bp) by comparison with standard molecular DNA ladder(3000bp),as seen in figure(2).



Figure(2): Detection of *K. pneumonia* using specific primers Lane L:(3000 bp DNA ladder), Lane:(no=8) specific primer band of *K. pneumonia* isolates. using 1.5% agarose gel, 70V, 45minutes.

3.4 Phenotypic identification of efflux pumps

The efflux pumps activity were detected in 18 *E.coli* isolates and 8 isolates of *K.pneumonia* using the ethidium bromide-agar cartwheel method (EtBr CW), relying on the ethidium bromide dye at different concentrations as a guide for phenotypic identification. The results showed the *E. coli* and *K. pneumonia* isolates were positive for phenotypic identification. As shown in figure (3) and table (5).



A- Positive efflux pumps **B-Negative efflux pumps**

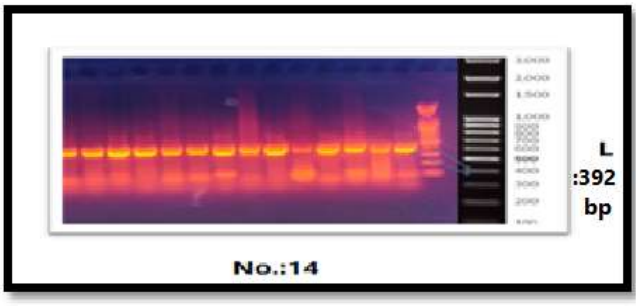
Figure (3): Phenotypic detection of efflux pumps in (n=18) of *Escherichia coli* isolates, (n=8) of *Klebsiella pneumonia* isolates using ethidium bromide- agar cartwheel method (EtBr CW), relying on the ethidium bromide dye, A-Positive efflux pumps, B- Negative efflux pumps.

Table (5):Number of phenotypic identification of efflux pumps in *E.coli* and *K.pneumonia*

Bacterial isolates	Number of isolates	phenotypic identification of efflux pumps	% Phenotypic identification of efflux pumps
E. coli	18	12	67
K. pneumonia	8	3	38

3.5 Detection of OqxA efflux pumps gene

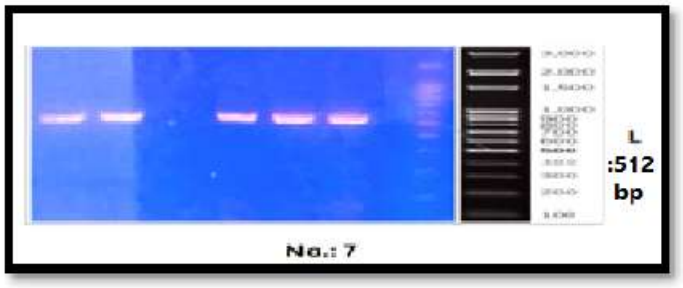
The DNA Extracted from (n=26) *E. coli and K .pneumonia* isolates were subjected to PCR for characterized of OqxA gene (392bp), as seen in figure (4).



Figure(4):Detection of OqxA efflux pump gene using Agarose electrophoresis patterns show PCR amplified products of the OqxA gene. Lane L: (3000bp DNA ladder), Lane :(No. : 14) OqxA gene bands of bacterial isolates using 0.8% agarose gel, 70 V, 60 minutes.

3.6 Detection of OqxB efflux pumps gene

The DNA Extracted from (n=26) *E. coli and K. pneumonia isolates* were subjected to PCR for characterized of OqxB gene (512bp) as seen in figure (5).



Figure(5):Detection of OqxB efflux pump gene using Agarose electrophoresis patterns show PCR amplified products of the OqxB gene. Lane L: (3000bp DNA ladder), Lane: (No.:7) OqxB gene bands of bacterial isolates using 0.8% agarose gel, 70 V, 60 minutes.

Table (6): Phenotypic and Molecular identification of efflux pumps

Test results	Molecular detection ofOqxA and OqxB efflux pumps in *E. coli*(%)	Molecular detection of OqxA and OqxBefflux pumps in *K.pneumonia*(%)
The result positive	13 (72)	4(50)
The result negative	5(28)	4(50)

DISCUSSION

E. coli and K.pneumonia revealed the most common bacteria that cause UTIs, with a percentage of isolation

reaching (51%) and (20%), respectively. E. coli was the most prevalence bacteria. this result in agreement with previous studies by (Al-Bidhani, 2018) in Iraq who observed the prevalence of E.coli in pregnant women with urinary tract infection and diabetes mellitus were 50%. Interestingly, the results in the current study are similar with the results carried out by (Khoshaba et al., 2020) in Iraq who found the prevalence of K. pneumonia in pregnant women with urinary tract infection and diabetes mellitus was 17.27%. The results in the current study are similar with the results carried out by (Naher et al., 2018) in Bangladesh who found the prevalence of E.coli, K. pneumonia in pregnant women with urinary tract infection and Diabetes mellitus was (57.9%), (21.1%), respectively. E.coli bacteria are the main cause of urinary tract infections (UTIs) infecting about 90% of patients suffering from UTIs in world (Levinson, 2016). The findings of this study are confirmed by numerous local and foreign ones that indicated that many urinary tract infections are caused by E.coli. The relation between diabetes in pregnant women and urinary tract infection might be due to the reduction of neutrophil response and drop of cytokine in the urine as well as the leukocyte concentration decline (Al-Bidhani, 2018). Cytokines secretion by the epithelial cells of the urinary tract which would have localized the infection is not happening during pregnancy, this would explain the rate of infection in non pregnancy is associated with diabetes compared to pregnancy (Hoepelman et al., 2003).The differences in the infection rate could be attributed to the variation in geographic and healthy conditions of the study design and the number of samples (Shuwaikh and Jassim, 2016). The abundance of E.coli bacteria could be explained by the fact that the bacteria is capable of adjusting to live in the urinary tract environment as well as tolerating unfavorable environmental conditions, not to mention that it possesses many virulence factors which are very powerful increasing its ability to cause an infection. The most significant factor of having such a high percentage of these bacteria in UTI is that they have a natural environment outlet and urinary outlet (Raeispour and Ranjbar 2018). The primer 16S rDNA is the one used in the E. coli detection. The gene is stable but varies a little with the bacterium's growth rate. The PCR technology was applied to identify the E. coli genes in all isolates based on a stable gene. A 100% rate in the uniqueness among the isolates was realized since the diagnostic E. coli bacteria gene sequences were uniform in all the isolates (1). Contrary to the results of (Lai et al. ,2016), (Maleki et al. ,2017), and opposite to the findings of (Jenkins et al., 2012) and (Hussein and Naser, 2023), this method is a quick and accurate technique that can be used for the detection of bacteria and exhibits the high sensitivity characteristic for the The current polymorphism has fixed parts which are arranged in the variable region which forms a basis for the identification both of the genus and species. It is just the mediator or the average one between the diverse or unique ones. The genetic structure that existed before that time differs very slightly presently (Srinivasan et al. ,2015); (Hidayat et al. ,2020). The K. pneumoniae was verified via 16S rDNA, a gene considered to be a stable one among the bacteria and one that has some heterogeneity after a long period of time. The rDNA found in the bacteria is specifically different, and it is unique per microdifference. The result showed that diagnostic gene sequence of K. pneumonia bacteria was detected in all isolates at frequency of (100%). The outcomes documented here are similar to the outcomes reported by the other study (Osman et al.,2014) in Egypt and (Mozan and Al-Amara, 2023) in Iraq. Genotyping is key to identify cases or outbreaks resulting from K. pneumoniae and to track sources and the incidence (Cheng et al., 2018). Phenotypic techniques are less dependable compared to genotypic ones because phenotypes may differ depending on the specific environmental conditions, like temperature, pH, and growth parameters. Therefore, using specific primer 16S rDNA for diagnosis is preferable to using biochemical and phenotypic methods, since it has several benefits: the gene is universal among bacteria and does not mutate frequently (Clarridge, 2004). Tatusova et al. (2014. In the current study, E.coli inpregnant women with UTI and DM were resistance to the following antibiotics: ampicillin, piperacillin, ticarcillin, trimethoprime/sulfamethoxazole (100%) and aztronam (56%), as seen in table (15). The results in the current study are consistent with the results reached by (Al-Hamdani and Abas, 2013) in Iraq who found that (100%), (62.5%) of E.coli isolates were resistance to ampicillin, piperacillin, respectively. The results in the current research are consistent with the results reached by (Mekapogu et al.,2016) in India and (Ashur et al.,2020) in Libya and (Mohapatra et al., 2022) in India found that (76%),(67%),(60%) of E. coli isolates were resistance to ampicillin, piperacillin, ticarcillin, respectively. The results in the current research are consistent with the results reached by (Ali et al., 2022) in Ethiopia and (Khalaf et al., 2021) in Iraqfound that (60%), (47.2%) of E.coli isolates were resistance to trimethoprim/sulfamethoxazole and aztronam, respectively. On the other hands, K. pneumonia were resistance to the following antibiotics: in the previous study amplicilation, revision of piperacillin and to/oicrillin (100% as indicated in table 16) as well as aztronam (63% as shown in table 16) were widely (159). This Southeast Asian research project once again comes back with the same results as the study of (Khoshaba et al., 2020) in Iraqi where the 100% of Knpneumonia isolates were found to be resistant to ampicillin, piperacillin, and ticarcillin. The results in the current research are consistent with the results reached by (Sarogamma and Ramakrishna, 2011) in found that(70%) of K. pneumonia isolates were resistance to aztronam. The reasons of antibiotic resistance by bacteria in general and especially in Enterobacteriaceae family from Gram-negative K. pneumonia, E .coli were either because of the production of enzymes that break down the beta-lactam ring present in the antibiotic formulation of the group of antibiotics beta-lactams (Al-Charrakh, 2011) or their possession of efflux pumps , in addition to their formation of mucous biofilm substance that

surrounds groups or colonies of bacterial cells to provide them with a kind of protections, Which largely prevents the antibiotics dose from penetrating and reaching the bacterial cell due to it being a sticky substance, or a change in the permeability of the bacterial cell membrane, as the membrane contains protein channels called porins obstruct or prevent the passage of the antigen molecule into the bacterial cell and its arrival to its site of action (Perron et al., 2012). In the following ways, Bacteria frequently resist certain antibiotics:alter thecell wall target protein, decrease the outer membrane's permeability and increases the expression of drug efflux pump (Holmes et al., 2012).. The results of molecular identification of the OqxA gene, showed that (72%) of E.coli in pregnant women with UTI and DM possessed the OqxA gene ,as shown in figures (5). The results of this research are consistent with the results achieved by (Olukemi et al., 2023) in Nigeria (88.9%), While the results of this researchdid not agree with the results reached (Gabret al., 2021) in Egypt, where (46.6%) of E coli isolates that possess this gene. On the other hands, our results revealed that (50%) of K.pneumonia possessed the OqxA gene. as shown in figures (5) The results of this study are consistent with the results reached by (Razavi et al.,2020) in Iran who showed that (47.27%) of K. pneumonia carried OqxA gene. Although the findings of this study were in contradiction with the results of (Rodriguez-Martinez et al., 2013) in Spain, who reported that (K.pneumonia isolates that possess this gene reached 76%), but they also showed that (zinc finger was not linked with the decreased MIC of colistin at the high level). The results of molecular identification of the OqxB gene, showed that (72%) of E.coli in Pregnant women with UTI and DM possessed the OqxB gene, as shown in figures (6).The results of this research are consistent with the results achieved by (Hansen et al., 2004;) in Denmark (77%). While the results of this research did not agree with the results reached by the researcher (Gabr et al., 2021) in Egypt (46.6%) of E. coli isolates carried OqxB gene. Furthermore, (50%) of K. pneumonia in Pregnant women with UTI and DM of current study possessed OqxB gene, as shown in figures (6). The outcome of this research is in line with the results of the researcher (Razavi et al.,2020) with the depression rate of 47.27% in Iran. Although the findings of this study differs with the results of the research (Rodriguez-Martinez et al., 2013) conducted in Spain whereby 75% of the bacteria isolates have this gene, the presence of antimicrobial peptides in the human body confers a defense mechanism against microbial infections. The K. pneumonia was confirmed to have the OqxA gene as well as the OqxB gene both together and in the ratio of 50%. This conforms to the results of a research (Razaviet al.,2020), conducted in Iran which has shown that the proportion of bacterial isolates which harbors both of these genes jointly is 47.27%.

REFERENCES

- Onyango, H. A., Ngugi, C., Maina, J. and Kiiru, J. (2018). Urinary Tract Infection among Pregnant Women at Pumwani Maternity Hospital, Nairobi, Kenya: Bacterial Etiologic Agents, Antimicrobial Susceptibility Profiles and Associated Risk Factors. Advances in Microbiology, 8(3), 175-187.
- Al-Bidhani, F. H. J. (2018). Isolation and Diagnosis of Bacteria Causing Urinary Tract Infection in Pregnant and Non pregnant Females with Diabetes Mellitus Type 2. Al-Mustansiriyah Journal of Science, 29(4), 291.
- Hoepelman, A. I. M., Meiland , R. and Geerlings, S. E.(2003). Pathogenesis and management of bacterial urinary tract infections in adult patients with diabetes mellitus. International Journal of Antimicrobial Agents, 22(2), 35- 43.
- Onoh, R., Umeora, O., Egwuatu, V., Ezeonu, P. and Onoh, T. (2013). Antibiotic Sensitivity Pattern of Uropathogens from Pregnant women with Urinary tract infection in Abakaliki, Nigeria. Infection and Drug Resistance, 6, 225-233.
- Emiru, T., Beyene, G., Tsegaye, W. and Melaku, S. (2013). Associated Risk Factors of Urinary Tract Infection among Pregnant women at FelegeHiwot Referral Hospital, Bahir Dar, North West Ethiopia. BMC Research Notes, 6, 292.
- Naher, N., Begum, F. and Hashem, N. (2018). Antibiotic Sensitivity in UTI among Diabetic Pregnant Women. Bangladesh Journal of Obstetrics and Gynaecology, 33(1), 54-58.
- Mahdi, B. M., Khudhur, H. B. and Abdul-Hussein, M. M.(2020). Bacterial Isolates of Urine and their Susceptibility to Antimicrobials. Macedonian Journal of Medical Sciences, 8(A) 84-88.
- Albadery, A. A., Al-Amara, S. M. and Al-Abdullah, A. A. (2023). Phenotyping and Genotyping Evaluation of E. coli Produces Carbapenemase Isolated from Cancer Patients in Al-Basrah, Iraq. Razi Vaccine & Serum Research Institute, 78(3), 849-855.
- Bengsston-Palme, J., Kristiansson, E. and Larsson, D. G. J. (2018). Environmental factors influencing the development and spread of antibiotic resistance. Federation of European Microbiological Societies microbiology reviews, 42(1), 68–80.

- Praveenkumarreddy, Y., Akiba, M., Guruge, K. S., Balakrishna, K., Vandana, K. E. and Kumar, V. (2020). Occurrence of antimicrobial-resistant Escherichia coli in sewage treatment plants of South India. Journal of Water Sanitation and Hygiene for Development, 10(1) ,48–55.
- Ali, S. A. and Al-Dahmoshi, H. O. M. (2022). Detection of Efflux Pumps Gene and Relation with Antibiotics Resistance in Uropathogenic Escherichia Coli (UPEC) Isolated from Patients with Cystitis. Iraqi Journal of Science, 63(6), 2388-2397.
- Moosavian, M., Sima, M. K., Khosravi, N. A. and Montazeri, E. A. (2021). Detection of OqxAB Efflux Pumps, a Multidrug -Resistant Agent in Bacterial Infection in Patients Referring to Teaching Hospitals in Ahvaz, Southwest of Iran. International Journal of Microbiology, 2021, 2145176.
- Webber, M. A. and Piddock, L. J. V. (2003). The importance of efflux pumps in bacterial antibiotic resistance. The journal of Antimicrobial Chemotherapy, 51(1), 9-11.
- Piddock, L. J. V. (2006). Multidrug-resistance efflux pumps—not just for resistance. Nature Reviews Microbiology, 4 (8),629-636.
- Piekarska, K., Wolkowicz, T., Zacharczuk, K., Rzeczkowska, M., Chrost, A., Bareja, E., Olak, M. and Gierczynsky, R. (2015). Co-existence of plasmid-mediated quinolone resistance determinants and mutations in gyrA and parC among fluoroquinolone-resistant clinical Enterobacteriaceae isolated in a tertiary hospital in Warsaw, Poland. International Journal of Antimicrobial Agents,45(3),238–243.
- Blair, J. M. A., Richmond, G. E. and Piddock, L. J. V. (2014). Multidrug efflux pumps in Gram-Negative Bacteria and Their Role in Antibiotic Resistance. Future Microbiology, 9(10), 1165–1177.
- Tonu, N. S., Sufian, M. A., Sarke, S., Kama, M. M., Rahman, M. H. and Hossain, M. M. (2011). Pathological Study On Colibacillosis In Chickens and Detection of Escherichia Coli By PCR. Bangladesh Society for Veterinary Medicine, 9(1), 17-25.
- Gamal, F. E., Gadallah, F., Zaghloul, M., Salama, E., Salama, S. and Khedr, A. (2017). Differentiation between vaccinal and field strains of E. coli using phenotype and genotype characterization. Journal of Immunology and Clinical Microbiology, 2 (3), 48-53.
- Osman, K. M., Hassan, H. M., Orabi, A. and Abdelhafez, A. S. T. (2014). Phenotypic, antimicrobial susceptibility profile and virulence factors of Klebsiella pneumonia isolated from buffalo and cow mastitic milk. Pathogens and Global Health, 108(4), 191-199.
- Khalaf, S. A., Mahmood, N. N. and Saleh, M. A. A. (2021). Detection of tetM, armA, blaPER-I and blaIMP genes in *E. coli* isolates among the gram negative bacteria that cause urinary tract infections. Journal of Physics Conference Series, 1999(1),012021.
- Khoshaba, J. Z., Sulaiman, S. M., Mardan, S. A. and Alazzawy, M. A. (2020). Relation of UTI with Type 2 Diabetes and Pregnancy in Women Attended Obstetrics, Gynecology and Pediatric Hospital in Kirkuk City. Medico Legal Update, 20(4),1599-1603.
- Levinson, W. (2016). Review of Medical Microbiology and Immunology. 14th Edition. McGraw-Hill education, Inc. PP 821.
- Shuwaikh, R. S. and Jassim, F. A. H. (2016). Types of bacteria that cause urinary tract infections and the extent of their resistance to antibiotics in some Baghdad Hospitals. Al-Qadisiyah Journal of Pure Sciences, 40 , 34-41.
- Raeispour, M. and Ranjbar, R. (2018). Antibiotic Resistance, Virulence Factors and Genotyping of Uropathogenic Escherichia coli Strains. Antimicrobial Resistance and Infection Control, 7(118), 1-9.
- Lai, Y. M., Zaw, M. T., Aung, T. S. and Lin, Z. (2016). Detection of Sequence Type 131 in Multi-Drug Resistant Uropathogenic*Escherichia coli* Isolates from Two Hospitals of Sabah. Asian journal of pharmaceutics, 10 (3), 421-424.
- Maleki, D., Jahromy, S. H., Karizi, S. Z. and Eslami, P.(2017). The Prevalence of acrA and acrB Genes Among Multiple-Drug Resistant Uropathogenic Escherichia coli Isolated from Patients with UTI in Milad Hospital, Tehran. Avicenna Journal of Clinical Microbiology and Infection, 4(1), 39785.
- Jenkins, C., Ling, C. L., Ciesielczuk, H. L., Lockwood, J., Hopkins, J. S., McHugh, T. D., Gillespie, S. H.andKibbler, C. C. (2012). Detection and \identification of bacteria in clinical samples by 16 srRNA gene sequencing: omparison of two different approaches in clinical practice. Journal of Medical Microbiology, 6(4)1, 483–488.

- Hussein, Z. M. and Naser, L. A. (2023). Phenotypic and Molecular Detection of Escherichia coli in Patients with Diabetic Foot Infection in Basrah, Iraq. CEENTRAL ASIAN JOURNAAL OF MEDICAL AND NATURAL SCIENCES ,4(3), 2660-4159.
- Srinivasan, R., Karaoz, U., Volegova, M., MacKichan, J., KatoMaeda, M., Miller, S.,Nadarajan , R., Brodie , E. L. and Lynch, S. V. (2015). Use of 16SrRNA Gene for Identification of a Broad Range of Clinically Relevant Bacterial Pathogens. Public Library of Science One, 10(2), e0117617
- Hidayat, T., Hadibarata, T. and AL-Farraj, D. A. (2020). Phylogenetic Analysis of Microbial Community in Selected Rivers in Johor Based on DNA Sequences. Journal of Engineering Sciences and Technology, 15(4), 2464-2471.
- Mozan, I. M. and Al-Amara, S. S. M. (2023). Frequencies New Delhi Metallo-β-Lactamase (NDM) in Klebsiella pneumoniae Isolates from Clinical Samples in Al-Basrah Governorate, Iraq. SCIENTIFIC JOURNAL OF MEDICAL RESEARCH , 7(27),15-18.
- Cheng, F., Li, Z., Lan, S., Liu,W., Li, X., Zhou, Z., Song, Z., Wu, J., Zhang, M. and Shan, W. (2018). Characterization of Klebsiella Pneumonia associated with cattle infections in southwest China using multi-locus sequence typing (MLST), antibiotic resistance and virulence associated gene profile analysis. Brazilian Journal of Microbiology, 49(1), 93-100
- Clarridge, J. E. (2004). Impact of 16S rDNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clinical microbiology reviews, 17(4), 840-862.
- Tatusova, T., Ciufo, S., Fedorov, B., O'Neill, K. and Tolstoy, I.(2014). RefSeg microbial genomes databse: new representation and annotation strategy. Nucleic Acids Research, 42, D553-D559.
- Al-Hamdani, M. A. and Abas, I. J. Study of plasmid profile and susceptibility patterns of Escherichia coli isolated from patients with urinary tract infection in Basra. Journal Thi-Qar Science, 4 (1), 1991-8690.
- Mekapogu, N. P., Gundela, S. and Avula, R. D. (2016). Diabetes Mellitus has no Significant Influence on the Prevalence of Antenatal Asymptomatic Bacteriuria. Journal of Clinical and Diagnostic Research, 10(4), DC16-DC20.
- Ashur, A. B., El magrahi, H., El kammoshir, A., Shomakhi, Y. and Alaawaj, W. (2020). A Study of Asymptomatic Bacteriuria among Pregnant Women with and Without Gestational Diabetes Mellitus in Al-Jalla Gynecology Hospital, Tripoli, Libya. *AlQalam Journal of Medical and Applied Sciences*, 4(1), 48-53.
- Mohapatra, S., Venugopa, S., J., Kalaivani, M., Kant, S., Tak, V., Panigrahy, R., Chunchanur, S., K., Kocher, D., Behera, B., Pundir, S. Chaudhuri, S., Gautam, H., Sood, S., Das, B. K., Kapil, A., Kumar, A., Kumari, R., Ambica, R., Hari, P., Malhotra, S. and Salve, H. R., (2022).Antibiotic resistance of uropathogens among the community-dwelling pregnant and nonpregnant female: a step towards antibiotic stewardship. BMC Infectious Diseases, 22, 939.
- Ali, A. H., Reda, D. Y. and Ormago, M. D. (2022). Prevalence and antimicrobial susceptibility pattern of urinary tract infection among pregnant women attending Hargeisa Group Hospital, Hargeisa, Somaliland. Scientific Reports, 12, 1419.
- Khoshaba, J. Z., Sulaiman, S. M., Mardan, S. A. and Alazzawy, M. A. (2020). Relation of UTI with Type 2 Diabetes and Pregnancy in Women Attended Obstetrics, Gynecology and Pediatric Hospital in Kirkuk City. Medico Legal Update, 20(4),1599-1603.
- Sarojamma, V. and Ramakrishna, V. (2011). Prevalence of ESBL- producing Klebsiella pneumonia isolate in Tertiary Cary hospital. International Scholarly Research Notices, 2011, 318348.
- AL-Charrakh , A. H., Yousif, S. Y. and AL-Janabi, H. S. (2011). Antimicrobial Spectrum of the action of bactriocines from Klebsiella isolation from Hilla/Iraq. Journal of Clinical Microbialogy, 2 (5), 1-11.
- Perron, G. G., Lee, A. E. G., Wang, Y. , Huang, W. E. and Barraclough, T. G. (2012) . Bacterial recombination promotes the evolution of multi- drug-resistance in functionally diverse populations. Proceeding Biological Sciences (England), 279(1735), 1477-1484.

- Holmes, B., Richards, D. M., Brogden, R. N. and Heel, R. C. (2012). A Review of its Antibacterial Activity, Pharmacokinetic Properties and Therapeutic Use. Piperacillin Drugs, 28 (5), 375–425.
- Olukemi, A. T., Olutoyosi, O., Stephen, N., Joshua, Oj. and Emmanuel, M. R. (2023). Assessment of OqxA and OqxB Resistance Genes in Escherichia coli and Klebsiella pnuemoniae Clinical Isolates Based on Polymerase Chain Reaction from Niger Delta University Teaching Hospital, Yenagoa, Bayelsa State. East African Scholars Journal of Medical Sciences, 6 (4), 141-146.
- Gabr, B. M., Zamzam , A. S. A., Eisa, E. A., El-Baradey, G. F. and Shams Eldeen, M. A.(2021). Detection of oqxA and oqxB efflux pump genes among nosocomial coliform bacilli: An observational cross-sectional study. Journal of Acute Disease, 10(3), 117-121.
- Razavi, S., Mirnejad, R. and Babapour, E. (2020). Involvement of AcrAB and OqxAB Efflux Pumps in Antimicrobial Resistance of Clinical Isolates of Klebsiella pneumonia. Journal of Applied Biotechnology Reports, 7(4), 251-257.
- Rodríguez-Martínez, J. M. , Díaz de Alba, P., Briales, A., Machuca, J., Lossa, M., Fernández-Cuenca,F., Rodríguez Baño,J., Martínez-Martínez, L. and Pascual, A. (2013). "Contribution of oqxAB efflux pumps to quinolone resistance in extended-spectrum-*β*-lactamase-producing *Klebsiella pneumoniae*." Journal of Antimicrobial Chemotherapy, 68 (1), 68–73.
- Hansen, L. H., Johannesen, E., Burmølle, M., Sørensen, A. H. and Sørensen, S. J.(2004). Plasmid-Encoded Multidrug Efflux Pump Conferring Resistance to Olaquindox in Escherichia Coli. Antimicrobial Agents and Chemotherapy, 48(9), 3332–3337.