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Original Research Paper

Evaluation the influence of Iron (III) chloride on Multidrug Resistance *Escherichia coli* Isolated from Cystitis Patients

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Abstract: Iron have a crucial role in the activity of TCA cycle enzymes, the electron transport chain, and oxygen metabolism within the body. The pathogens such as bacteria have a challenge in acquiring iron as it is typically bound to heme or by proteins like transferrin or lactoferrin in the body. uropathogenic E. coli uses various strategies to capture iron, siderophores is one of these strategies. Urinary tract infection (UTI) is predominantly caused by *Escherichia coli*, which has antimicrobial resistance in Al-Najaf city. Antibiotics resistance is one of the most common and severe global public health threats, antibiotics overuse and misuse can result in antibiotic resistance. Therefore, in this present work tries to investigate the effect of iron (FeCl3) on growth of antibiotic resistant *Escherichia coli* collected from different ages of outpatient. total of (300) urine sample were collected from patients clinically complaining of signs and symptoms of UTIs, from the hospitals of the Najaf Center (Al-Sader Teaching Hospital, Al-Hakeem General Hospital, Al Zahraa' Teaching hospital, AL-Furat Al-Awsat hospital). Antibiotics Susceptibility Test of identified *Escherichia coli* were performed using the methods of Kirby-Bauer on Mueller Hinton agar according to CLSI ,2021. FeCl3 preparation performed using serial dilution method to prepare solution known concentration. A total of 45 isolates of Escherichia coli are recovered from (300) patients suffering from urinary tract infections. The data showed in the presence of condensed concentration FeCl3 resulted in a clear inhibited in the rate of Escherichia coli growth compared to other diluted concentration of iron, Iron at concentrations of 10^{-3}, 10^{-4}, and 10^{-5} showed a more prominent effect, while cultures supplemented with iron at concentrations of 10^{-1} and 10^{-2} showed low growth. The Results showed that iron has toxic and inhibitory effects on growth rate of *Escherichia coli* at high concentration and but it had Positive effect for stimulation E.coli growth in the presence other iron concentration (10^{-1}, $10^{-2,}$ 10^{-3}, 10^{-4}, 10^{-5}). When it comes to antibiotics, all isolates were resistance to Ampicillin and Ampiclox, while the all the isolates were sensitive to meropenem. A set of 45 urine sample of *Escherichia coli* isolated from outpatient facilities in Al- Najaf center, The study uncovered that FeCl3 has a significant impact on the growth of E. coli. It was found to have an inhibitory effect at high concentrations, while it had a growth-stimulating effect at low concentrations. The study found that there is a high prevalence of Escherichia coli resistant to amipiclox and ampicillin. It also noted that the prevalence of resistance varies depending on the geographic location.

Keywords: *Escherichia coli*, urinary tract infection, $FeCl_3$, antibiotic resistance

1. Introduction

Iron is an essential element for the proper functioning of proteins and co-factors involved in various cellular processes. It plays a crucial role in the activity of TCA cycle enzymes, the electron transport chain, and oxygen metabolism within the body [1]. it is a critical element in the structure of microbial protein cofactors, where it serves important functions such as catalyzing reactions, facilitating redox processes, transferring electrons, and regulating various cellular activities [2]. The growth and development of bacteria require iron at a concentration of 10-6, However, higher concentrations of iron can be harmful to the bacteria, exhibiting bactericidal or antimicrobial effects on the microorganisms[3]. The infections of urinary tract, by uropathogenic E. coli (UPEC) that employ various strategies, one of which is the synthesis of high affinity and low molecular weight proteins called siderophores, these siderophores play a crucial role in the bacteria's ability to capture iron, which is essential for their survival and proliferation in the urinary tract [4]. the pathogens such as bacteria have a challenge in acquiring iron as it is typically bound to heme or by proteins like transferrin or lactoferrin in the body, this limitation can hinder their ability to thrive and cause infection[5] In aqueous solutions and in free oxygen, iron appear in the ferric oxidation state. the solubility of This form of iron is poorly and requires binding to proteins or hydrophilic chelators in order to be biologically available[6]. Iron is crucial for the functioning of living cells, and excessive boundless iron can be hurtful due to its ability to generate reactive oxygen species (ROS). These ROS led to damage the cellular components, leading to inflammatory processes associated with various health conditions[7]. Urinary tract infections (UTIs) are a common and significant concern that can impact individuals of any gender, with a higher prevalence among women[8]. UTIs occur when bacteria or other infectious agents penetrate the urinary tract and proliferate, leading to symptoms like frequent and painful urination. These infections can impact various parts of the urinary tract, including the kidneys, bladder, urethra, and prostate [9]. Cystitis is an inflammatory condition of the urinary bladder that can be caused by various factors such as infection, physical trauma, radiation, and chemotherapeutic agents[10]. UTIs can be occur when there is a breach in the host's defense mechanisms, allowing microbes to adhere, multiply, and persist within the urinary tract[11]. UTIs are further classified according to the presence of predisposing conditions for infection, (uncomplicated)(are simple urinary tract infections that can be managed with outpatient antibiotics and have good outcomes[12]. They do not have any risk factors that complicate the infection and typically resolve with first-line antibiotics) or complicated (complicated UTI as an infection that carries a higher risk of treatment failure[13]. These infections typically require longer courses of treatment, different antibiotics, and require additional workup to identify underlying anatomical abnormalities, immune status, and potential sources of infection[14]. all UTIs in immunocompromised patients, males, patients, and those associated with fevers, stones, sepsis, urinary obstruction, catheters, or pregnant involving the kidneys are considered complicated infections)[15]. Urinary tract infections represent a major public health challenge, given that they may stem from a range of pathogens, not limited to Gram-negative and Gram-positive bacteria, but also including certain fungi, parasites, and viruses. Seeking medical advice is crucial if UTI symptoms are present, as timely treatment is vital to avoid potential complications. [16]. Bacterial infections are the leading cause of urinary tract infections (UTIs), typically stemming from Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis, and Staphylococcus saprophyticus. These bacteria can result in discomfort and health complications, underscoring the significance of recognizing symptoms and seeking appropriate medical care. [17]. Escherichia coli, is a bacterial organism that belongs to the family Enterobacteriaceae. This organism is a significant cause of both hospital-acquired and community-acquired infections in humans. It can be isolated from a variety of clinical specimens, with urine and blood being the most frequently observed sources[18]. it is the most common causative bacteria associated with urinary tract infections up to 80%.[19]. [20]. Uropathogenic Escherichia coli (UPEC) is a highly virulent strain of Escherichia coli that falls under the category of extraintestinal pathogenic Escherichia coli (ExPEC). It is known for causing both uncomplicated and complicated urinary tract infections (UTIs), impacting the urinary system outside the intestinal tract [21]. Escherichia coli 's pathogenicity in the urinary tract involves several key factors. These include the bacterium's ability to adhere to uroepithelial cells, invade host tissues, and produce a variety of virulence factors, These mechanisms contribute to the bacterium's ability to cause infection and disease in the urinary tract [22]. Antibiotic resistance is a prevalent and serious global public health threat. It is estimated that approximately 700,000 people die each year as a result of antibiotic-resistant infections, and this number could escalate to ten million per year by 2050. This highlights the urgent need for coordinated efforts to address this growing problem and develop alternative solutions to combat antibiotic resistance [23]. Antibiotic resistance is a serious issue that can arise from the overuse and misuse of antibiotics. Even when used properly, antibiotics can lead to

increased rates of resistance in bacteria. Misuse of antibiotics, such as using broad-spectrum antibiotics when they are not necessary or not administering them correctly, can further contribute to this problem [24]. Taking antibiotics for the wrong reasons, such as for colds or other viral infections, can be ineffective and lead to dangerous side effects that are unnecessary. Additionally, it contributes to the development of bacteria that are more difficult to destroy over time, which can impact not only the individual taking the antibiotics but also their family and society as a whole. The lack of public control over antibiotic use, combined with the emergence of drug-resistant infections [25].. Hence the aim of this study to determine the antibiotic susceptibility pattern and effect FeCl3 on growth rate of Escherichia coli isolated from cystitis patient collected from in and around the Al-Najaf city of Iraq.

2. Methodology

Samples collection

During the period from September to December 2023. A total of (300) urine sample were collected from patients clinically complaining of signs and symptoms of UTIs. The age range was 1-69 years. 194 (65%) were females and 106 (35%) were males, suffering from urinary tract infections the isolation done by using the bacteriological culture technique. The samples were collected from the hospitals of the Najaf Center (Al-Sader Teaching Hospital, Al-Hakeem General Hospital, Al Zahraa' Teaching hospital, and AL-Furat Al-Awsat hospital).

Bacterial isolation

Transport the urine sample to laboratory which Isolation and identification of *Escherichia coli* were achieved by inoculated on MacConkey broth and after show bacterial growth then culture on MacConkey agar and eosin methylene blue agar. the colonies grown on cultural media were observed and described based on their physical characteristics the form, size, margin, color, and odor of the colonies can provide valuable information about the type bacteria present.

Identification of bacteria

Motility and standard biochemical tests IMVIC (indole test, methyl red test, Vogus Proskauer Test, Citrate Utilization Test), finaly identify by VitecK2 system.

Antibiotics Susceptibility Test

Identified *Escherichia coli* was performed using the methods of Kirby-Bauer on Mueller Hinton agar according to CLSI,2021. Using a sterile wire loop, four to 5 isolated colonies of *Escherichia coli* were transported to 5 ml of sterile normal saline in test tube and a cell density adjusted to the turbidity of McFarland, after prepare the standardized suspension of diagnosed bacterial, a sterile cotton swab was dipped. Rotating the swab hard against the inside of the tube above fluid level helped to drain the extra fluid, then the swab Inoculate the dried surface of a MH agar plate by streaking the swab three times over the entire agar surface; rotate the plate culture approximately 60 degrees at each time to ensure an even distribution of the inoculum on agar surface. Leaving the lid slightly ajar, allow the cultured plate to sit at room temperature for 3 to 5 minutes, this permits the surface of the agar plate to dry before proceeding to the next step. The chosen antimicrobial-impregnated disks, put on the surface of the inoculated medium by using sterile forceps to dispense each antimicrobial disk one at a time, Then the inoculation plates, replace the lid, invert the plates, and place them in a 37°C air incubator for 18 to 24 hours. Following incubation, the inhibition zone sizes were measured to the nearest millimeter using a ruler or caliper. Control of *Escherichia coli* isolated from 2-month infant stool used as control bacteria, knowing that the infant does not take any antibiotic.

Antibiotics discs

The following antibiotic disc were used: Levofloxacin (LEV, 5 gm), Norfloxacin (NOR, 10 gm), Imipenem (IMP, 10 gm), Meropenem (MEM, 10 gm), Aztreonam (ATM, 30), Nalidixic acid (NA, 30 gm), Gentamicin High level (HLG, 120 gm), Amikacin (AK, 10 gm), Netilmicin (NET, 30 gm), Trimethoprim / sulfamethoxazole (SXT, 25 gm), Erythromycin (E, 30 gm), Ceftazidime (CAZ, 30 gm), Cefotaxime(CTX, 30, 50 gm), cefepime (FEP, 30), Ceftriaxone (CTR, 30 gm), Nitrofurantoin (F,300 gm), Ampicillin(AM,25 gm), Piperacillin (PRL, 75 gm), Ampiclox (APX, 30 gm).

Cultivation of bacterial isolates in media supplemented with FeCl3

All bacterial isolates were tested to determine the effect of ferric chloride on the growth rate of the bacteria.

Preparation of FeCl3 stock solutions and culture medium

The EMB medium was prepared in accordance with the manufacturer's guidelines and then sterilized by autoclaving at 121°C for 15 minutes. The effect of varying concentrations of iron (III) chloride on the growth of *E.coli* has been studied by the preparation serial dilution from (Fecl3 98 %) stock solution Ben-David and Davidson [26]. To prepare the diluted solution, extract 1 mL from the stock solution and transfer it to tube 1, which contains 9 mL of distal water. This will result in solution1. Replicate this procedure by

transferring 1 mL from solution1 to tube 2. Repeat the aliquoting and resuspension steps until the final tube is reached, effectively reducing the stock concentration by a factor of 10 at each stage as show in the table 1.

Table 1: iron concentration used in this study

Iron	**concentration**					
$FeCl_3$	condensed	con.1 (10^{-1})	con.2 (10^{-2})	con. 3 (10^{-3})	con. 4 (10^{-4})	con. 5 (10^{-5})

The inhibitory or growth-stimulating of iron concentration was determined by spreading method [27]. the culture medium (EMB) was prepared and then poured on agar plate, 1 ml of different iron concentrations (concentrated, 10^{-1}, 10^{-2} ,10^{-3} ,10^{-4} ,10^{-5}) was added to each plate directly, gently mixed, rotated and allowed to solidification at room temperature. The turbidity of the bacterial suspension was adjusted to 0.5 McFarland standard turbidity, which is equivalent to 1.5 × 10^8 cells/ml. A one ml from each standardized bacterial suspension was added to 9 ml distal water and serial dilution were prepared. Finally, a working suspension containing 10^7 bacterial cells per ml was resulted. A one ml of last dilution of bacterial suspension added to EMB agar plate with different concentration of iron, by glass rod spreader the suspension has spread and incubated for 24 h at 37°C. A series of control agar plate containing media and bacterial suspension, but without iron addition step was included, and also incubated for 24 h at 37°C. The bacterial colony was counted by colony counter instrument based on spread count method.

Statistical analysis

The statistical analysis was carried out by using the computer software (graph pad prism version 6) and a mean value and standard error (SE) have been obtained for each value. Statistically significant P values less than 0.05 were considered for the statistical analysis.

3. Results and discussion

Out of a total of 300 urine samples, 45 samples (15%) were diagnosed with urinary tract infection, and the main cause was the *E.coli*. These samples were distributed according to the age and sex of the patients. The age groups most affected by urinary tract infections with *E. coli* were 30-39 year (31.2%), 40-49 year (24.4%), 20-29 year (20%), 50-59 year (8.9 %), 60-69 year (6.7 %), 10-19 year and 1-9 year (4.4%) respectively. Additionally, they were also distributed according to sex, with female being more affected by urinary tract infections (78 %) than male (22 %) as show in the table 2.

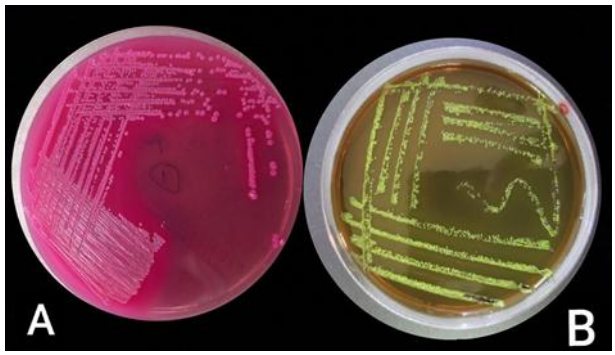

Fig 1. *Escherichia coli* diagnostics on agar media: (A) Growth on MacConkey agar: (B) Growth on EMB agar

Iron source have important role in bacterial infections as invading microbes often face iron-limiting conditions in the body fluids of hosts. During infections of urinary tract, Escherichia coli use various strategies to acquire iron from the surrounding environment, with the synthesis of siderophores being an important survival tactic. Siderophores are low molecular weight, high affinity iron-chelating agents that enable bacteria to thrive in iron-limited environments[28].

The study showed that 300 urine sample gave 87(29 %) no growth, 213 (71%) positive growth results for bacterial UTI, out of the 45 samples from urine, the main cause was the bacteria *E. coli*. As for the rest of the samples, the predominant bacteria were of a different type than *E.coli* as shown in the table 3.

Table 2: The distribution of patients depends on both age and sex factors.

Age	**No. of patients**	**sex**		**No. of patient**	**sex**			
		male	**female**		**percentage %**	**male**	**percentage %**	**female**	**percentage %**
1-9 years	21	6	15	2	4.4 %	0	0%	2	4%
10-19 years	33	11	22	2	4.4 %	0	0%	2	4%
20-29 years	50	17	33	9	20 %	1	2%	8	18 %
30-39 years	75	22	53	14	31.2 %	5	11 %	9	20 %
40-49	62	26	36	11	24.4	3	7%	8	18

years					%				%
50-59 years	36	14	22	4	8.9 %	0	0%	4	9%
60-69 years	23	10	13	3	6.7 %	1	2%	2	4%
Total	300	106	194	45	100 %	10	22 %	35	78 %
		300							

On EMB agar, they have distinct metallic green sheen colonies, and on MacConkey agar, they formed pink to red colored colonies. These bacteria can grow at 37°C, after staining by gram stain, the essential characteristics for diagnosing this bacterium have been identified, Its show straight, cylindrical, Gram-negative bacteria. The bacterial cells are rod-shaped with rounded ends and are 0.5 µm in diameter and 2.0 µm in length. They can occur singly or in pairs under microscope as shown in figure 1.

Table 3: The numbers and Percentage of urine sample isolated from patient with urinary tract infection.

Urine culture	No. of urine sample	Conc. %
Positive growth	213	71%
No growth	87	29%
Total	300	100%
Total number infected with *Escherichia coli*	45	15%

in this present work tries to investigate the effect of iron(FeCl3) on E. coli growth. In The presence of condensed concentration FeCl3 resulted in a clear inhibited in the rate of bacterial growth compared to other diluted concentration of iron. The Results showed that iron has toxic and inhibitory effects on growth rate of *Escherichia coli* at high concentration and but it had Positive effect for stimulation E.coli growth in the presence other iron concentration (10^{-1}, $10^{-2,}$ 10^{-3}, 10^{-4}, 10^{-5}). EMB cultures supplemented with iron at concentration (10^{-3}, 10^{-4}, 10^{-5}) showed a more prominent effect compared to cultures supplemented with iron at concentration (10^{-1}, 10^{-2}), and the differences were statistically significant. Our study agreement with Chatterjee that found the iron nano-particles appeared significant inhibitory effects on the growth of the bacteria strains that were exposed to them [29].
The data in the table 4 and figure 2 and figure 3 shows the resistance rates for various antibiotics in a sample of 45 cases. Ampicillin and Ampiclox had the highest resistance rate at 100% (45/45), followed by Nalidixic acid had a resistance rate of 53% (24/45), while ceftazidime, erythromycin, and Aztreonam had a resistance rate of 51% (23/45). Piperacillin followed closely at 49% (22/45), and cefepime at 37% (17/45). Netilmicin had a resistance rate of 31% (14/45), while trimethoprim/sulfamethoxazole had a rate of 24% (11/45). Ceftriaxone had a resistance rate of 20% (9/45), and both Cefotaxime (50) and Cefotaxime (30) had rates of 17% (8/45) and 13% (6/45) respectively. Amikacin had a resistance rate of 11% (5/45), while both Norfloxacin and Nitrofurantoin had rates of 6.6% (3/45). Levofloxacin and Imipenem had a resistance rate of 4.4% (2/45), Gentamicin at 2.2% (1/45), and meropenem showed no resistance in the samples.

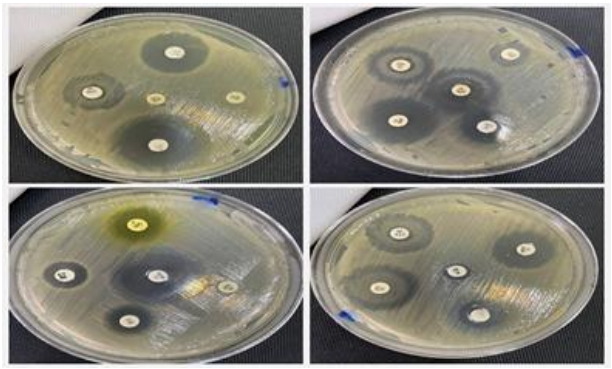

Fig 2. Antibiotics resistant test for *Escherichia coli* isolated from patients.

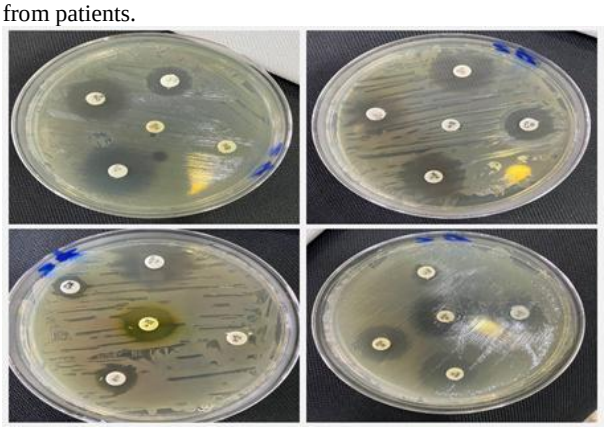

Fig 3. antibiotics resistant test for *Escherichia coli* isolated from infant (as control).

Incubation of selected strains of *E.coli* in EMB agar with five distinct concentrations of Iron(III) chloride , (condensed , 10^{-1}, 10^{-2} ,10^{-3} ,10^{-4} ,10^{-5}) as show in figure (4), Upon continuous monitoring of cellular density in both test and control groups, it was observed that *E.coli* grow better in presence iron in all five concentrations, When it was found that lower concentration led to increased bacterial growth. In this method, one milliliter of the final dilution (10^{-7}) of a bacterial suspension is

cultured on an EMB agar plate with a known concentration of iron (condensed, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}). In another study [30], it was found that higher concentrations of iron and certain other trace elements caused a notable decrease in the surface hydrophobicity of E. coli when interacting with uroepithelial cells. But It appears that the results are inconsistent with previous findings(Bruins) [31], iron has generally been considered non-cytotoxic when increased level of concentration. Matsuo showed Iron is crucial for S. cerevisiae in low concentration as it helps regulate oxidative stress within the cells. By maintaining the balance of reactive oxygen species, iron plays a vital role in protecting the cells from oxidative damage [32]. Minandri found presence of FeCl3 increase the growth rate and used as a growth stimulant for gene expression for P. aeruginosa in certain environmental conditions[33]

The results of the research showed similarity with the researcher Kalantari, who found that the toxic effect of high concentrations of iron (ferrous and ferric ion) on E.coli and *bacillus cereous* bacteria[34], and showed the grow of E. coli at presence of various concentrations of iron At presence of 0.1 mM/L and 0.5 mM/L concentration of Fe (II and III) bacteria grew approximately as well as control while the bacterial number decreased to 31.3 % when 1 mM/ L concentration of Fe (III) was used and no growth at presence of 1 mM/L concentration of iron. The results align with findings from other research, suggesting that Fe (III) is a damaging substance that affects an extra cytoplasmic target of gram-negative bacteria [35].

Based on this study and through this experiment, $FeCl_3$ has been proven that the best bacterial growth is achieved at a concentration of 10^{-3} ,10^{-4}, 10^{-5}, while the moderate growth at concentration 10^{-1}, however, the higher concentration of iron (condensed $FeCl_3$) was found to be inhibitory to the isolated bacteria's growth. this result compared with the control cultured with no iron supplements**.** Comparing the results from the statistical test using GraphPad Prism version 6 clearly indicate that there were significant differences in the growth rates of the bacteria due to the varying concentrations of $FeCl_3$ supplementation. It was observed that supplementing the media with $FeCl_3$ had a significant growth-promoting effect on the tested bacteria across all five concentrations, with a statistically significant difference ($P<0.0001$, $P<0.0011$) as shown in figure 6.

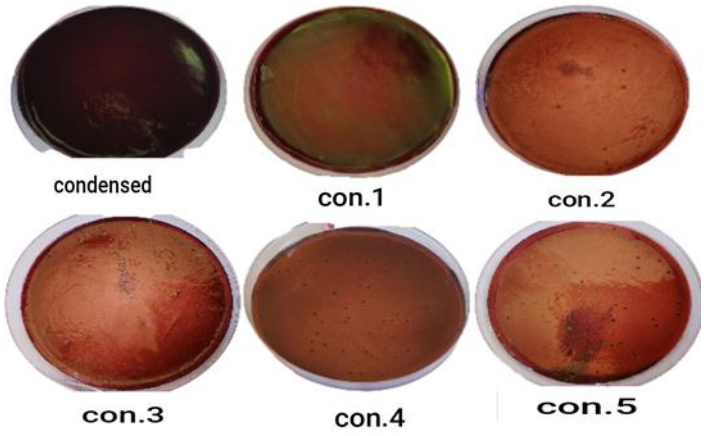

Fig 4. *E.coli* growth on EMB medium supplemented with different concentration of $FeCl_3$.

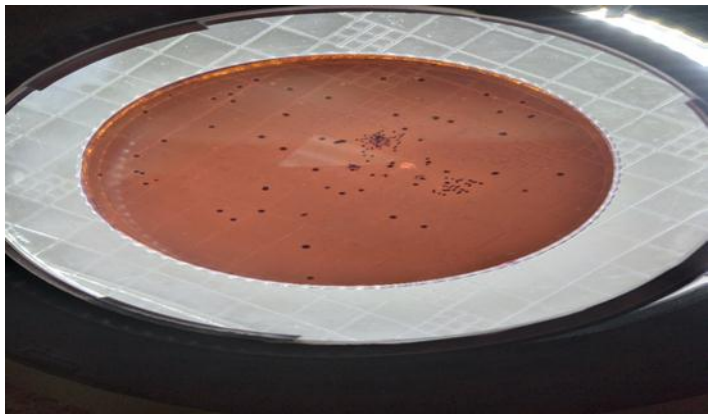

Figure 5: *E.coli* on EMB medium under light of colony counter instrument

Table 4: Antibiotic susceptibility test for 45 isolates from *Escherichia coli.*

Type of antibiotic class (subclass)	Antibiotic disk	Con. (µg/U)	No. of resistance isolates (%)	No. of Sensitive isolates (%)
Fluroquinolones	Levofloxacin	5	2 %	43 %
	Norfloxacin	10	3 %	42 %
β-lactams	Imipenem (Carbapenems)	10	2 %	43 %
	Meropenem (Carbapenems)	10	0 %	45 %
	Aztreonam (Monobactams)	30	23 %	42 5
quinolone	Nalidixic acid	30	24 %	21 %
Aminoglycosides	Gentamicin	120	1 %	44 %
	Amikacin	10	5 %	40 %
	Netilmicin	30	14 %	31 %
Sulfonamides (antifolate)	Trimethoprim / sulfamethoxazole	25	11 %	34 %
Macrolides	Erythromycin	30	23 %	22 %
cephalosporin	Ceftazidime	30	23 %	22 %
	Cefotaxime	50	8 %	37 %
	Cefotaxime	30	6 %	39 %
	cefepime	30	17 %	28 %
	Ceftriaxone	30	9 %	36 %
Nitrofuran antibiotics (Furans)	Nitrofurantoin	300	3 %	42 %
penicillin's	Ampicillin	25	43 %	2 %
	Piperacillin	75	22 %	23 %
	Ampiclox	30	45 %	0 %

Based on the input results of this study comparing the growth rates of bacteria cultured in media with different iron concentration. The bacteria cultured in media supplemented with (10^{-5}) FeCl3 showed a statistically significant increase in growth rates, compared to those cultured in media with 10^{-1} iron supplements (P<0.0001), it seems that a low concentration of FeCl3 has a positive impact on the growth of bacteria, while a high concentration inhibits or decreases the growth rate. This suggests that maintaining an optimal level of Fe is important for promoting bacterial growth.

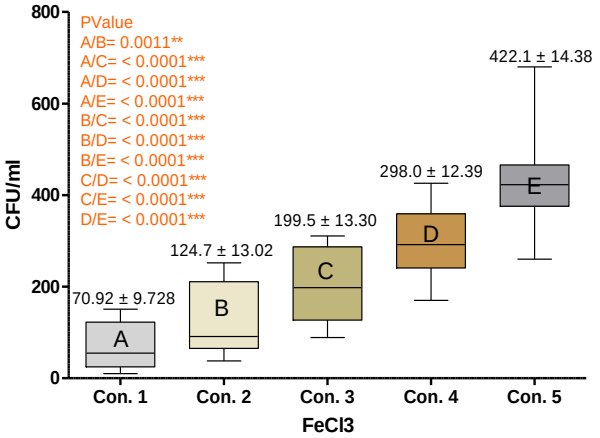

Fig 6. Growth rate of *E. coli* in the presence $FeCl_3$ in culture medium

Antibiotic resistance is a significant clinical issue in the treatment most of infections. One of the most important multidrug-resistant (MDR) opportunistic Gram-negative bacteria, *Escherichia coli* which causes a variety of disease with high mortality and morbidity through hospital-acquired infections and nonhospital-acquired infections. susceptibility patterns to antibiotics vary by geographic area, and *Escherichia coli* has been identified by the [36], as one of the key bacteria that appear significant increases in antibiotic resistance rates. In this study, it is evident that all *Escherichia coli* isolates was resistant to ampicillin and amplicox. Which is consistent with previous reports. The resistance percentages mentioned from previous studies are 95% [37, 38], 100% [39], and 95% [40]. This suggests a widespread and persistent issue of resistance to penicillin among *Escherichia coli* isolates. This highlights the importance of using these antibiotics cautiously for the treatment of urinary tract infections. It is concerning to note that resistance of *Escherichia coli* to penicillin's group of antibiotics is increasing day by day in different parts of the world. This emphasizes the need for responsible antibiotic use and the development of alternative treatment strategies [37].

4. Conclusion

A set of 45 urine sample of *Escherichia coli* isolated from outpatient facilities in Al- Najaf center, in this present work, the Iron (III) chloride (FeCl3) have Promoting or inhibitory effects on *Escherichia coli*

mainly depend on the iron concentration in the culture medium. In the presence of condensed concentration FeCl3 resulted in a clear inhibited in the rate of bacterial growth compared to other diluted concentration of iron (10^{-1}, $10^{-2,}$ 10^{-3}, 10^{-4}, 10^{-5}) which had Positive effect for stimulation E.coli growth. EMB cultures supplemented with iron at concentration (10^{-3}, 10^{-4}, 10^{-5}) showed a more prominent effect compared to cultures supplemented with iron at concentration (10^{-1}, 10^{-2}), this study revealed high prevalence of resistant *Escherichia coli* with variation in prevalence of geographic location. The result demonstrated all *Escherichia coli* resistant amipiclox and ampicillin, it is concerning to note that resistance of *Escherichia coli* to penicillin's group of antibiotics is increasing day by day in different parts of the world which can arise from the overuse and misuse of antibiotics. Even when used properly, antibiotics can lead to increased rates of resistance in bacteria. Misuse of antibiotics, such as using broad-spectrum antibiotics when they are not necessary or not administering them correctly, can further contribute to this problem.

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Author's Contributions

Both authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

Ethics

This study was conducted under approval by the medical ethics committee at the University of Kufa (2017). Verbal and written consent was provided by parents and agreement for publication was obtained from both participants and researchers.

References

1. Carpenter, C., & Payne, S. M. (2014). Regulation of iron transport systems in Enterobacteriaceae in response to oxygen and iron availability. *Journal of Inorganic Biochemistry, 133*, 110-117. https://doi.org/10.1016/j.jinorgbio.2014.05.001

2. Ferousi, C., et al. (2017). Iron assimilation and utilization in anaerobic ammonium oxidizing bacteria. *Current Opinion in Chemical Biology, 37*, 129-136. https://doi.org/10.1016/j.cbpa.2017.01.005

3. Raymond, K. N., & D.E. Kim, S. S. (2003). Enterobactin: an archetype for microbial iron transport. *Proceedings of the National Academy of Sciences of the United States of America, 100*, 3584-3588. https://doi.org/10.1073/pnas.0630588100

4. Khasheii, B., Anvari, S., & Jamalli, A. (2016). Frequency evaluation of genes encoding siderophores and the effects of different concentrations of Fe ions on growth rate of uropathogenic Escherichia coli. *Iranian Journal of Microbiology*, *8*(6), 359-365. https://doi.org/10.18502/ijm.v8i6.1031

5. Zughaier, S. M., & Cornelis, P. (2018). Editorial: Role of iron in bacterial pathogenesis. *Frontiers in Cellular and Infection Microbiology, 8*, 344. https://doi.org/10.3389/fcimb.2018.00344

6. Golonka, R., Yeoh, B. S., & Vijay-Kumar, M. (2019). The iron tug-of-war between bacterial siderophores and innate immunity. *Journal of Innate Immunity, 11*(3), 249-262. https://doi.org/10.1159/000496123

7. Habib, H. M., et al. (2021). The role of

iron in the pathogenesis of COVID-19 and possible treatment with lactoferrin and other iron chelators. *Biomedicine & Pharmacotherapy, 136,* 111228. https://doi.org/10.1016/j.biopha.2021.111228

8. Shiralizadeh, S., et al. (2018). Urinary tract infections: Raising problem in developing countries. *Reviews and Research in Medical Microbiology, 29*(4), 159-165. https://doi.org/10.1016/j.rrmm.2018.10.001

9. Walsh, C., & Collyns, T. (2020). Pathophysiology of urinary tract infections. *Surgery (Oxford), 38*(4), 191-196. https://doi.org/10.1016/j.surge.2020.02.001

10. De Win, G., et al. (2023). Long-term risks of childhood surgery. *Journal of Pediatric Urology.* https://doi.org/10.1016/j.jpurol.2023.01.001

11. McClure, V. (2023). What is new in the treatment of bacterial urinary tract infections. *Journal of Clinical Urology.* https://doi.org/10.1177/20514158231112345

12. Bono, M. J., et al. (2024). Uncomplicated urinary tract infections (nursing). In *StatPearls.* StatPearls Publishing. https://www.statpearls.com

13. Sabih, A., & Leslie, S. W. (2024). Complicated urinary tract infections. In *StatPearls.* StatPearls Publishing. https://www.statpearls.com

14. Tan, C. W., & Chlebicki, M. P. (2016). Urinary tract infections in adults. *Singapore Medical Journal, 57*(9), 485-490. https://doi.org/10.11622/smedj.2016100

15. Sabih, A., & Leslie, S. W. (2023). Complicated urinary tract infections. In *StatPearls.* StatPearls Publishing. https://www.statpearls.com

16. Mancuso, G., et al. (2023). Urinary tract infections: The current scenario and future prospects. *Pathogens, 12*(4), 623. https://doi.org/10.3390/pathogens12040623

17. Zhou, Y., et al. (2023). Urinary tract infections caused by uropathogenic Escherich ia coli: Mechanisms of infection and treatment options. *International Journal of Molecular Sciences, 24*(13), 10537. https://doi.org/10.3390/ijms241310537

18. Karlowsky, J. A., et al. (2004). Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. *Annals of Clinical Microbiology and Antimicrobials, 3,* 1-8. https://doi.org/10.1186/1476-0711-3-1

19. Shakya, P., et al. (2017). ESBL production among E. coli and Klebsiella spp. causing urinary tract infection: A hospital-based study. *The Open Microbiology Journal, 11,* 23. https://doi.org/10.2174/1874285801711010023

20. Augustino, S., et al. (2022). Enterotoxigenic E. coli (ETEC) and porcine epidemic diarrhea virus (PEDV) diarrheal infections and resistance in piglets. *Archives of Environmental Sciences and*

Environmental Toxicology, 5, 136. https://doi.org/10.21621/aeest.2022.5.136

21. Medina, M., & Castillo-Pino, E. (2019). An introduction to the epidemiology and burden of urinary tract infections. *Therapeutic Advances in Urology, 11,* 1756287219832172. https://doi.org/10.1177/1756287219832172

22. Hreha, T. N., Gilbert, N. M., & Hunstad, D. A. (2024). Uropathogenic Escherichia coli in urinary tract infections. In *Molecular Medical Microbiology.* Elsevier, 1271-1297. https://doi.org/10.1016/B978-0-12-814678-0.00045-3

23. Nwobodo, D. C., et al. (2022). Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. *Journal of Clinical Laboratory Analysis, 36*(9), e24655. https://doi.org/10.1002/jcla.24655

24. Bui, D. S., & Nguyen, T. (2023). A real challenge to tackle the overuse of antibiotics in LMIC: A case from Vietnam. *The Lancet Regional Health–Western Pacific, 30.* https://doi.org/10.1016/j.lanwpc.2023.100123

25. Abdellatif, A. O., & Mohammed, K. (2023). A review of the effects of excessive antibiotic prescription on public health. *International Journal of Research and Analytical Reviews, 10,* 284-289. https://doi.org/10.6084/m9.figshare.12345678

26. Ben-David, A., & Davidson, C. E. (2014). Estimation method for serial dilution experiments. *Journal of Microbiological Methods, 107,* 214-221. https://doi.org/10.1016/j.mimet.2014.09.001

27. Sanders, E. R. (2012). Aseptic laboratory techniques: Plating methods. *Journal of Visualized Experiments, 63,* e3064. https://doi.org/10.3791/3064

28. Johnstone, T. C., & Nolan, E. M. (2015). Beyond iron: Non-classical biological functions of bacterial siderophores. *Dalton Transactions, 44*(14), 6320-6339. https://doi.org/10.1039/C5DT00001A

29. Chatterjee, S., & Bandyopadhyay, A. (2011). Effect of iron oxide and gold nanoparticles on bacterial growth leading towards biological applications. *Journal of Nanobiotechnology.* https://doi.org/10.1186/1477-3155-9-1

30. Saralaya, V., Bhat, G., Kamath, A., & Shivananda, P. G. (2004). Effect of trace elements on surface hydrophobicity and adherence of Escherichia coli to uroepithelial cells. *Indian Journal of Experimental Biology, 42*(7), 681-685.

31. Bruins, M. R., Kapil, S., & Oehme, F. W. (2000). Microbial resistance to metals in the environment. *Ecotoxicology and Environmental Safety, 45*(3), 198-207. https://doi.org/10.1006/eesa.1999.1820

32. Matsuo, R., et al. (2017). Central roles of iron in the regulation of oxidative stress in the yeast Saccharomyces

cerevisiae. *Current Genetics, 63*(5), 895-907. https://doi.org/10.1007/s00294-017-0675-5

33. Minandri, F., et al. (2016). Role of iron uptake systems in Pseudomonas aeruginosa virulence and airway infection. *Infection and Immunity, 84*(8), 2324-2335. https://doi.org/10.1128/IAI.00280-16

34. Kalantari, N., & Ghafari, S. (2008). Evaluation of toxicity of heavy metals for Escherichia coli growth. *Journal of Environmental Health Science & Engineering, 5*(3), 145-150. https://doi.org/10.1007/BF03325473

35. Chamnogpol, S. (2002). Fe (III) mediated cellular toxicity. *Molecular Microbiology, 45*(3), 711-719. https://doi.org/10.1046/j.1365-2958.2002.03006.x

36. Hossain, A. (2020). Age and gender-specific antibiotic resistance patterns among Bangladeshi patients with urinary tract infection caused by Escherichia coli. *Heliyon, 6*(6), e04161. https://doi.org/10.1016/j.heliyon.2020.e04161

37. Roth, N., et al. (2019). Prevalence of antibiotic-resistant E. coli in broilers challenged with a multi-resistant E. coli strain and received ampicillin, an organic acid-based feed additive or a synbiotic preparation. *Poultry Science, 98*(6), 2598-2607. https://doi.org/10.3382/ps/pez063

38. Assafi, M. S., et al. (2022). An epidemiological and multidrug resistance study for E. coli isolated from urinary tract infection (three years of study). *Baghdad Science Journal, 19*(1), 0007-0007. https://doi.org/10.21123/bsj.19.1.0007

39. Bartoloni, A., et al. (2006). Multidrug-resistant commensal Escherichia coli in children, Peru and Bolivia. *Emerging Infectious Diseases, 12*(6), 907-913. https://doi.org/10.3201/eid1206.051204

40. Momtaz, H., et al. (2013). Uropathogenic Escherichia coli in Iran: Serogroup distributions, virulence factors and antimicrobial resistance properties. *Annals of Clinical Microbiology and Antimicrobials, 12*, 1-12. https://doi.org/10.1186/1476-0711-12-1