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Study of Antibacterial Chemical Substances and Molecular Investigation among Sulfamethoxazole-trimethoprim (SXT)-Resistant *Escherichia coli* Isolates

Ruaa Ali Muhammed Ali[1], Jamal Mohammed Ridha Alshara[2], Nabil Salim Saaid Tuwaij*[3], Huda Jameel Baker Al-khilkhali[3]

Abstract

Background: *Escherichia coli* (*E. coli*) remains one of the leading agents of urinary tract infection (UTIs), it has become resistant to many drugs. Current work aimed to evaluate some chemical substances as antibacterial agents and molecular study of virulence factors associated with UTIs.
Methods: This work involved 133 urine specimens obtained from females' patients suffering from UTIs, Methods of well diffusion and disk diffusion were achieved to assay the effect of some chemical substances and antibiogram profiles toward Sulfamethoxazole-trimethoprim (SXT)-resistant *E. coli* respectively. Virulence genes were done based on the technique of Polymerase Chain Reaction (PCR).
Results: The results recorded 49/133 (36.84%) *E. coli* among women suffering UTIs, 28/49 (57.14%) were resistant to SXT drug. imipenem, meropenem, and nitrofurantoin were recorded more effectively. Chemicals substances at the concentration 0.3 (g/ml) recorded percentages of inhibition, reaching 9.143±1.442, 15.36±0.5914, and 21.82±0.8699 for NaHCO3, Ch4c, and Viroxide Super™ respectively. PCR demonstrated that 28/28 (100%) of SXT-resistant *E. coli* isolates were harbored *Sul-2*, *FeoB* and *PapC* genes, while 14/28 (50%), 15/28 (53.57%), 19/28 (67.85%) and 26/28 (92.85%) in *U250* (*pet*), *FumC*, *Sul-1* and *IutA* genes, respectively. *Sul-3* gene was not observed.
Conclusions: Observed a high percentage of *E. coli* that were resistant to SXT drug, and having several virulence genes, poses a real threat, it requires a real pause to create substitutions to limit the spreading of this threat.

Keywords: Chemical substance, PCR, SXT-resistant *E. coli*, UTIs.

Introduction

Urinary tract infections (UTIs) represent a popular reason for morbidity in females and it considers a significant cause of hospital visitation worldwide. the most prevalent bacteria are *Escherichia coli* (1,2). Antibiotic medical care is vital within the UTI treatment however in recent years it's changing into more difficult thanks to the increasing resistance of UTIs to habitually applied antibiotics. Drug of Sulfamethoxazole-trimethoprim (SXT) is another vital and wide used first-line antibacterial agent within the treatment of uncomplicated urinary tract infections. Recently, resistance to the drug of SXT among clinical isolates of *E. coli* observed increased, as well as patients have been

1: The Gifted Students' School in Najaf, Ministry of Education, Iraq.
2: Faculty of Dentistry, University of Kufa, Kufa, Iraq.
3: Faculty of Science, University of Kufa, Kufa, Iraq.
***Corresponding author: Nabil Salim Saaid Tuwaij; Tel: +9647801280263; E-mail: nabeel.tuwaij@uokufa.edu.iq.**
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infected with an isolate of SXT-resistant *E. coli* and cured by this drug may they more effect into clinical failure (3-5).

There are several microorganisms have capable to create and release virulence factors, which are mainly proteins. In bacterial pathogens, these components are encoded by particular genes on the chromosome or mobile genetic elements (6). *Escherichia coli (E. coli)* can acquire an admixture of mobile genetic agents through a horizontal exchange in related bacteria or in bacteria from different families, which consist of genes that encode virulence factors allowing them to become an emergent human pathogen (7). Therefore, one of the objectives of current work was to detect levels of SXT-resistant in *E. coli* isolates which were responsible for UTIs and to estimate evaluate three chemical substances as antibacterial agents as well as antimicrobial susceptibility patterns to determine the suitable treatment, also this work involved molecular detection for some important virulence factor in this pathogen.

Materials and Methods

Patients and E. coli Diagnosis

This study included 133 non-recurring women suffering from UTIs symptoms who visited some hospitals and laboratories in the City of Najaf, during the period from August to November 2021. Mild stream urine was taken from all patients and immediately cultured on Blood agar, MacConkey agar and chromogenic agar (Pronadisa) then overnight incubated aerobically at 37 °C under sterile conditions. Bacterial growth was diagnosed firstly through morphological character, as well as some biochemical tests (IMViC), (8) and finally identified according to the Vitek-2 system.

Antibacterial agents' susceptibility

Initially, all 49 isolates of *E. coli* were tested to screen its SXT susceptibility, then only SXT-resistant *E. coli* isolates were involved in this work. Briefly, the method of disk diffusion that described through Kirby-baur method were approval to determine antibiogram profile of SXT-resistant *E. coli* isolates to differs categories of antimicrobial agents as shown in table 2 (9). Under sterile condition, all isolates were lawn on Mueller-Hinton agar after adjacent turbidity with McFarland tube (0.5) and incubated at 37 °C aerobic overnight. Guide of CLSI (10) was employed to characterize the isolates sensitivity, intermediate, and resistance.

Chemical substances and measuring antibacterial activity

Present study involved tested the activity of three various chemical substances toward SXT-resistant *E. coli* isolates, these substances Sodium bicarbonate, Ch4c (chalcone derivative compound) that obtained it from Department of Chemistry, Faculty of Science, University of Kufa, this derivative compound prepared and creative base on Adam *et al*., (11), and current study involved using chemical disinfectant of Viroxide Super™ (Neogen®).

Based on the method of agar well diffusion The activity of three chemical substances by three concentrations for each substance (0.1,0.2, and 0.3 g/ml) as antibacterial agents were evaluated for 28 isolates of SXT-resistant *E. coli*. All isolates were lawn on the medium of Mueller-Hinton agar after adjacent turbidity with McFarland tube (0.5), depending on the micropipette add 50 µl from each concentration for all chemical substances under the antiseptic condition within the well has a five-milliliter diameter which done on the medium and incubated at 37 °C aerobic overnight. Using a ruler, the zone of inhibition was measured.

DNA extraction and PCR assay

An extraction kit for whole-genome DNA (Favogen, Korea) was used to extract the total DNA from 28 isolates of SXT-resistant *E. coli*. the extraction has been accomplished as claimed by instructions of the manufacturer's company. All DNA was stored at -20 °C using a deep-freezing device, the PCR apparatus has

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applied to expose all the genes enrolled in Table 1. Gel document devices (Cleaver, United Kingdom) were employed to screen and analysis of positive bands of genes after migration through agarose (1%) (iNtRoN, Korea).

Table 1. Oligo-sequences of primer applied in the current work.

Gene	Primer Sequence 5' to 3'	Annealing (°C)	Size of product (bp)	Reference
Sul1-F	GTGACGGTGTTCGGCATTCT	54.7	921	12
Sul1-R	TCCGAGAAGGTGATTGCGCT			
Sul2-F	CGGCATCGTCAACATAACCT	51.5	721	12
Sul2-R	TGTGCGGATGAAGTCAGCTC			
Sul3-F	CAGATAAGGCAATTGAGCATGCTCTGC	55	569	13
Sul3-R	GATTTCCGTGACACTGCAATCATT			
FeoB-F	AATTGGCGTGCATGAAGATAACTG	59	470	14
FeoB-R	AGCTGGCGACCTGATAGAACAATG			
IutA-F	ATGAGCATATCTCCGGACG	51	587	15
IutA-R	CAGGTCGAAGAACATCTGG			
PapC-F	GTGGCAGTATGAGTAATGACCGTTA	63	200	16
PapC-R	ATATCCTTTCTGCAGGGATGCAATA			
U250 (pet)-F	TGACTCTGCATGGATTGAGC	58	250	17
U250 (pet)-R	GACGCATCACTCAGTACAGT			
FumC-F	ATCCACGCGCTTGCTTTAAC	53	1060	18
FumC-R	TACGCTCACGGTTTGGTTCA			

Statistical analysis

Based on the program of GraphPad prism V.7, the data was expressed as (Mean±standard division) as well as (P-value) and it applied for comparison among diameters of inhibition zone**.**

Results

Specimens and bacterial growth

Data of present work reported among 133 urine specimens observed that 99 (74.43%) were bacterial growth involved 26 (19.54%) and 73 (54.88%) returned to gram-positive and gram-negative respectively, while 34 (25.56%) of urine specimens were no growth.

Identification of E. coli and SXT susceptibility

Results of present work recorded high rate of *E. coli* isolates involved 49/133 (36.84%) among women suffering UTIs, at same time, 28/49 (57.14%) of these isolates were resistance to SXT drug compared with 21/49 (42.85%) of isolates were sensitive.

Antibiogram profile of SXT -resistant E. coli isolates

Current work also involved study of antibiogram profile for 28 isolates of SXT-resistant *E. coli*, data in table 2 recorded different level of resistance according to type of antimicrobial agents. However, 24 (85.7%), and 26 (92.8%) of SXT-resistant *E. coli* isolates were resistance to piperacillin, and amoxicilllin-clavulanic acid respectively, while this rate decreases to 7(25%) by piperacillin/tazobactam drug.

Isolates of SXT-resistant *E. coli* gave moderate resistance against cephalosporins and monobactam drugs involved 22 (78.5%) for cefixime and cefotaxime while 20(71.4%) and 15 (53.5) for cefepime and aztreonam respectively. Imipenem, meropenem and nitrofurantoin recorded good effective against SXT-resistant *E. coli* isolates. At same respect, table 2 revealed that drugs of azithromycin, ciprofloxacin, ofloxacin,

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amikacin, tetracycline, doxycycline, and chloramphenicol caused different rate of resistance among SXT-resistant *E. coli* isolates involved 8(28.5), 17(60.7), 12(42.8), 9(32.1), 25(89.2), 19(67.8) and 10(35.7), respectively.

Table 2. Antibiogram profile of 28 isolates of SXT-resistant *E. coli*.

antimicrobial agent	Resistance (%)	Intermediate (%)	Sensitive (%)
Piperacillin	24(85.7)	0(0)	4(14.2)
Piperacillin/Tazobactam	7(25)	2(7.1)	19(67.8)
Amoxicillin – Clavulanic Acid	26(92.8)	1(3.5)	1(3.5)
Cefixime	22(78.5)	0(0)	6(21.4)
Cefotaxime	22(78.5)	0(0)	6(21.4)
Cefepime	20(71.4)	0(0)	8(28.5)
Aztreonam	15(53.5)	1(3.5)	12(42.8)
Imipenem	5(17.8)	2(7.1)	21(75)
Meropenem	5(17.8)	2(7.1)	21(75)
Azithromycin	8(28.5)	0(0)	20(71.42)
Ciprofloxacin	17(60.7)	4(14.2)	7(25)
Ofloxacin	12(42.8)	4(14.2))	12(42.8)
Amikacin	9(32.1)	9(32.1)	10(35.7)
Tetracycline	25(89.2)	0(0)	3(10.7)
Doxycycline	19(67.8)	3(10.7)	6(21.4)
Nitrofurantoin	5(17.8)	0(0)	23(82.1)
Chloramphenicol	10(35.7)	0(0)	18(64.2)

Evaluation of some chemical substance as antibacterial against SXT-resistance E. coli isolates

Results of this method recorded different inhibition zone among SXT-resistance *E. coli* isolates based on the type of chemical substance and base on the concentration of the chemical substances. However, all three chemical substances gave a good effect against 28 isolates of SXT-resistance *E. coli* at a concentration of 0.03 (g/ml) involved 9.143± 1.442, 15.36±0.5914 and 21.82±0.8699 for NaHCO3, Ch4c and Viroxide Super™ (Neogen®) respectively. All isolates were resistant to Sodium bicarbonate substance at 0.01 g/ml and do not have any inhibition zone. Disinfectant of Viroxide Super™ was more effect on the growth of bacteria at all three concentration (0.01, 0.02 and 0.03 g/ml) while Ch4c gave good effect in both 0.02 g/ml and 0.03 g/ml concentrations (Table 3).

Table 3. Antibacterial activity of chemical substances against 28 isolates of SXT-resistance *E. coli.*

Concentration	NaHCO3 (g/ml)	Ch4c (g/ml)	Viroxide Super™ (g/ml)	p value
0.01	0.0±0.0 **cb**	0.750±0.5211 **b**	9.036±0.3933 **a**	< 0.0001
0.02	2.821± 0.7401**c**	8.679±1.098 **ab**	10.36±0.2313 **a**	< 0.0001
0.03	9.143±1.442 **c**	15.36±0.5914 **b**	21.82±0.8699 **a**	< 0.0001

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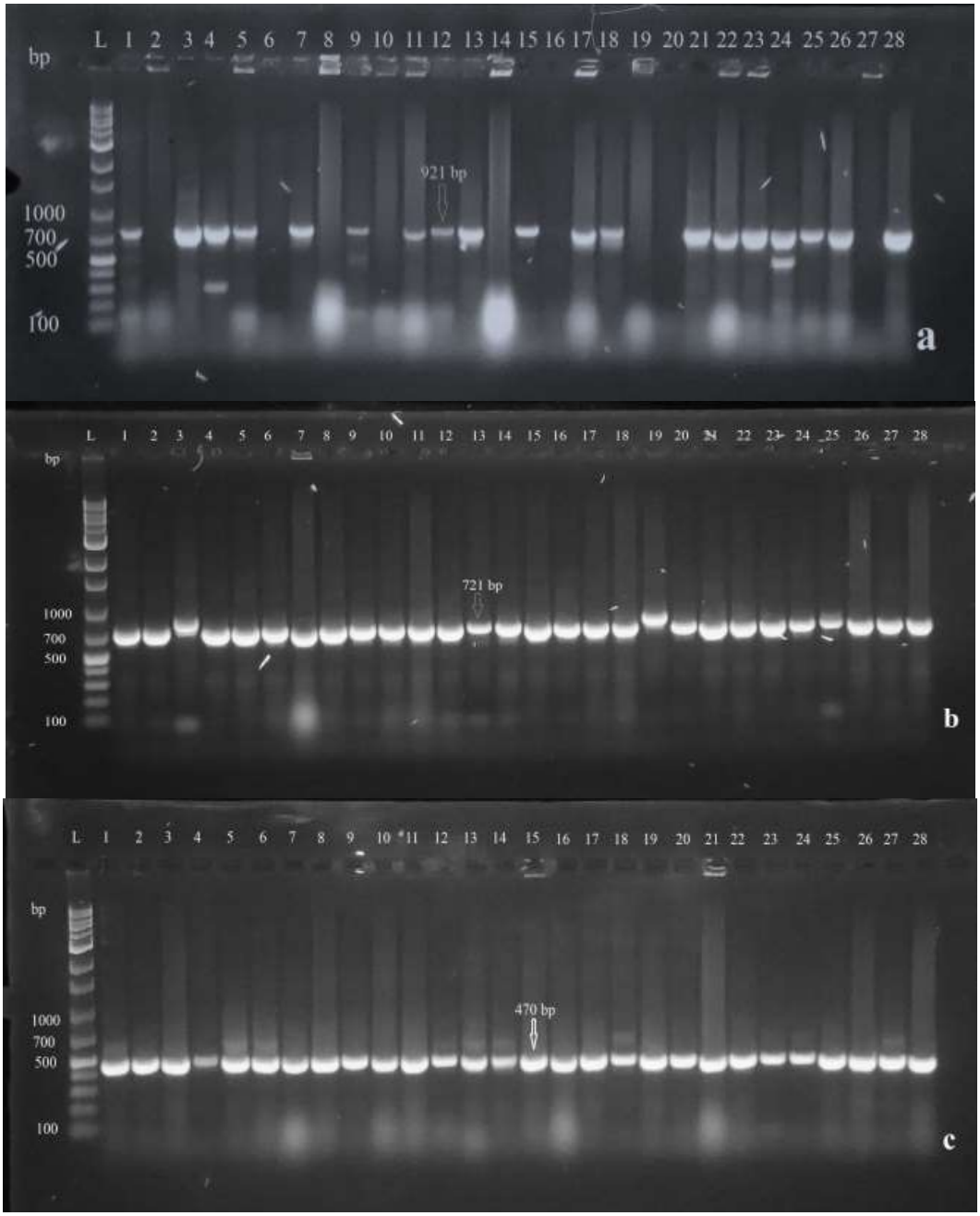
Molecular study

Results of molecular investigation about *sul-1*, *Sul-2* and *Sul-3* among 28 isolates of SXT-resistant *E. coli* were revealed high frequency of *Sul-1* and *Sul-2* genes involved 19/28 (67.85%) and 28/28 (100%) respectively, while *Sul-3* was no observed in this work (Figs. 1a and 1b). The virulence genes among 28 isolates of SXT-resistant *E. coli* recorded that 28/28 (100%) for *FeoB* and *PapC* genes (Figs. 1c and 1d) while involved 15/28 (53.57%), 26/28 (92.85%) and 14/28 (50%) in *FumC*, *IutA* and *U250* (*pet*) genes, respectively (Figs.1e,1f, and 1g).



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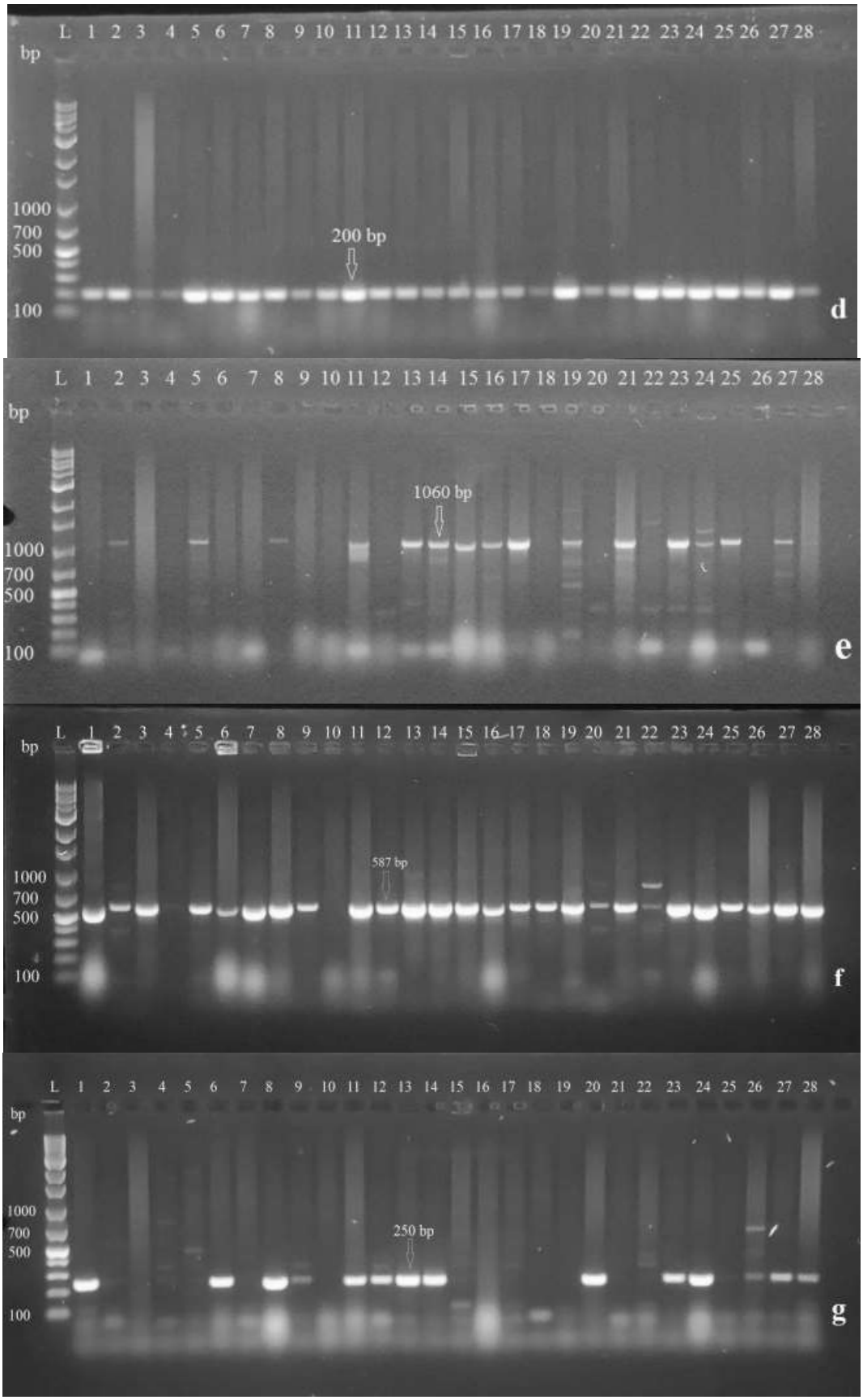

Fig. 1. PCR amplification of DNA SXT-resistant *E. coli* isolates using specific primers for (a), *Sul-2* (b), *FeoB* (c), *PapC* (d), *FumC* (e), *IutA* (f) and *U250* (*pet*) (g) genes, lane L represent DNA ladder, 1-28 represent bacterial numbers through agarose gel (1%) stained with ethidium bromide (0.5 µg/ml).

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Discussion

Data of current work was close to another local study done by Abbas and Al-Mathkhury (19), they recorded that rate of *E. coli* isolate was a prevalent reach 42.5% among other genus of gram negative and gram-positive isolates which recovered from women suffering UTIs. At same respect, several previous studied were observed that *E. coli* was the most pathogen caused UTIs among patient (20, 21). The data of current work was consistent with findings of several previous studies, they observed the rate of *E. coli* isolate was highest among bacterial agents which caused UTIs (22, 23). This may be for several reasons especially it's formed a part of microbiota in the urinary tract as well as have many virulence factors that permit it to cause a huge range of infection.

Patients of UTIs require to be diagnosed early and treated with antimicrobial agents' therapy as soon as possible to reduce the damage of the kidney. In the past few decades, resistance to antimicrobial has increased considerably (24, 25). Present work recorded a rising rate of SXT-resistant among *E. coli* isolates, in fact, drug of SXT has been widely used as an antimicrobial agent in UTIs treatment, which may demonstrate many reported' recorded resistance elevation among isolates of *E. coli* to this agent that led to complicated treatment options (26). Results in Table 2 were revealed different spectrum of resistance for antimicrobial agents, however There were a high rate of antibiotic resistance among SXT-resistant *E. coli* isolates for piperacillin, amoxicillin – clavulanic acid, cefixime, cefotaxime, aztreonam and tetracycline. Locally, there are confronting diverse challenges that be able to promote the development or expansion of drugs resistance this may be repeated or misemployment of drugs in humans, animals, as well as administration of an antibacterial agent without recipe have contributed to increased resistance to these drugs. While the drugs of piperacillin/tazobactam, imipenem, meropenem and nitrofurantoin were more effect against isolates of SXT-resistant *E. coli* and the findings in this study were in accord with those reports in other parts of the worldwide, indicating the agents have good effective against *E. coli* isolates (27).

High concentration of all three chemical substances recorded a good effect on SXT-resistant *E. coli* isolates, However, previous works were done by Gutiérrez-Huante *et al*., (28) and Dobay *et al.*, (29) indicated that NaHCO3 reduces the time generation of bacteria growth due to its effect on the pH and this harmony with the results of current work. In the same respect, present data agree with the results of Adam *et al*., (11) they recorded a positive effect of the Ch4c derivative compound against bacterial growth. PCR data demonstrated the existence of sul-1 and *Sul-2* genes correlated positively with SXT resistance. However, previous work done by Abbassi, *et al*., (30) observed that 46.2%, 23.8, and 8.9% of *E. coli* isolates were harbored Sul-1, *Sul-2*, and *Sul-3* respectively. Another study was done by Tabidehchi and Amini, (31) recorded that 76.6% and 61.6% of *E. coli* isolates were harbored *Sul-1* and *Sul-2*, respectively.

Genes associated with main virulence factors such as adhesins, toxins, as well as systems of iron acquisition among isolates of *E. coli* usually have been associated with increased pathogenicity of this pathogen and caused human infection especially UTIs (32, 33). Genes of *U250* (pet toxin) and *fum C* were detected in the present study and this was conducted with previous reports that indicated that these genes are important virulence factors associated with UTIs (34, 35). At same respect, current work observed that *PapC* gene was exist in all isolates of SXT-resistant *E. coli*, however, this result could be related to pathogeneses and confirms its crucial role during colonization of the urinary tract. This finding was conducting with previous works achieved by López-Banda *et al*., (36) and Mahmoud *et al*. (37) they indicated that *PapC* more common among *E. coli* isolates.

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Possession of iron has a necessity for *E. coli* existence in an environment that is as iron bounded as the urinary tract (38). Metabolism of Iron in several microorganisms are dependent on a ferrous iron transporter (FeoB) (39). Current data was agreement with a result of a recent work done by Kocúreková et al., (14) recorded that genes of iron-uptake FeoB and iutA were 94% and 58% respectively, among *E. coli* isolates. While Rocha et al. observed that iutA gene was (45.9%) among *E. coli* isolates (40).

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References

1. Medina M, Castillo-Pino E. An introduction to the epidemiology and burden of urinary tract infections. Ther Adv Urol. 2019;11:3-7.
2. Odoki M, Almustapha Aliero A, Tibyangye J, Nyabayo Maniga J, Wampande E, Drago Kato C, et al. Prevalence of bacterial urinary tract infections and associated factors among patients attending hospitals in Bushenyi district, Uganda. International journal of microbiology. 2019;1-8.
3. Bartoletti R, Cai T, Wagenlehner FM, Naber K, Bjerklund Johansen TE. Treatment of urinary tract infections and antibiotic stewardship. Eur Urol Suppl. 2016;15(4):81-87.
4. Bischoff S, Walter T, Gerigk M, Ebert M, Vogelmann R. Empiric antibiotic therapy in urinary tract infection in patients with risk factors for antibiotic resistance in a German emergency department. BMC Infect Dis. 2018;18(1):56.
5. Kot B. Antibiotic resistance among uropathogenic *Escherichia coli*. Pol J Microbiol. 2019;68(4):403-415.
6. Pakbin B, Brück WM, Rossen JW. Virulence factors of enteric pathogenic *Escherichia coli*: A review. Int J Mol Sci. 2021;22(18):9922.
7. Sarowska J, Futoma-Koloch B, Jama-Kmiecik A, Frej-Madrzak M, Ksiazczyk M, Bugla-Ploskonska G, et al. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: recent reports. Gut pathogens. 2019;11:1-6.
8. Collee J G, Fraser AG, Marmin BP, Simmons AM, Mccartney. Practical Medical Microbiology - 14th Edition (Churchill Livingstone, London). 1996;385-404.
9. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45(4):493-6.
10. Performance Standards for Antimicrobial Susceptibility Testing. 30th ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute; 2021, p: 316.
11. Adam RW, Al-Labban HM, Kadhim IN, Aljanaby, AAJ. Synthesis, characterization, and antibacterial activity of some new pyrimidine derivatives from chalcone derivatives. Drug Invention Today. 2019;11(7):1732-1739.
12. Tamocha L, Oyinloye M, Rahube TO. Airborne bacterial diversity and antibiotic resistance patterns in operating theatre and paediatric wards in Botswana hospitals. African Journal of Microbiology Research. 2019;13(21):341-352.
13. El-Kazzaz SS, Mashaly GE, El-Sabbagh AM, Abd Elmagid DS. Resistance genes to sulphonamide in commensal *Escherichia coli* isolated from stool of patients in Mansoura University Children Hospital. African Journal of Microbiology Research. 2016;10(33):1363-1370.
14. Kocúreková T, Karahutová L, Bujňáková D. Antimicrobial Susceptibility and Detection of Virulence-Associated Genes in *Escherichia coli* Strains Isolated from Commercial Broilers. Antibiotics. 2021;10(11):1303.
15. Kaczmarek A, Budzynska A, Gospodarek E. Prevalence of genes encoding virulence factors among Escherichia coli with K1 antigen and non-K1 *E. coli* strains. J Med Microbiol. 2012;61(Pt 10):1360-1365.
16. Ghazvini H, Taheri K, Edalati E, Miri A, Sedighi M, Mirkalantari S. Virulence factors and antimicrobial resistance in uropathogenic *Escherichia coli* strains isolated from cystitis and pyelonephritis. Turk J Med Sci. 2019;49(1):361-367.

[DOI: 10.52547/rbmb.11.1.166]

17. Gutiérrez-Lucas LR, Mendoza-Hernández G, González-Pedrajo B, Eslava-Campos C, Bustos-Martínez J, Sainz-Espuñes T. Identification of the autotransporter Pet toxin in *Proteus mirabilis* strain isolated from patients with urinary tract infections. Advances in Biological Chemistry. 2012;2(3):283.
18. Al-Yasseen AK, Alatrash AK. The effect of sodium azide on swimming and swarming phenomenon of *Proteus mirabilis*. International Journal of ChemTech Research. 2017;10(6):865-872.
19. Abbas HM, Al-Mathkhury HJ. Isolation and identification of bacteria from Iraqi women with recurrent urinary tract infection. Plant Archives. 2020;20(2):1838-1842.
20. Alanazi MQ, Alqahtani FY, Aleanizy FS. An evaluation of *E. coli* in urinary tract infection in emergency department at KAMC in Riyadh, Saudi Arabia: retrospective study. Ann Clin Microbiol Antimicrob. 2018;17(1):3.
21. Tuwaij NS. Molecular Profile of Aminoglycoside, Fluoroquinolone, and Class 1 Integron Genes among Gentamicin-Resistant *Escherichia coli* in Najaf City, Iraq. International Journal of Research in Pharmaceutical Sciences. 2020;11(2):2558-2567.
22. Bunduki GK, Heinz E, Phiri VS, Noah P, Feasey N, Musaya J. Virulence factors and antimicrobial resistance of uropathogenic *Escherichia coli* (UPEC) isolated from urinary tract infections: a systematic review and meta-analysis. BMC Infect Dis. 2021;21(1):753.
23. Alsamarai AM, Khorshed SA, Ali H. Urinary tract infection in female in Kirkuk city, Iraq: Association between risk factors and bacterial type. Our Dermatology Online. 2017;8(3):242.
24. Yun KW, Kim HY, Park HK, Kim W, Lim IS. Virulence factors of uropathogenic *Escherichia coli* of urinary tract infections and asymptomatic bacteriuria in children. Journal of Microbiology, Immunology and Infection. 2014;47(6):455-461.
25. Rezaei S, Hadadian S, Khavari-Nejad RA, Norouzian D. Recombinant tandem repeated expression of s3 and sδ3 antimicrobial peptides. Rep Biochem Mol Biol. 2020;9(3):348-356.
26. Aabed K, Moubayed N, Alzahrani S. Antimicrobial resistance patterns among different *Escherichia coli* isolates in the Kingdom of Saudi Arabia. Saudi journal of biological sciences. 2021;28(7):3776-3782.
27. Rafalskiy V, Pushkar D, Yakovlev S, Epstein O, Putilovskiy M, et al. Distribution and antibiotic resistance profile of key Gram-negative bacteria that cause community-onset urinary tract infections in the Russian Federation: RESOURCE multicentre surveillance 2017 study. J Glob Antimicrob Resist. 2020;21:188-194.
28. Gutiérrez-Huante M, Martínez H, Bustamante VH, Puente JL, Sánchez J. Bicarbonate enhances the *in vitro* antibiotic activity of kanamycin in *Escherichia coli*. Lett Appl Microbiol. 2015;60(5):440-6.
29. Dobay O, Laub K, Stercz B, Kéri A, Balázs B, Tóthpál A, at al. Bicarbonate inhibits bacterial growth and biofilm formation of prevalent cystic fibrosis pathogens. Front Microbiol. 2018;9:2245.
30. Abbassi MS, Kilani H, Abid I, Sáenz Y, Hynds P, Lengliz S, et al. Genetic Background of Antimicrobial Resistance in Multiantimicrobial-Resistant *Escherichia coli* Isolates from Feces of Healthy Broiler Chickens in Tunisia. Biomed Res Int. 2021:1269849.
31. Tabidehchi SD, Amini K. Detection of sul1, sul2, and int1 Genes in the Uropathogenic *Escherichia coli* Strains by Multiplex-PCR and their Antibiotic Susceptibility Patterns. Sadra Medical Journal. 2016;4(3):195-204.
32. Terlizzi ME, Gribaudo G, Maffei ME. UroPathogenic *Escherichia coli* (UPEC) infections: virulence factors, bladder responses, antibiotic, and non-antibiotic antimicrobial strategies. Front Microbiol. 2017;8:1566.
33. Handrová L, Čuvalová A, Kmeť V. The relationship between biofilm formation, genes of virulence and iron metabolism in *Escherichia coli*. Annales Universitatis Paedagogicae Cracoviensis Studia Naturae. 2018;24-32.
34. Espinosa-Antúnez VK, Castrillón-Rivera LE, Vega-Memije ME, Valenzuela-Vargas MT, Sainz-Espuñes T, Luna-Herrera J, et al. Proteus mirabilis expressing Plasmid-encoded toxin (Pet) causes urinary tract alterations. TIP.

[DOI: 10.52547/rbmb.11.1.166]

Revista especializada en ciencias químico-biológicas. 2019;22:1-10.
35. Himpsl SD, Shea AE, Zora J, Stocki JA, Foreman D, Alteri CJ, et al. The oxidative fumarase FumC is a key contributor for *E. coli* fitness under iron-limitation and during UTI. PLoS pathogens. 2020;16(2):e1008382.
36. López-Banda DA, Carrillo-Casas EM, Leyva-Leyva M, Orozco-Hoyuela G, Manjarrez-Hernández ÁH, Arroyo-Escalante S, et al. Identification of virulence factors genes in *Escherichia coli* isolates from women with urinary tract infection in Mexico. Biomed Res Int. 2014;2014:959206.
37. Mahmoud AT, Ibrahem RA, Salim MT, Gabr A, Halby HM. Prevalence of some virulence factors and genotyping of hospital- acquired uropathogenic *Escherichia coli* isolates recovered from cancer patients. Journal of Global Antimicrobial Resistance. 2020;23:211-216.Skaar EP. The battle for iron between bacterial pathogens and their vertebrate hosts. PLoS pathogens. 2010;6(8):e1000949.
38. Hagelueken G, Duthie FG, Florin N, Schubert E, Schiemann O. Expression, purification and spin labelling of the ferrous iron transporter FeoB from *Escherichia coli* BL21 for EPR studies. Protein Expr Purif. 2015;114:30-6.
39. Rocha AC, Rocha SL, Lima-Rosa CA, Souza GF, Moraes HL, Salle FO, et al. Genes associated with pathogenicity of avian *Escherichia coli* (APEC) isolated from respiratory cases of poultry. Pesquisa Veterinária Brasileira. 2008;28(3):183-186.

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