

# Possible Synergism between Ampicillin and Some Pain-reducing Drugs against *Escherichia coli*

Thoraa Abdul Amir Drees<sup>1</sup>, Falah Hasan Obayes AL-Khikani<sup>2</sup>, Haider Hussein Yas Khudair<sup>3</sup>, Haider Abdul Hussein Nuri<sup>4</sup>, Ali Amer Hussein<sup>5</sup>

Received on: 10 August 2023; Accepted on: 01 September 2023; Published on: 21 December 2023

## ABSTRACT

**Background:** *Escherichia coli* (*E. coli*) is one of the most common human bacterial pathogens implicated in antibiotic resistance, especially in the last decades. The antimicrobial combination is important to reduce these resistances. So the aim of this study is finding a possible synergism between ampicillin and other drugs to treat *E. coli* infections.

**Materials and methods:** The present study was conducted for the period from January 2023 to March 2023 on bacteria *E. coli*. The tested bacteria isolated and identified by using standard bacteriological methods. Detection antibiotic sensitivity pattern of isolates was determined by agar well diffusion method. The antibiotics (flagyl, paracetamol, dexamethasone, diclofenac sodium, and ampicillin) were added separately in one dish, and then added in another dish combined with the ampicillin to know the effect of antibiotics on the growth of bacteria before and after the addition of ampicillin.

**Results:** Twenty *E. coli* were isolated from various body sites. The study showed that females were more infected (70%) with *E. coli* than males (30%). Flagyl, paracetamol, and dexamethasone were resistant to all isolates (100%). But diclofenac sodium was resistant to 85% isolates. Resistance to ampicillin was 50%. Ampicillin showed synergy after combination with other agents but still nonsignificant ( $p > 0.05$ ). The addition of flagyl increased the effect of ampicillin to 15.35 mm while it was 13.75 mm before the combination ( $p = 0.53$ ). The addition of paracetamol increased the effect of ampicillin to 15 mm while it was 13.75 mm before the combination ( $p = 0.63$ ). The addition of dexamethasone increased the effect of ampicillin to 15.55 mm, it was 13.75 mm before addition ( $p = 0.51$ ). The combination of diclofenac increased the effect of ampicillin to 14.95 mm, it was 13.75 mm before addition ( $p = 0.64$ ).

**Conclusion:** Flagyl, paracetamol, and dexamethasone were resistant to all isolates (100%). But diclofenac sodium was resistant in 85% of all isolates. Ampicillin showed synergy after adding other agents but still non-significant ( $p > 0.05$ ).

**Keywords:** Ampicillin, *Escherichia coli*, Dexamethasone, Diclofenac sodium, Flagyl, Paracetamol, Synergism.

*Bengal Physician Journal* (2023): 10.5005/jp-journals-10070-8020

## INTRODUCTION

*Escherichia coli* (*E. coli*) is the dominant non-pathogenic facultative flora of the human intestine. However, some *E. coli* strains have evolved the ability to cause gastrointestinal, urinary, or central nervous system diseases even in the most robust human hosts. *Escherichia coli* can cause intestinal and extra-intestinal infections ranging from mild to life-threatening infections.<sup>1</sup> The severity of infection depends on many factors including virulence properties and antimicrobial resistance.<sup>2,3</sup>

Febrile urinary tract infections (UTIs), defined as UTI with systemic symptoms, frequently occur in women and are predominantly caused by *E. coli*.<sup>4</sup>

Guidelines recommend treating UTIs that require hospitalization with a 7–14 days course of antibiotics that usually consists of empiric intravenous treatment preferably followed by an oral step-down treatment targeted to the susceptibility pattern of the causal uropathogens. Optimal treatment of UTI is hampered by the increase in resistant Gram-negative bacteria count.<sup>5–7</sup>

Flagyl metronidazole extended-release tablets is an oral formulation of the synthetic nitroimidazole antimicrobial agent, 2-methyl-5-nitro-1H-imidazole-1-ethanol. Metronidazole is the major component appearing in the plasma, with lesser quantities of metabolites.<sup>8</sup>

Dexamethasone rapidly diffuses across all tissues. It even crosses the placenta and enters breast milk. Repeated, prolonged

<sup>1,3–5</sup>Department of Medical Laboratories, Al-Furat Al-Awsat, Technical University, Babylon Technical Institute, Iraq

<sup>2</sup>Al-Furat Al-Awsat Technical University, Babylon Technical Institute; Department of Medical Laboratory Technology, College of Medical Technology, The Islamic University, Najaf; Department of Microbiology, Al-Shomali General Hospital, Babylon Health Directorate, Babylon, Iraq

**Corresponding Author:** Falah Hasan Obayes AL-Khikani, Al-Furat Al-Awsat Technical University, Babylon Technical Institute; Department of Medical Laboratory Technology, College of Medical Technology, The Islamic University, Najaf; Department of Microbiology, Al-Shomali General Hospital, Babylon Health Directorate, Babylon, Iraq, Phone: +9647817307280, e-mail: falahgh38@gmail.com

**How to cite this article:** Drees TAA, AL-Khikani FHO, Khudair HHY, et al. Possible Synergism between Ampicillin and Some Pain-reducing Drugs against *Escherichia coli*. *Bengal Physician Journal* 2023;10(3): 79–82.

**Source of support:** Nil

**Conflict of interest:** None

administration is associated with an increased risk of intrauterine growth restriction.<sup>9</sup>

Diclofenac sodium is a potent non-steroidal anti-inflammatory drug with pronounced analgesic properties, is used in the long-term

treatment of rheumatoid arthritis and osteoarthritis. Its biological half-life has been reported as 1–2 hours.<sup>10</sup>

Paracetamol is used as an analgesic and antipyretic drug. It is the preferred alternative analgesic–antipyretic to aspirin (acetylsalicylic acid), particularly in patients with coagulation disorders, individuals who cannot tolerate aspirin, as well as in children. Paracetamol is activated in the kidney by a nicotinamide adenine dinucleotide phosphate (NADPH)-dependent cytochrome P450 mechanism to an arylating agent which can bind covalently to cellular macromolecules.<sup>11</sup>

Ampicillin is authorized for use as a veterinary drug against bacterial infections in a wide range of companion and food animals. Ampicillin may have a synergistic action with aminoglycosides and with the  $\beta$ -lactamase inhibitors clavulanic acid and sulbactam.<sup>12–15</sup>

The aim of the study was to evaluate the antimicrobial activity of ampicillin and some pain-reducing agents on the growth of *E. coli*. As well as detection the possible synergism between ampicillin and these drugs against *E. coli* clinically isolates.

## MATERIALS AND METHODS

Bacteria were isolated from blood stream, UTI, and wounds. Twenty clinical strains of *E. coli* were acquired from the Al-Shomali General Hospital, and were also isolated from admitted patients. Brain–heart infusion broth, blood agar, macConkey, and eosin methylene blue agars were used for bacterial growth. Bacteria were diagnosed based on bacteriological standard methods.

Agar well diffusion method was widely used to evaluate the antimicrobial activity; the agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire agar surface on the Muller Hinton agar medium. Then, a hole with a diameter of 5 mm was punched aseptically with a sterile cork borer, and a volume (50  $\mu$ L) of the antimicrobial agent at desired concentration was introduced into the well after they were compared with the McFarland standards. Then, agar plates were incubated under suitable conditions depending upon the test microorganism at 37°C for 18–24 hours. The antimicrobial agent diffuses in the agar medium and zone of inhibition was measured in millimeters.

On January 1, 2023, the study protocol was accepted by the Ethical Committee of the Babylon Health Directorate. Furthermore, the patients' verbal consent was obtained before taking the sample. During the sampling, precautions were taken to ensure the safety of the participants. This work was also carried out by the Iraqi Ministry of Health's Ethics Committee and followed all national rules.

For statistical analysis, SPSS software 26 (SPSS Inc., Chicago, USA) was used. Means and standard deviations were used to represent the data. *T* test was used to examine the data with a normal distribution. Chi-square used for non-parametric variables, *p* value of less than 0.05 was considered significant.

## RESULTS

The gender distribution among patients appeared that females were more prevalent than males, 70% and 30% respectively with significant differences ( $p < 0.05$ ; Fig. 1).

*Escherichia coli* was isolated from different sources: 10 (50%) from UTI, 5 (25%) from wounds, 5 (25%) from blood stream (Fig. 2).

Figure 3 showed that flagyl was resistant to all isolates (100% resistance). As well as paracetamol was resistant to all isolates (100% resistance). Dexamethasone was resistant to all isolates

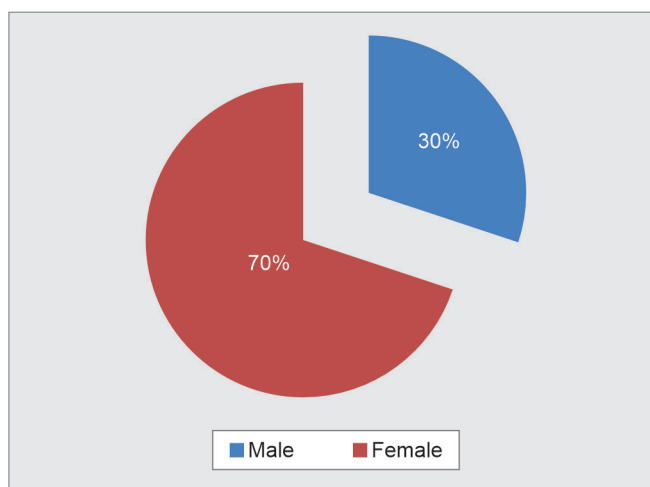


Fig. 1: The gender distribution among patients

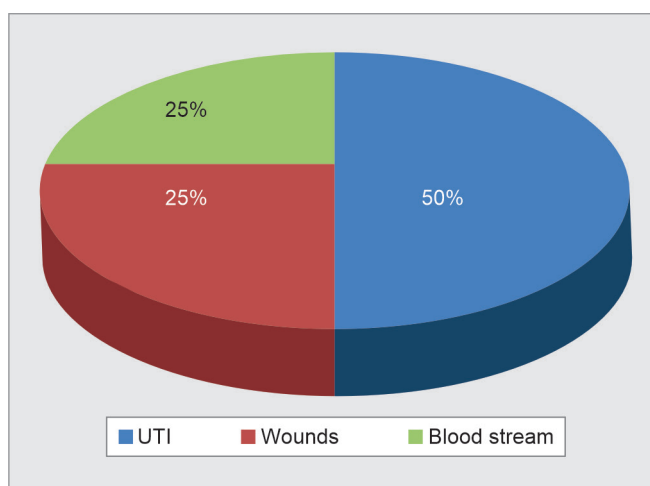


Fig. 2: The sources of *Escherichia coli*

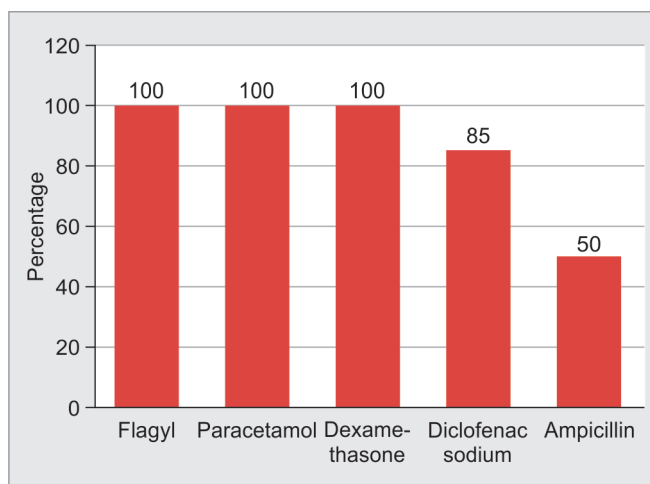


Fig. 3: Resistance of isolates to different antimicrobial agents

(100% resistance). But diclofenac sodium was resistant in 85% of all isolates (sensitive in 15%; 3 of 20). Resistance to ampicillin was 50% of all isolates.

**Table 1:** Comparison of ampicillin sensitivity before and after the addition of different antibiotics

Antibiotic	N	Mean (mm)	Std. Deviation	P-value
Ampicillin (AM)	20	13.75	8.65	0.53
AM plus flagyl	20	15.35	7.49	
Ampicillin	20	13.75	8.65	0.63
AM plus paracetamol	20	15.00	7.65	
Ampicillin	20	13.75	8.65	0.51
AM plus dexamethasone	20	15.55	8.74	
Ampicillin	20	13.75	8.65	0.64
AM plus diclofenac	20	14.95	7.71	

The mean inhibition of ampicillin on the growth of *E. coli* was 13.75 mm. However, the addition of flagyl increased the effect to 15.35 mm, but still non-significant ( $p = 0.53$ ). The addition of paracetamol increased the effect to 15.00 mm with no significant difference (0.36). Also, the addition of dexamethasone increased the effect to 15.55 mm without significant difference ( $p = 0.51$ ). The addition of diclofenac sodium increased the effect to 14.95 mm, still it was non-significant ( $p = 0.64$ ; Table 1).

## DISCUSSION

*Escherichia coli* is one of the most common causative agents of bacterial infection, is one of the most frequent causes of UTI, and is among the most important pathogens causing bloodstream infections, otitis media, and wound infections. Antimicrobial resistance in *E. coli* has been reported worldwide and increasing rates of resistance among *E. coli* is a growing concern in both developed and developing countries.<sup>16,17</sup>

This study showed that women are more prone to *E. coli* isolates than men as 70% and 30%, respectively. *Escherichia coli* infection in women are high because they are more susceptible to UTIs than men due to anatomical differences, such as a shorter urethra and perineal contamination of the urinary tract with fecal microorganisms.<sup>18</sup>

Urinary tract infection is less common in men than in women because the male urethra is long, making it difficult for bacteria to spread to the bladder. Women are more prone to UTIs than men because the urethra is much closer to the anus and is shorter than in males.<sup>19</sup> Furthermore, women lack the bacteriostatic properties of prostatic secretions. Among the elderly, UTI frequency is roughly equal in women and men.<sup>20</sup> This is due, in part, to an enlarged prostate in older men. As the gland grows, it obstructs the urethra, leading to increased frequency of urinary retention.<sup>21-23</sup>

Another study found that *E. coli* is the predominant etiology of UTI, a total of 160 *E. coli* isolates were collected from the urine samples of female (62.5%) and male (37.5%) patients suffering from UTI caused by *E. coli*.<sup>24</sup>

In the current study, flagyl was resistant to all isolates (100% resistance). As well as paracetamol was resistant to all isolates (100% resistance). Dexamethasone was resistant to all isolates (100% resistance). But diclofenac sodium was resistant in 85% of all isolates (sensitive in 15% 3 of 20). Resistance to ampicillin was 50% of all isolates.

*Escherichia coli* was found to be highly susceptible to ertapenem (97.6%) and imipenem (96.4%) but resistant to ampicillin (87.8%). For wound and cervical swabs, *E. coli* was 100% resistant to ampicillin and cefepime but 100% sensitive to ertapenem and imipenem. It was found that *E. coli* isolates from blood samples were 100%

resistant to ampicillin, ceftriaxone, and cefoxitin, and around 75% of them were sensitive to ertapenem, ciprofloxacin, and levofloxacin. Finally, *E. coli* isolated from other clinical samples were highly sensitive to ertapenem, imipenem, levofloxacin, nitrofurantoin, and ceftazolin.<sup>25</sup>

Approximately one in three women will require antimicrobial treatment for an UTI before the age of 24 years, and 40–50% of women will have an UTI during their lifetime. Urinary tract infection in male patients are considered complicated. *Escherichia coli* is the most common cause of UTIs. A study mentioned that 553 (8.7%) patients out of 7056 were shown to be urine culture positive (68% females and 32% males). The most isolated bacterium was *E. coli* with frequency rate of 59%.<sup>26</sup>

The current study showed the effect of following antibiotics (flagyl, paracetamol, dexamethasone, diclofenac sodium, and ampicillin) on 20 sample of *E. coli* and it includes the results of applying them separately to the bacteria, and their results after adding ampicillin, which shows synergy and thus an increase in the effect on the growth of bacteria. The mean inhibition of ampicillin on the growth of *E. coli* was 13.75 mm. However, the addition of flagyl increased the effect to 15.35 mm. The mean inhibition of ampicillin on the growth of *E. coli* was 13.75 mm. However, the addition of paracetamol increased the effect to 15.00 mm.

*Escherichia coli* showed a high resistant to flagyl, paracetamol, and dexamethasone, and less resistant to the diclofenac sodium and ampicillin. This resistance may be because *E. coli* cells with complex cell wall consisting of two-layer of lipids. This cell wall plays many functional roles in protection, transport, mobility, sensor, detoxification, and energy production.<sup>27</sup>

These results are partially agreement with those of Krishna et al.,<sup>28</sup> who reported the *E. coli* resistance to antibiotics (ampicillin, ciprofloxacin, and fosfomycin).

Several studies showed that the administration of single antibiotics resulted in the appearance of resistant bacteria, while combinations reduced their emergence.<sup>29-31</sup>

## CONCLUSION

Antimicrobial resistance is an alarming concern especially in commonly reported disease and diseases associated with Gram-negative bacilli such as *E. coli*. Flagyl, paracetamol, and dexamethasone were resistant to all isolates (100%). But diclofenac sodium was resistant in 85% of all isolates. Ampicillin showed synergy after adding other agents but still non-significant ( $p > 0.05$ ).

## REFERENCES

1. Braz VS, Melchior K, Moreira CG. *Escherichia coli* as a multifaceted pathogenic and versatile bacterium. *Front Cell Infect Microbiol* 2020;10:548492. DOI: 10.3389/fcimb.2020.548492.
2. AL-Khikani F. Factors affecting flowering of *Pseudomonas aeruginosa* in urine. *Microbes Infect Dis* 2022;3(4):956–957.
3. AL-Khikani F. Virulence factors in *Pseudomonas aeruginosa*: The arms race between bacteria and humans. *Microbes Infect Dis* 2021;4:24. DOI: 10.21608/MID.2021.99954.1202.
4. Flores-Mireles AL, Walker JN, Caparon M, et al. Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. *Nat Rev Microbiol* 2015;13(5):269–284. DOI: 10.1038/nrmicro3432.
5. Doesschate T, Kuiper S, van Nieuwkoop C, et al. Fosfomycin vs ciprofloxacin as oral step-down treatment for *Escherichia coli* febrile urinary tract infections in women: A randomized, placebo-controlled, double-blind, multicenter trial. *Clin Infect Dis* 2022;75(2):221–229. DOI: 10.1093/cid/ciab934.

6. AL-Khikani FH, Ayit AS. *Pseudomonas aeruginosa* a tenacious uropathogen: Increasing challenges and few solutions. *BBRJ* 2022;6(3):311. DOI: 10.4103/bbrj.bbrj\_256\_21.
7. Al-Khikani FH, Kadem BJ. Unusual false-negative serum human chorionic gonadotropin detected by qualitative immunoassay: A case report of two Iraqi women. *J Med Sci Res* 2020;3(3):238. DOI: 10.4103/JMISR.JMISR\_30\_20.
8. Salas-Herrera IG, Pearson RM, Johnston A, et al. Concentration of metronidazole in cervical mucus and serum after single and repeated oral doses. *J Antimicrobial Chemotherapy* 1999;28(2):283–289. DOI: 10.1093/jac/28.2.283.
9. Kakodkar PS. Routine use of dexamethasone for postoperative nausea and vomiting: The case for. *Anaesthesia* 2013;68(9):889–891. DOI: 10.1111/anae.12308.
10. Barzegar-Jalali M, Alaei-Beirami M, Javadzadeh Y, et al. Comparison of physicochemical characteristics and drug release of diclofenac sodium–eudragit RS100 nanoparticles and solid dispersions. *Powder Technol* 2012;219:211–216. DOI: 10.1016/j.powtec.2011.12.046.
11. McMurtry RJ, Snodgrass WR, Mitchell JR. Renal necrosis, glutathione depletion, and covalent binding after acetaminophen. *Toxicol Appl Pharmacol* 1978;46(1):87–100. DOI: 10.1016/0041-008x(78)90139-4.
12. Al-Janabi AA, Al-Khikani FH. Prophylaxis and therapeutic ability of inactivated dermatophytic vaccine against dermatophytosis in the rabbits as an animal model. *Turk J Pharm Sci* 2021;18(3):326. DOI: 10.4274/tjps.galenos.2020.81226.
13. AL-Khikani FH, Ayit AS. Correlation study between urinary tract bacterial infection and some acute inflammatory responses. *BBRJ* 2019;3(4):236. DOI: 10.4103/bbrj.bbrj\_122\_19.
14. Alhusayni AA, Al-Khikani FH, Aljaburi HK, et al. Antibiotic susceptibility profile of bacterial uropathogens in Al-Shomali General Hospital, Babylon, Iraq. *Journal of Preventive, Diagnostic and Treatment Strategies in Medicine* 2022;1:240. DOI: 10.4103/jpdtsm.jpdtm\_45\_22.
15. AL-Khikani FH, Kadim BJ, Ayit AS, et al. Evaluation cephalosporins resistance in pathogenic bacteria isolated clinically. *World News Nat Sci* 2020;31:65. DOI: 10.4103/jpdtsm.jpdtm\_376\_20.
16. Bell JM, Turnidge JD, Gales AC, et al. Prevalence of extended spectrum beta-lactamase (ESBL) - producing clinical isolates in the Asia-Pacific region and South Africa: Regional results from SENTRY Antimicrobial Surveillance Program (1998–99). *Diagn Microbiol Infect Dis* 2002;42(3):193–198. DOI: 10.1016/s0732-8893(01)00353-4.
17. AL-Khikani FH, Kadim MM. Secondary unculturable bacteria associated with Sars-Cov-2: More information are required. *Med J Dr DY Patil Univ* 2022;15(7):S136–S137. DOI: 10.4103/mjdrdypu.mjdrdypu\_698\_21.
18. Mohiuddin AK. UTI prevalence among population with chronic conditions. *J Med Res Case Rep* 2019;1(2):89.
19. Ebie MY, Kandakai-Olukemi YT, Ayanbadejo J, et al. Urinary tract infections in a Nigerian military hospital. *Nigerian J Microbiol* 2001; 15(1):31–37.
20. Leitner L, Sybesma W, Chanishvili N, et al. Bacteriophages for treating urinary tract infections in patients undergoing transurethral resection of the prostate: a randomized, placebo-controlled, double-blind clinical trial. *BMC Urol* 2017;17:1–6. DOI: 10.1186/s12894-017-0283-6.
21. Al-Khikani FH, Almosawey HA, Abdullah YJ, et al. Potential antiviral properties of antifungal drugs. *J Egypt Women Dermatol Soc* 2020;17:185. DOI: 10.4103/JEWD.JEWD\_40\_20.
22. AL-Khikani FH. Pulmonary mycoses treated by topical amphotericin B. *BBRJ*. 2020;4(2):123. DOI: 10.4103/bbrj.bbrj\_12\_20.
23. Obayes AK, Hasan F. The forgotten role of methenamine to prevent recurrent urinary tract infection: Urgency for reuse 100 years after discovery. *Pharmaceut Biomed Res* 2020;6(4):247–250. Available from: <http://dx.doi.org/10.18502/pbr.v6i4.5110>.
24. Staji H, Rassouli M, Jourablou S. Comparative virulotyping and phylogenomics of *Escherichia coli* isolates from urine samples of men and women suffering urinary tract infections. *Iranian journal of basic medical sciences* 2019;22(2):211. DOI: 10.22038/ijbms.2018.28360.6880.
25. Naqid IA, Hussein NR, Balatay A, et al. Antibiotic susceptibility patterns of uropathogens isolated from female patients with urinary tract infection in Duhok province, Iraq. *Jundishapur J Health Sci* 2020;12(3):76. DOI: 10.5812/jjhs.105146.
26. Mansour Amin, Zohreh Pourangchi. Study of bacteria isolated from urinary tract infections and determination of their susceptibility to antibiotics. *Jundishapur Journal of Microbiology* 2009;7:118–123.
27. Xu C, Kong L, Liao Y, et al. Mini-review: Antibiotic-resistant *Escherichia coli* from farm animal-associated sources. *Antibiotics* 2022;11(11):1535. DOI: 10.3390/antibiotics11111535.
28. Krishna A, Zere T, Mistry S, et al. Evaluation of a sequential antibiotic treatment regimen of ampicillin, ciprofloxacin and fosfomycin against *Escherichia coli* CFT073 in the hollow fiber infection model compared with simultaneous combination treatment. *Antibiotics* 2022;11(12):1705. DOI: 10.3390/antibiotics11121705.
29. AL-Khikani FH. Antimicrobial resistance profile among major bacterial pathogens in Southern Babil, Iraq. *Galician Med J* 2020;27(3):20. DOI: 10.21802/gmj.2020.3.6.
30. Al-Khikani F, Ayit A. The antibacterial action of safranin and gentian violet. *Rambam Maimonides Med J* 2022;13:76. DOI: 10.5041/RMMJ.10475.
31. AL-Khikani FH. Non culturable bacteria associated with COVID-19: More details are demanded. *Microb Infect Dis* 2021;2:611–612. DOI: 10.21608/MID.2021.92689.1186.