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The Role of Some Organic Acids in The Eradication of Antibiotics Resistance of *E. Coli* Isolated from Urinary Tract Infections

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ABSTRACT

Background: The organic acids like succinic acid and malic acid have an antibacterial impact on *E.coli* infection.
Objective: The aim of the present study is the detection of *E.coli* in urine samples using PCR and antibiotic susceptibility testing of strains obtained from people and food using ampicillin and erythromycin, in addition to testing malic and succinic acids against uropathogenic *E.coli*.
Material and methods: This study describes a biochemical and polymerase chain reaction *E.coli* test. PCR can accurately verify *E.coli* colonies on culture plates. The Minimum Inhibitory Concentration (MIC) method assessed organic acids and antibiotics for bactericidal action.
Results: The study showed that malic acid has a higher ratio of inhibition that increase from 0.06 to hit 0.14 in concentration 0.3 mg/ml, while Ampicillin's ratio of inhibition fluctuates the same level until rise gradually to reach 0.04 in concentration 0.09 mg/ml. Succinic acid's ratio increased to 0.35 in concentration 0.4 mg/ml, while in 0.5 mg/ml hit 0.4 and dropped to 0.1 in the concentration of 0.6 mg/ml, in contrast to Erythromycin that have a higher ratio of inhibition increased gradually to reach 1.5 in concentration 0.7 mg/ml.
Conclusion: Urinary tract infections caused by *E.coli* may be challenging to treat empirically in Ile-Ife due to antibiotic resistance. In order to reduce the selection pressure that could lead to the spread of E. coli in the environment and to increase the likelihood of a successful treatment, culture and susceptibility tests must be performed prior to administering any antibiotics.

Keywords: Urinary tract infection, Escherichia coli, Succinic acid, Malic acid, Antibiotic resistance, University of Baghdad.

INTRODUCTION

One of the most prevalent bacterial diseases in humans is urinary tract infection (UTI), which is most usually brought on by *E.coli* [(1)]*;* The main source of nosocomial and community-acquired diseases [(2)].

When exposed to antimicrobials, commensal *E.coli* in the gastrointestinal system is particularly capable of passing on its genes for resistance to antibiotics to a variety of other microorganisms, including pathogenic bacteria [(3)]. This is supported by the findings of a great deal of study that has been carried out over the years [(4,5)].

Colonization and/or infection of humans is possible through unintentional or occupational contact, as well as through the intake of food that has been tainted with pathogens. There is a widespread dissemination of antibiotic-resistant microorganisms throughout the food chain, most notably in animal products [(6)].

There has been an increase in the number of *E.coli* isolates that are resistant to treatment, and a number of studies have discovered epidemiological linkages between these strains and humans as well as food [(7,8,9)].

Urinary tract infections that are brought on by *E.coli* in Ile-Ife may be difficult to treat empirically with only nitrofurantoin as a treatment due to the extensive resistance to regularly used antibiotics in the region.

Before beginning treatment, it is important to do susceptibility and culture tests on the bacterium that is causing the infection in order to determine whether or not it is susceptible to the antimicrobial agents that will be used [(10)].

For severe UTIs such pyelonephritis, amoxicillin-clavulanic acid is the treatment of choice. Amoxicillin-clavulanic acid resistance rates among UPEC vary greatly between regions. In the European countries, the levels of resistance to this antimicrobial range from 5.3% (Germany) to 37.6% (France) [(11)].

Seven distinct antibiotics were evaluated, and each one was shown to be effective against a different isolate. Each of the isolates that was examined had some level of resistance to the various antibiotics that were utilized in the examination process. The majority of bacteria tested positive for resistance to ampicillin, but just a small percentage of bacteria tested positive for resistance to gentamicin [(12)].

Researchers discovered that the organic acids in cranberry juice can prevent *E.coli* from colonizing the bladder in a mouse model of a urinary tract infection (UTI). The study was conducted on mice [(13)].PCR

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enables the detection of target genes with great speed, specificity and sensitivity [14].

Therefore, PCR-based methods have been developed to detect *E.coli* in clinical [15], the rapid concurrent detection of virulence factors by mu ltiplex PCR is a practical technique for identifying *E.coli* that causes urinary tract i nfections [16].

The aim of this study is using PCR methods to detect *E.coli* in urinary tract infection samples and also attempted to examine the antibiotic susceptibility of *E.coli* strains isolated from people and food in addition to determining potentially pathogenic isolates.

MATERIAL AND METHODS

Isolation and identification of *E.coli*

The 40 urine samples of UTIs were obtained from patients for this investigation from 3 hospitals in Baghdad, Iraq. The patients' ages ranged from 1 to 60 years, of both genders. Urine samples were taken from a midstream location and transferred in two hours to the lab in a standardized, sterile container. The samples were grown for 24 hours at 37°C using MacConkey agar and Eosin Methylene blue agar media. Biochemical tests were used to distinguish between pink lactose-positive colonies and green metallic sheen colonies [17]. The API 20E system validated the biochemical test results (BioMerieux, France).

DNA extraction

After 24 hours of incubation at 37°C with a single colony, genomic DNA was extracted from *E.coli* bacterial cells by according to the technique provided by the manufacturer of a DNA extraction kit made by Promega in the United States. Spectrophotometric analysis was used to determine both the amount of DNA present and its level of purity.

Detection of antibiotic resistant genes by PCR

Table 1 summarizes the *Uid* A gene's PCR detection res ults. In accordance with the directions of each earlier investigation, the thermal cycler conditions for the Uid A gene were carried out. The PCR products were electrophoresed in an agarose gel (1%), with ethidium bromide at a concentration of 0.5 g/ml for forty minutes at a voltage of ninety volts, and the bands were then seen using a device called a Gel Doc 2000 transmittance system [18].

Table 1. Oligonucleotide primer for *E. coli* gene.

Gene	Primer	DNA sequence (5′ → 3′)	Size (bp)	Thermocycling conditions	References
UidA	*Uid* A F	5′-ATGGAATTTCGCCGATTTTGC-3′	194	95°C for 5 min, 40 cycles of 95°C for 30 s, 60°C for 1 min, 72°C for 1 min, and final extension at 72°C for 7 min	Heijnen and Medema (2006)
	UidA R	5′-ATTGTTTGCCTCCCTGCTGC-3′			

Determination of MIC for Succinic acid and Malic acid

The minimum inhibitory concentration was determined by assaying the ability of bacteria *E.coli* to grow in broth cultures containing different concentrations of the succinic acid and malic acid respectively. The following dilutions 0:10; 1:9; 2:8; 3:7; 4:6; 5:5; 6:4; 7:3; 8:2; 9:1 were prepared in test tubes as succinic acid: nutrient broth. They were inoculated with 0.1ml of bacteria ($1.5*10^5$ cfu/ml) then incubated at 37°C for 24hrs. Growth intensity of each tube was observed by inculcation on nutrient agar and incubation at 37°C for 24hrs. Then recorded as light (+), medium (++), heavy (+++), and no growth (-). Growth was estimated by using spectrophotometer, and optical density (OD.600) nm for each dilution. Results were matched with the growth intensity mentioned by Midolo *et al.*, (1995) [19]. The same procedure was repeated for malic acid. The lowest concentration of the extract that prevented growth of pathogenic bacteria was considered as the minimum inhibitory concentration [20].

Determination of MIC for Erythromycin and Ampicillin

The minimal inhibitory concentration was obtained by observing the ability *of E.coli* bacteria to grow in broth cultures containing various doses of Erythromycin and Ampicillin. As erythromycin: nutrient broth, the following dilutions were made in test tubes: 0:10; 1:9; 2:8; 3:7; 4:6; 5:5; 6:4; 7:3; 8:2; 9:1.

They were inoculated with 0.1ml of bacteria (1.5*105 cfu/ml) then incubated at 37°C for 24hrs. Inculcation on nutrient agar and incubation at 37°C for 24 hours was used to determine the growth intensity of each tube, which was then classified as light (+), medium (++), heavy (+++), and no growth (-). For each dilution, growth was measured using a spectrophotometer and optical density (OD.600) nm. The growth intensity was matched with the results [19]. The administration of ampicillin followed the same pattern. The minimum inhibitory concentration (MIC) was found by testing the extract at a variety of concentrations against a number of different types of dangerous bacteria [20].

Ethical considerations:

The Institute of Genetic Engineering and Biotechnology at Baghdad University and the Ethics Committee at hospitals in Baghdad have both given their blessing to the initiative. Each participant in the meeting signed a document indicating their agreement. The Declaration of Helsinki, established by the World Medical Association, was followed in all studies involving human subjects.

Statistical analysis

The collected data were introduced and statistically analyzed by utilizing the Statistical Package for Social Sciences (SPSS) version 20 for windows. Qualitative data were defined as numbers and percentages. Quantitative data were described as means and standard deviations (SD).

RESULTS

Twenty different bacterial isolates, 12 from females and 8 from males, were collected from individuals exhibiting clinical signs of UTI. Out of 45 patient samples, 78% of *E.coli* isolates were found to originate from females and 22% from males, making *E.coli* the most often isolated species.

A total of 40 samples were tested for the presence of *E.coli* using a battery of morphological and biochemical techniques (**Table 2**). The API 20E system confirmed the results of the biochemical testing (BioMerieux, France). However, the presence of the uidA gene in *E.coli* strains has been confirmed by PCR, providing evidence that the organism does in fact exist. The bands began at 194 beats per minute (**Figure 1**).

Table 2: Biochemical tests of *E.coli* isolated from UTI **samples**

Biochemical test	Escherichia coli reaction
Gram staining	G-ve, Small Rod
Citrate utilization	-
Oxidase	-
Indole	+
Methyle red	+
Voges-Proskauer	-
Catalase	+
Lactose	+
Urea hydrolysis	+
Nitrate reduction	+
Casein hydrolysis	+
Gelaatin hydrolysis	+

+ = 90 to 100% of the isolates were positive; − = 0 to 10% of the isolates were Positive.

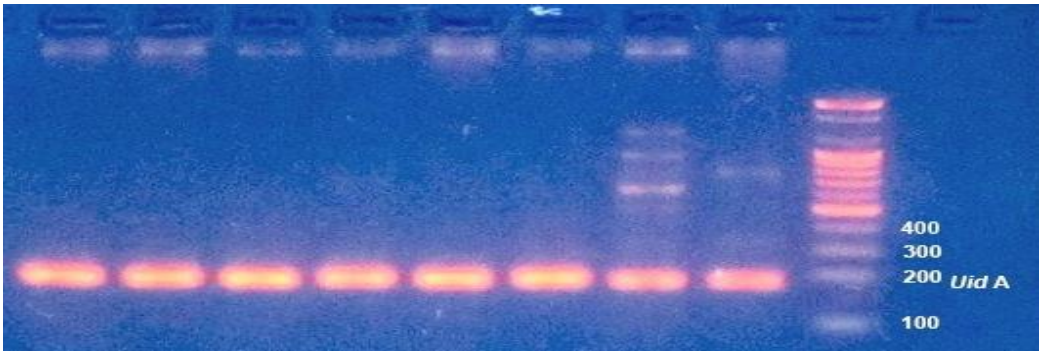


Figure 1: Electrophoresis of agarose gels containing UidA gene amplification products. l00bp ladder (M), lane Successful outcome in lanes 2-8, with positive bands totaling 194 bp Lane 10: Negative control *E.coli*.

Table 3 has shown that MIC's of the succinic acid for the *E.coli* bacteria. Results of the table indicated that the first two concentrations (1:9 and 2:8) had no effect against bacteria while heavy growth of bacteria was noticed after incubation. However, the growth was reduced at the following two concentrations (6:4 and 7:3) for tested bacteria. The situation was different at the concentration 8:2 since no bacterial growth was observed. The last two concentrations of succinic acid (9:1) were sufficient to cease growth of *E.coli*.

Table (3): Minimum inhibitory concentrations (MIC's) of succinic acid against *E.coli*.

Bacteria	Concentration of succinic acid: media								
	9:1	8:2	7:3	6:4	5:5	4:6	3:7	2:8	1:9
E.coli	-	-	+	+	++	++	++	+++	+++

- : no growth, + : light growth, ++ : medium growth, +++ : heavy growth.

Table 4 has shown that MIC's of the malic acid for the *E.coli* bacteria. Results of the table indicated that the first two concentrations (1:9 and 2:8) had no effect against bacteria while heavy growth of bacteria was noticed after incubation. However, the growth was reduced at the concentration 6:4 for tested bacteria. The situation was different at the concentration 7:3 since no bacterial growth was observed. The last two concentrations of malic acid (8:2, 9:1) were sufficient to cease growth of *E.coli*.

Table (4): Minimum inhibitory concentrations (MIC's) of malic acid against *E.coli* .

Bacteria	Concentration of malic acid: media								
	9:1	8:2	7:3	6:4	5:5	4:6	3:7	2:8	1:9
E.coli	-	-	-	+	+	++	+++	+++	+++

- : no growth, + : light growth, ++ : medium growth, +++ : heavy growth.

Table 5 shows MIC of the Ampicillin for the *E. coli* bacteria. Results of the table indicated that the first two concentrations (1:9 and 2:8) had no effect against bacteria while heavy growth of bacteria was noticed after incubation. However, the growth was reduced at the concentration 6:4 for tested bacteria. The situation was different at the concentration 7:3 since no bacterial growth was observed. The last two concentrations of Ampicillin (8:2, 9:1) were sufficient to cease growth of *E.coli*

Table (5): Minimum inhibitory concentrations (MIC's) of Ampicillin against *E. coli*.

Bacteria	Concentration of Ampicillin acid : media								
	9:1	8:2	7:3	6:4	5:5	4:6	3:7	2:8	1:9
E.coli	-	-	-	+	+	++	+++	+++	+++

- : no growth, + : light growth, ++ : medium growth, +++ : heavy growth.

The MIC of Erythromycin for *E. coli* bacteria is shown in **Table 6**. The table showed that the first two concentrations (1:9 and 2:8) had no effect on bacteria, whereas considerable bacterial growth was observed following incubation. However, the growth of tested bacteria was inhibited at the following two concentrations (6:4 and 7:3). At the concentration of 8:2,

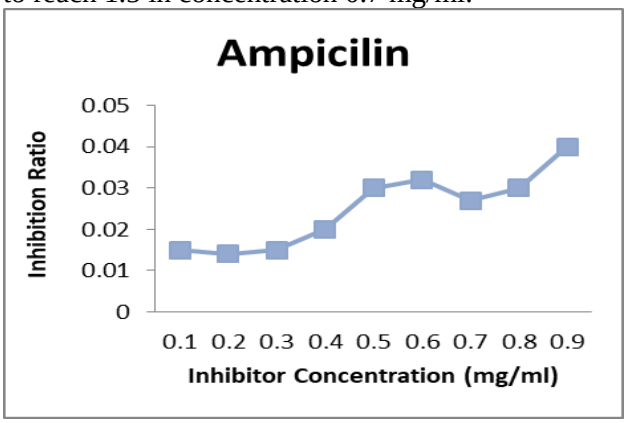
the situation was different, as no bacterial growth was seen. The last two amounts of Erythromycin (9:1) were enough to stop *E. coli* from growing.

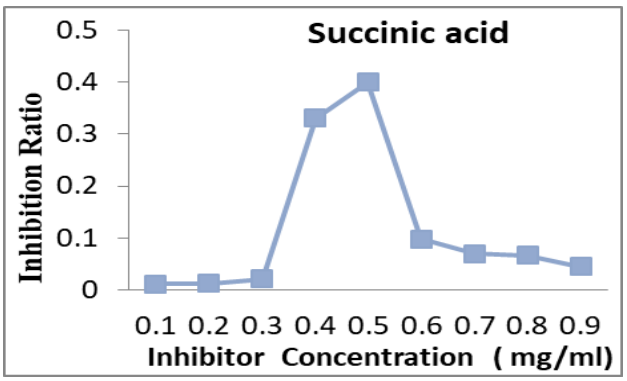
Table (6): Minimum inhibitory concentrations (MIC's) of Erythromycin against tested bacteria.

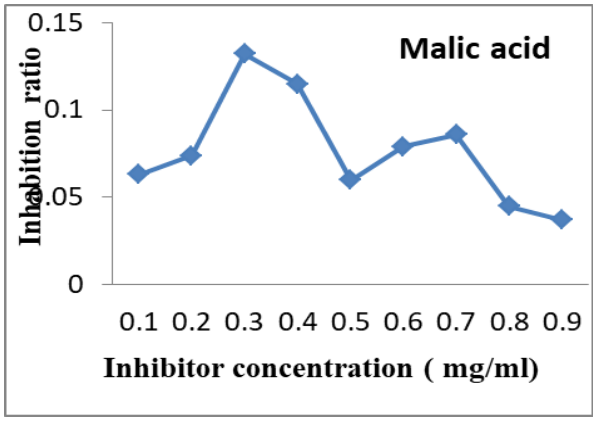
Bacteria	Concentration of Erthromycin acid : media								
	9:1	8:2	7:3	6:4	5:5	4:6	3:7	2:8	1:9
E- coli	-	-	+	+	++	++	++	+++	+++

- : no growth, + : light growth, ++ : medium growth, +++ : heavy growth.

Figure 1 has shown that the malic acid and succinic acid high inhibition ratio than Ampicillin and Erythromycin. Generally, there is a significant difference between the Ampicillin and malic acid in antimicrobial activity, in comparison to Ampicillin, malic acid have a higher ratio of inhibition that increase from 0.06 to hit 0.14 in concentration 0.3 mg/ml of malic acid and dropped back to 0.06 in concentration 0.5 mg/ml, while the ratio of inhibition in Ampicillin fluctuate the same level until arise gradually to reach 0.04 in the concentration of 0.09 mg/ml. The comparison between succinic acid and Erythromycin showed that there are many differences in antimicrobial activity, the ratio of inhibition in succinic acid showed no inhibition and maintain the same level from concentration 0.1 to 0.3 mg/ml and Increased to 0.35 in concentration 0.4 mg/ml while in 0.5 mg/ml hit 0.4 and dropped to reach 0.1 in the concentration of 0.6 mg/ml, in contrast to Erythromycin that have a higher ratio of inhibition increased gradually to reach 1.5 in concentration 0.7 mg/ml.







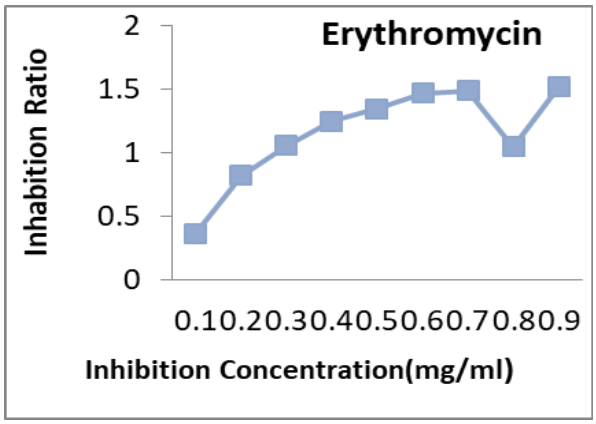


Figure (1): The relationship between the types of inhibitor and inhibition concentration for two organic compounds (malic acid and succinic acid) and two antibiotics (Ampicillin and Erthromycin).

DISCUSSION

According to the findings of a recent study, the treatment of urinary tract infections caused by *E.coli* could be difficult due to the widespread resistance of antibiotics. Before administering any treatment, it is important to conduct susceptibility and culture tests on the pathogen that is responsible for the infection. This will allow for the prevention of medication that does not work and the mitigation of selection pressures that favor the spread of *E.coli* in the environment. Using a recently developed real-time PCR assay for the detection of *E.coli*, *E.coli* O157, and the

shiga-like toxin genes Stx1 and Stx2, it is now possible to type *E.coli* colonies that have been produced in culture plates in a manner that is both speedy and accurate [(21)]. Both ampicillin (90.1% resistant) and cefotaxime (80% resistant) were ineffective against the vast majority of gram-negative bacterial isolates, which demonstrated high levels of resistance to both antibiotics. *E.coli* was the primary contributor to the problem in 64.2% of the cases that were reported **[(22)].**

The inhibitory ratio of malic acid and succinic acid is larger than that of ampicillin and erythromycin. There are several distinctions in antibacterial activity between succinic acid and erythromycin, as was discovered by the analysis, and between ampicillin and malic acid, in general, Against New Delhi metallo-lactamase-1 *E.coli*, we tested the antibacterial activity of citric acid, fumaric acid, malic acid, oxalic acid, succinic acid, and tartaric acid both on their own and in combination with colistin. There was a unique inhibitory zone that corresponded to each acid **[(23)]**.

However, the synthesis of antibiotics and other innovative drugs is a time-consuming and expensive process that must go through numerous phases before being subjected to clinical testing. Therefore, one possible alternative for treating germs that are resistant to antibiotics is the reuse of drugs that have already been granted approval for usage **[(24)]**.

There are different compounds recommended as alternative of antibiotics such Green Synthesis of Cu/CuO Nanoparticles **[(25)]**, Berberine **[(26)]**, and others. Based on what has been discussed so far, it recommended to testing some organic acids against a wide range of harmful antibiotics resistance bacteria, viruses, and parasites such as *Clostridium perfringens* **[(27)]**, *Brucella melitensis* **[(28)]**, *Proteus vulgaris* **[(29,30)]**, *Staphylococcus aureus* **[(31)]**, *Pseudomonas aeruginosa* **[(32)]**, *Toxoplasma* spp. **[(33,34)]** and SARS-Cov-2 **[(35)].**

Author Contributions

All authors agreed to have their work published in this journal, contributed to or revised it for key intellectual substance, and assumed responsibility for all parts of the work from its inception to its completion.

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REFERENCES

1. **Foxman B (2014):** Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. Infect Dis Clin., 28(1):1-13.
2. **Von Baum H, Marre R (2005):** Antimicrobial resistance of Escherichia coli and therapeutic implications. Int J Med Microbiol., 295(6-7):503-11.
3. **Smith J, Drum D, Dai Y *et al.* (2007):** Impact of antimicrobial usage on antimicrobial resistance in commensal Escherichia coli strains colonizing broiler chickens. Appl Environ Microbiol., 73(5):1404-14.
4. **Angulo F, Nargund V, Chiller T (2004):** Evidence of an association between use of anti-microbial agents in food animals and anti-microbial resistance among bacteria isolated from humans and the human health consequences of such resistance. J Vet Med., 51(8-9):374-9.
5. **Wang H, Manuzon M, Lehman M *et al.* (2006):** Food commensal microbes as a potentially important avenue in transmitting antibiotic resistance genes. FEMS Microbiol Lett., 254(2):226-31.
6. **Riano I, Moreno M, Teshager T *et al.* (2006):** Detection and characterization of extended-spectrum β-lactamases in Salmonella enterica strains of healthy food animals in Spain. J Antimicrob Chemother., 58(4):844-7.
7. **Manges A, Smith S, Lau B *et al.* (2007):** Retail meat consumption and the acquisition of antimicrobial resistant Escherichia coli causing urinary tract infections: a case–control study. Foodborne Pathog Dis., 4(4):419-31.
8. **Johnson J, Sannes M, Croy C *et al.* (2007):** Antimicrobial drug–resistant Escherichia coli from humans and poultry products, Minnesota and Wisconsin, 2002–2004. Emerg Infect Dis., 13(6):838.
9. **Thorsteinsdottir T, Haraldsson G, Fridriksdottir V *et al.* (2010):** Prevalence and genetic relatedness of antimicrobial-resistant escherichia coli isolated from animals, foods and humans in Iceland. Zoonoses Public Health, 57(3):189-96.
10. **Olorunmola F, Kolawole D, Lamikanr A (2013):** Antibiotic resistance and virulence properties in Escherichia coli strains from cases of urinary tract infections. Afr J Infect Dis., 7(1):1-7.
11. **Kot B (2019):** Antibiotic Resistance among Uropathogenic. Pol J Microbiol., 68(4):403-15.
12. **Kiiti R, Komba E, Msoffe P *et al.* (2021):** Antimicrobial Resistance Profiles of Escherichia coli Isolated from Broiler and Layer Chickens in Arusha and Mwanza, Tanzania. Int J Microbiol., 2021. doi: 10.1155/2021/6759046
13. **Jensen H, Struve C, Christensen S *et al.* (2017):** Cranberry juice and combinations of its organic acids are effective against experimental urinary tract infection. Front Microbiol., 8:542. doi: 10.3389/fmicb.2017.00542, 8, 542.
14. **Nissen M, Sloots T (2002):** Rapid diagnosis in pediatric infectious diseases: the past, the present and the future. J Pediatr Infect Dis., 21(6):605-12.
15. **Takeshi K, Ikeda T, Kubo A *et al.* (1997):** Direct detection by PCR of Escherichia coli O157 and enteropathogens in patients with bloody diarrhea. Microbiol Immunol., 41(10):819-22.

16. **Salih E, Nader M, Rasheed M (2015):** Rapid Detection of Uropathogenic Escherichia Coli virulence factors in Iraqi patients by multiplex polymerase chain reaction. World J Pharmace Res., 4(12):507-12.
17. **Moulin-Schouleur M, Schouler C, Tailliez, P *et al*. (2006):** Common virulence factors and genetic relationships between O18: K1: H7 Escherichia coli isolates of human and avian origin. J Clin Microbiol., 44(10):3484-92.
18. **Kerrn M, Klemmensen T, Frimodt-Møller N *et al*. (2002):** Susceptibility of Danish Escherichia coli strains isolated from urinary tract infections and bacteraemia, and distribution of sul genes conferring sulphonamide resistance. J Antimicrob Chemother., 50(4):513-6.
19. **Midolo P, Lambert J, Hull R *et al*. (1995):** In vitro inhibition of Helicobacter pylori NCTC 11637 by organic acids and lactic acid bacteria. J Appl Bacteriol., 79(4):475-9.
20. **Nikaido H, Vaara M (1985):** Molecular basis of bacterial outer membrane permeability. Microbiol Rev., 49(1):1-32.
21. **Heijnen L, Medema G (2006):** Quantitative detection of E. coli, E. coli O157 and other shiga toxin producing E. coli in water samples using a culture method combined with real-time PCR. J Water Health, 4(4):487-98.
22. **Hasan M, Ghaima K, Abdulhassan A *et al*. (2021):** Prevalence and Antimicrobial Susceptibility of Gram-negative Bacteria in Pediatric Patients with Urinary Tract Infections in Baghdad Hospitals. Ann Romanian Soc Cell Biol., 25(6):6102-9.
23. **Kumar R, Chandar B, Parani, M (2018):** Use of succinic & oxalic acid in reducing the dosage of colistin against New Delhi metallo-β-lactamase-1 bacteria. Indian J Med Res., 147(1):97-101.
24. **Mrtatha K, Alzrkani R, Abdulkareem A (2022):** Uropathogenic Escherichia coli Antibiotic Resistance and in silico Virtual Screening Using Pharmit Technique. Iraqi J Biotechnol., 21(1):26-41.
25. **Saeed N, Ibrahim L, Abdulameer S *et al.* (2022):** Green Synthesis of Cu/CuO Nanoparticles and Their Antibacterial Effects against Streptococcus Mutans. Egypt J Hosp Med., 89(2):6923-7.
26. **Khalid T,Ghaima K (2022):** Effect the Natural Efflux Pump Inhibitor (Berberine) in Multidrug Resistant Kleibsiella pneumoniae Isolated from Urinary Tract Infections in Several Baghdad Hospitals. Egypt J Hosp Med., 89(2):6882-8.
27. **Hashim S, Fakhry S, Rasoul L *et al.* (2021):** Genotyping toxins of *Clostridium perfringens* strains of rabbit and other animal origins. Trop J Nat Prod Res., 5(4):613-6.
28. **Awadh H, Hammed Z, Hamzah S *et al*. (2022):** Molecular identification of intracellular survival related *Brucella melitensis* virulence factors. Biomedicine (India), 42(4):761-5**.**
29. **Abdul-Gani M, Laftaah B (2017):** Purification and characterization of chondroitinase ABC from *Proteus vulgaris*, an Iraqi clinically isolate. Curr Sci., 113(11):2134-40.
30. **AL-Imam M, AL-Rubaii B (2016):** The influence of some amino acids,vitamins and anti-inflammatory drugs on activity of chondroitinase produced by *Proteus vulgaris* caused urinary tract infection. Iraqi J Sci., 57(4A):2412-21.
31. **Fakhry S, Hammed Z, Bakir W *et al*. (2022):** Identification of methicillin-resistant strains of *Staphylococcus aureus* isolated from humans and food sources by use mecA 1 and mecA 2 genes in Pulsed-field gel electrophoresis technique. Bionatura, 7(2):44. doi: 10.21931/RB/2022.07.02.44.
32. **Shehab Z, AL-Rubaii B (2019):** Effect of D-mannose on gene expression of neuraminidase produced from different clinical isolates of *Pseudomonas aeruginosa*. Baghdad Sci J., 16(2):291-8.
33. **Jiad A, Ismael M, Salih T *et al*. (2022):** Genotyping and evaluation of interleukin-10 and soluble HLA-G in abortion due to toxoplasmosis and HSV-2 infections. Ann Parasitol., 68(2):385-90.
34. **Jiad A, Ismael M, Muhsin S *et al*. (2022):** ND2 Gene Sequencing of Sub fertile Patients Recovered from COVID-19 in Association with Toxoplasmosis. Bionatura, 7(3):45. doi: 10.21931/RB/2022.07.03.45.
35. **Rasoul L, Nsaif M, Al-Tameemi M *et al*. (2022):** Estimation of primer efficiency in multiplex PCR for detecting SARS-Cov-2 variants. Bionatura, 7(3):48. doi: 10.21931/RB/2022.07.03.49.