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Evaluation Honey Concentration and Antibiotics Disc Against E.Coli Isolated From Urinary Tract Infection

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Abstract:

Background: *Escherichia coli is a nonspore creating, Gram-negative bacterium, usually move via peritrichous flagella. Escherichia coli is the most shared reason of critical urinary tract contagions as well as urinary tract sepsis.*

Methods: *In this study 100 patients with have sever renal infection were engage from Medical Al-Sader City and Al-Fourat Hospital in Najaf, city from 1 December (2022) to 1 April (2023) , The Chief identification depends on Gram stain, cluster morphology on dissimilar media and some biochemical tests , also used the Vitek 2 system to make confirmatory documentation.Then make observe resistance to antibiotics and antimicrobial activity assay by natural honeys .*

Results*: E. coli were found to be 52 (52%), while the other bacteria have percent 40(40%), the remaining samples 8 (8%) have no growth for bacteria.*

The antibiotics susceptibility test study of all isolates are examined, there was differences among isolates it has been found that more isolates were exhibited high resistance to Nalidixic acid and Ceftriaxone 34(65.3) %, while the isolates showed had moderate resistance rates to Ofloxacin and levofloxacin between 30 (57.6) % to24 (46.1) % and Ciprofloxacin 22(42.3) % showed the lowest resistance among fluoroquinolones ,the study showed most isolates were sensitive 44 (84.6)% to the antibiotic Amikacin and only 8(15.3) % have resistance to it. The antimicrobial activity of some natural honeys sample proved have effective in vitro Inhibitor activity against antibiotic hardy bacteria of E. coli causing several serious infections to humans ,the series dilution assays for honey samples on Escherichia coli isolates that have antibiotic resistant ,the result showed the MIC was measured the lowest attention of honey in which bacterial growing is inhibited saw on the nutrient agar surface of bacteria in ***H1*** *the* ***MIC*** *is between (****1:2,1:4 and 1:8****) v/v,also* ***H2*** *the* ***MIC*** *is between* ***(1:2,1:4,1:8 and 1:16)*** *v/v on resistant isolates of E.coli , The result from fifth dilution to eighth dilution showed high growth of colonies in nutrient agar when compare with the control .*

Conclusion:

- *In this study suggest that E.coli is the most corporate causes of infection renal in Iraqi patients and high levels of MDR of E.coli isolates in Najaf hospitals, E. coli isolates highly active against nalidixic acid and Ceftriaxone while weakly active against Amikacin .*
- *Honey created since some sources displays great antimicrobial activity,and different honey types vary in antimicrobial power due to their unique bioactiv compounds.*
- *The most important advantages absence of side special effects for patients (important downside of antibiotics and low prices of treatments).*

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I. Introduction

Escherichia coli, a Gram-negative bacterium that does not form spore, usually move via peritrichous flagella. It is the most conjoint culprit of critical urinary tract infections as sepsis. Additionally, E. coli has stayed implicated in newborn meningitis and abscess formation across various organ systems. *E. coli* may also cause acute enteritis in humans and animals Know as 'traveler's diarrhea', a dysentery-like disease affecting humans, and haemorrhagic colitis often referred to as 'bloody diarrhea'. The tenacity of *E. coli* in environments will certainly be aided through its aptitude to grow as a biofilm. (Flores *et al***.**2015)

UTI can take in contagion from urethra to the kidneys. Signs in case of cystitis include tender and common urination whereas condition as high fervor and wing pain are seen in pyelonephritis another symptoms of UTI include fiery feeling during urination, harm of bladder control, amplified of urination particularly in small amounts, low spinal pain, gloomy, and bloody or smelly urine (Subedi N. and Pudasaini S. 2017).

Enterobacteriaceae attend to accumulate the multidrug-resistance (MDR) genes, also develop different mechanisms against numerous antibiotics, consequently limiting therapeutic choices towards UTIs (Abbas *et al.* 2022)

Quinolones, particularly fluoroquinolones are antibiotics that have broad-spectrum frequently important treat urinary tract infections (UTIs) initiated by Enterobacteriaceae (Azargun *et al.* 2018).

Honeybees

Honey is formed after honeybees nursing on flower juice. The collected substances are mixed with other specific compounds from the honeybees and dropped as wax honeycomb and permitted to complete over time. The structure of honey hinge on the basis of the plants that bees nourish on it (Eteraf-Oskouei and Najafi 2013) honey has been used in the dealing of bacterial impurities, chills, cough, and various infectious diseases. making of hydrogen peroxide (H2O2), bee defensin-1, height osmolarity, and low pH seem to be important for honey's antibacterial efficacy. When using honey for therapeutic purposes, ensuring its quality is vital. Honey can contain various microorganisms, such as *Clostridium botulinum* and *C. tetani* endospores is frequently ignored, so sterilizing honey meant for medicinal use is essential to prevent potential infections. (Chou, J. W.,et al,2018).

Additionally, nectar constructing plants are often treated with herbicides and pesticides,can contaminate honey. Environmental impurity, weighty metals, and the use of antibiotics in beekeeping may impact honey quality. Therefore, establishing a clear definition of honey purity and safety is essential "medical grade honey" It is essential to ensure the safety and efficacy of honey used in treatments. Medical-grade honey must take at the resulting criteria:

1. **Organic and Pure**: Free from contaminants and poisonous substances.
2. **Sterilized**: Gamma-sterilized under standardized conditions to eliminate harmful microorganisms.
3. **Safe for Medical Use**: Suitable for implementation in medical therapies.
4. **Regulated Manufacture and Storing**: Adheres to severe legal, safety also quality standards.
5. **Physicochemical Compliance**: Meets specific criteria necessary for its use in wound care (Hermanns, R. *et al.*2018).

Honey, is primarily collected from sugars- glucose, sucrose, and fructose to make up approximately 80% of its weight, the residual 20% consisting of water. Additionally, honey contains vitamins, flavonoids, amino acids, enzymes, minerals, and phenolic acids. It also possesses anti-inflammatory properties (Tonks et al. 2003)

The catalase enzyme content, active ingredient composition, and overall effects of dissimilar types of honey can vary significantly dependent on the plant classes and region of origin (Llla Nagy-Radványi,*et al.*2024).

II. Materials and Methods

Patients and Clinical Specimens' Collection:

Patients and Samples: In this study 100 samples of the urine were collected from patients suffering from sever renal infection whose range of age is (20-70 years) from both sexes and at specific time from 1 December (2022) to 1 April (2023), were engaged to Al-Sader Medical City and Al-Fourat Hospital in Najaf City, Iraq.

In this study, infection and colonization were distinguished according to the Centers for Disease Control and Prevention (CDC, 2008) criteria. "We adhered to Iraqi and international ethical and privacy regulations throughout our study. Prior to input,we provided written informed consent. The study received IRB approval (617/2020) from the College of Science at the University of Kufa in Iraq, in agreement with the principles for the Statement of Helsinki for the protection of humanoid research subjects

Isolation with Identification of Bacterial Isolates

After the samples were collected from Mid-stream urine specimens put in sterilized screw-cap container, then transported quickly to department of bacteriology laboratory by use loop full (0.01 milliliter) of urine specimens after mix well had inoculated onto appropriate media, such as use MacConkey agar, Eosin _methylene blue (E.M.B) agar and blood agar, also examining microscopically after staining with Gram-stain. The primary diagnosis of isolates was recognized through Gram staining, remark of colony morphology on various media, also showing several biochemical tests.

The final confirmed identification process utilizes kits of the Vitek2 system for bacteria isolates, with GP-ID cards for Gram-positive bacteria and GN-ID cards for Gram-negative bacteria, providing ID message confidence levels ranging from very good to outstanding, with a prospect percentage of 95 to 99.

Antimicrobial sensitivity testing (AST) was shown by using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. Standard discs with distinct concentrations were utilized to determine the confrontation of isolates. The dishes were incubated for 18-24 huores , then area of inhibition was measured in millimeters (mm).

The areas were read as sensitive or resistant according to (CLSI ,2020), Antimicrobial sensitivity testing were performed with six types of antibiotics disc, which show effects on *Escherichia coli* isolates including Nalidixic acid (NA 30 ug) , Cefotaxime (CRO 10 ug), Amikacin (AK 10 ug), Oflaxicin (OFX 5 ug), Ciprofloxacin (CIP 10 ug) and levofloxacin(LEV 5 ug).

Antibacterial activity assay

The bacterial strains were grownup overnight in nutrient broth and incubated at 37°C for 18-24 hours and compared with (0.5) McFarland standard tube, in this study was performed to evaluate the antibacterial potential of the honey samples

Broth macrodilution : The bacterial broth in macrodilution assay, put 10 sterilized plane tube in rack point from1 to 8 leave two tube positive control refer to tube holding culture broth with bacterial inoculum, the negative control tend to culture broth with honey sample ,then(1 ml) of the sterile culture nutrient broth to each tube then add (1ml)% of the honey samples to tube number 1, then make macrodilution assay by take 1ml from first tube to the second ,to the third …..to the last tube take 1ml removed out with altered the tips step by step and then make (1:2, 1:4, 1:8,1:16,1:32, 1:64,1:128 and 1:256 v/v).all the tube putted in vortex aperture , The bacterial regular inoculum (1ml) was decanted into the tubes expect tube of the honey control and incubated at 37°C for 18- 24 houres. Samples occupied from the tubes into petridishes covering by nutrient agar to confirm bacterial growing. Inhibition of bacterial growing was observable as a clear broth and the existence of growth was noticed by the existence of turbidity. The experiment was repeated three times. (Kacanioova et al., 2011)

The Minimum Inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) is the lowest concentration of honey capable of inhibiting bacterial growth. It was determined using incubated tubes from the macrodilution assay, where a 100 µl sample from each tube was inoculated onto petridishes covering nutrient agar. The plates were then incubated at 36.5°C for 24 hours. The MIC defined as the lowermost honey concentration at which no bacterial growing was detected on the agar surface, indicating 99.9% bacterial demise. (Ahmad Aamer *et al*.2015).

III. Results and Discussion

Table 1: show Among 100 clinical specimens 92 sample have positive results, among them 52(52%) isolates are belonged to *Escherichia coli* while other 40(40%) isolates belonged to other bacterial genera including *Pseudomonas, Proteus, Klebsiella* and another, and another 8(8) % consider negative results, not occur growth.

Table 1: percentage of bacteria that isolated from clinical cases

Isolated No.	Positive culture		Negative culture
	Escherichia coli	Other bacteria	
100	52	40	8
100%	52%	40%	8%

In this study, *E. coli* accounted for 52% of the positive isolates. A study conducted in Lusaka, Zambia, reported a similar prevalence, with *E. coli* constituting 47.8% of isolates from clinical samples70, also findings indicate that 40% of the isolates were from genera such as *Pseudomonas*, *Proteus*, and *Klebsiella* (Maisa Kasanga,*et al*.2024) . In a study from Ethiopia, *Klebsiella pneumoniae* was identified in various clinical specimens, with a notable prevalence in blood samples (42.2%). (Belay Tafa Regassa *et al*.2023)

Bacterial Identification

Bacterial isolates identification by cultured morphological and microscopically charecteristic,The aptitude to ferment lactose gives choice use MacConkey agar to distinguish *E. coli* ,non-lactose fermenting so pink, round medium-sized colonies are picked as *E. coli* doubtful colonies and on Eosin _methylene blue (E.M.B) agar observed with green metallic sheen show in (figure 1) also, and examining microscopically after staining with Gram-stain.

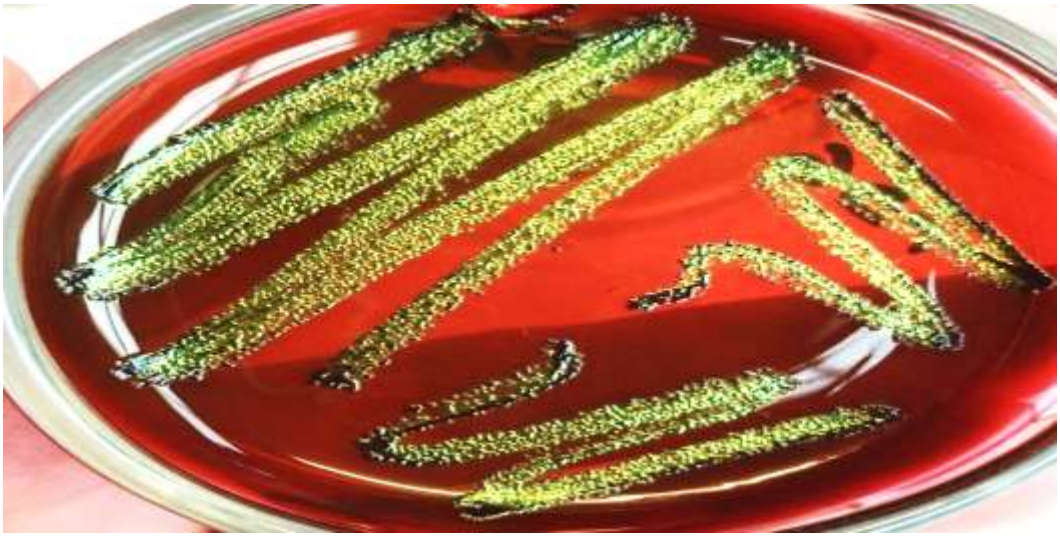

figure 1: metallic sheen of *E. coli* colonies on Eosin _methylene blue agar.

Biochemical tests: **table 2 show resultes for some biochemical tests are performed to identification of *E coli* isolates:**

Catalase test: By streaking the nutrient medium with the designated bacterial colonies and incubated at 37ºC for18- 24 hrs, then transfer limtied amoun of the growth by the loop place it on a clean slide then added a droplet of (3% H_2O_2), positive result show once the gas foams look **, Oxidase test** : A strip of filter paper is saturated with a little freshly made reagent Oxidase reagent, the colony is picked up with a sterilized wood rod and smeared over the filter paper. A Negative result is showed by not appeared purple color within 5-10 seconds, **Indole Test**: Peptone water was inoculated with culture and incubated at 37C^{0}for 24h, 3-5 droplets of Kovacs reagent (0.5 ml) then forming a red colour ring showing a positive result detected the Indole production, **Methyl red test :** The tubes of the (MR-VP broth) are inoculation with the designated bacterial colonies and incubated at 37^{o}C for 24 hrs. then5 droplets of methyl red reagent are added to it, appearance red color tend to a positive result and a complete analysis of glucose . by the same procedure employ **Vogas-prokauer** test but added Barritt's reagent to broth and Negative result refer to unable to production of acetyl-methel carbinol, **Simmon citrate** test was inoculated with a single colony of young culture and incubated in 37C^0 for 24 h, a used for

determining the ability of bacteria to utilize citrate as the sole carbon source by change to blue color and streak of growth appearance indicated a positive result while still green color tend to Negative result, (Forbes *et al*., 2007).

Table 2: Show the biochemical tests for *E coli*

Biochemical Tests	Results
Gram stain	G –ve, rod
Catalase test	Positive
Oxidase test	Negative
Indole test	Positive
Methyl Red	Positive
Vogas-prokauer	Negative
Simmon's citrate	Negative

Antibiotics susceptibility test Disc diffusion method These results are shown in(table 3) The antibiotics susceptibility study of all isolates are examined, there was differences of antibiotic susceptibility among isolates it has been found that more isolates were entirely resistant to Nalidixic acid and Ceftriaxone 34 (65.3)%, also 30(57.6) %to Oflaxicin, while the isolates showed nearly resistant to Ciprofloxacin and levofloxacin between 22(42.3)% to 24 (46.1) % ,the study showed most isolates were sensitive 44(84.6)% to the antibiotic Amikacin and only 8(15.3) % were resistance to it. trend to increasing antimicrobial resistance similar pattern for aminoglycosides.

Table 3: Antibiotics susceptibility test

Isolates of E.coli bacteria	Nalidixic- acid	Ofloxacin	Levofloxacin	Ciproflox-acin	Ceftriaxone	Amikacin
Resistance percentage	34(65.3)%	30(57.6) %	24(46.1) %	22(42.3)%	34(65.3)%	8(15.3)%
Sensitive percentage	18(34.6) %	22(42.3) %	28(53.8) %	30(57.6)%	18(34.6) %	44 (84.6)%

an older quinolone including nalidixic acid, has seen increased resistance over time. A study highlighted that *E. coli* isolates from Iraq exhibited significantly higher resistance to various antibiotics, compared to isolates from the USA,Ofloxacin, a fluoroquinolone in Iraq research indicates that *E. coli* strains show higher resistance to fluoroquinolones compared to those in other regions and rising resistance levels, Levofloxacin show lower resistance as the same study reported that 76.2% of *E. coli* isolates from Iraq were fluoroquinolone-resistant, underscoring the challenge in using these antibiotics.(Debarati Choudhury, *et al*.2024)

The Minimum Inhibitory concentration (MIC) of honey

The results are presented in (Table 4)

Two honey patterns were option **H1: Honey sample from north of Iraq in AL-Najaf Governorate** and ***H2: Honey sample from south of Iraq in Duhok Governorate** .the result showed honey has strong antimicrobial activity by using the series dilution assays for honey samples at higher concentrations **(1:2, 1:4, 1:8)** on *Escherichia coli* isolates that have antibiotic resistant**,**where t is H2 is slightly more effective than H1 because have differ unique bioactve compound otherwise dilution after 1:16 lose activity gradually **.**

Table 4: Effect of two honey concentrations patterns (H1 and H2) on bacterial growth of antibiotic-resistance for *E. coli* isolated

Honey sample	Honey Concentration (v/v)									
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	Honey control	Broth culture contol
*H1	-	-	+	++	+++	+++	+++	+++	-	+++
*H2	-	-	-	++	+++	+++	+++	+++	-	+++

***H1**: **Honey sample from north of Iraq,** ***H2**: **Honey sample from south of Iraq**

(-) : Refer to No growth of colonies in nutrient agar **(+) :** Refer to trace growth

(++) : Refer to moderate growth, **(+++):** Refer to heavy growth

These resulte tend to potential of honey as a natural antibacterial agent and support its traditional use in infection management

This study match research on the antibacterial properties of honey at various concentrations. Studies have demonstrated that higher concentrations of honey exhibit significant inhibitory effects on bacterial growth, while dilution reduces this efficacy. For instance, a comparative study found that honey concentrations of 50%, 75%, and 100% effectively inhibited bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella* species.

Similarly, Different concentrations (50, 75 and 100 %) of honey were studied in-vitro using Staphylococcus aureus, Escherichia coli, Citrobacter, Klebsiella spp. Salmonella typhi, Pseudomonas aeruginosa, Bacillus spp, and Enterococcus faecium. The data showed the inhibitory effect of honey with clear zones of inhibition.(Saranraj, P. (2017).

In similar study proven evaluates the antibacterial activity of 4types of Indian and Yemeni honey at concentrations of 80% and 50% (w/v) against various bacterial pathogens, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus and* Methicillin-Resistant *Staphylococcus aureus* (MRSA), employs the agar well diffusion method, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays to assess antibacterial efficacy. Results indicate that all tested honey samples exhibit antibacterial activity, with higher concentrations yielding stronger inhibitory effects. also Yemeni honey demonstrated more activity as antibacterial properties compared to Indian honey. (Saeed ,M. (2020).

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