Susceptibility of *Klebsiella spp.* to the crude extract of *Thuja orientalis*

Ammar Jawad Kadhim[*1], Dhuha Badr Mahmood[1] and Sulaiman Dawod Sulaiman[1]

1Department of Biology, Al-Farabi University College Baghdad, Iraq

[*] Corresponding author e-mail: ammarjkadhim92@gmail.com

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Abstract

The present study was conducted to investigate the antibacterial activity of *Thuja orientalis* extract against fourteen isolates of Klebsiella from urinary tract infection patients. Antibiotic susceptibility test was done against eight antibiotics to determine the bacterial resistance. The highest bacterial resistance was against Cefixime and Trimethoprim (100%), while the lowest resistance was against Ciprofloxacin, Levofloxacin and Imepenem (28.57%). Finally the effect of *Thuja oriantalis* plant extract was tested and results showed that, this plant extract has marked inhibitory effect against all Klebsiella isolates.

Keywords: *Thuja orientalis, Klebsiella,* Urinary tract infection (UTI), Antibacterial activity.

1. Introduction

Klebsiella spp. is very notable among human pathogenic bacteria. They are non-motile, gram-negative, facultative anaerobic, lactose fermenting, encapsulated, rod-shaped bacteria, found as mouth, intestine, and skin normal flora (Ryan and Ray, 2004). *Klebsiella* spp. are pervasive, occurring naturally in (soil, vegetables, and water). In humans, *Klebsiella* spp. cause different infections, such as (pneumonia, septicemia, urinary tract infections, abdominal infections, and soft tissue infections) (Podschun and Ullmann, 1998). Antibiotics are utilized against illnesses brought about by *Klebsiella spp.*, but these pathogens are becoming progressively antibiotic-resistant, and many are now labeled as Multidrug-resistant (MDR) (Yan *et al.*, 2001; Ktari *et al.*, 2006). Thus it is significant for researchers to figure out alternative medicines. A urinary tract infection (UTI) is an infection in any part of the urinary system. UTI is the occurrence of a certain bacterial number in urine, generally more than 10^5cell/ml. Symptomatic UTI was classified according to the site and severity as a- pyelonephritis, b- urosepsis syndrome or upper UTI, with infection of the kidney, and c- cystitis or lower UTI, with bacteria in the bladder (Smelov *et al.*, 2016). The causative agents that are most common in UTIs are *Klebsiella* spp., *Escherichia coli*, *Enterococcus faecalis*, members of the *Proteae* tribe, Group B Streptococci (GBS), *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus*, and *Staphylococcus aureus*, in addition to *Candida* spp. (Stefaniuk *et al.*, 2016).

UTI is one of the most common infections in humans, with more than 150 million cases worldwide (Terlizzi *et al.*, 2017). Additionally to that, UTIs also represent 36% of all healthcare-associated infections (HAI) (Parker *et al.*, 2017). Based on epidemiology, community-onset UTI is viewed widely as a disease of women only. In America, it is estimated that at least half of women will suffer a UTI during their lifetimes (Lane and Takhar, 2011).

The incidence of UTI in middle age is almost limited to females but specific populations of males exhibit an increased risk of UTI. The sex differences seen in UTI epidemiology have been traditionally related to anatomic and, less conclusively, hygienic factors, which are the permeability of colonization by microbes of the surrounding vaginal and perineal environments, a shorter distance from the anus to the urethral meatus, and shorter length of the urethra in females (Olson, 2018).

Since ancient times, medicinal plants have been utilized because of their advantageous impacts on the treatment and control of various disorders. As a result of reported cost-effectiveness, fewer side effects, lack of bacterial resistance, and easy availability, even at the beginning of the 21st century, medicinal plants have gained much more popularity and reliability worldwide (Das *et al.*, 2015).

Thuja orientalis is a typical ornamental evergreen tree belonging to the family Cupressaceae, originally native to Northwest China. It is a highly resinous shrub and aromatic generally developed in gardens located in temperate and semi-temperate places. *T. orientalis* is a large, hardy evergreen shrub or a tree of a small to medium size scarcely exceeding 20 m in nature. Its shape is pyramidal, and dense, but usually exhibits, a more open and scattering form preferring moist, well-drained soil and full sun. Its bark is grayish with brown highlights and contains thin but deep furrows. Younger bark is reddish-brown and exfoliates in long, thin strips (Kaya *et al.*, 2014).

T. orientalis is being utilized as a natural medication from old times and sorted as one of the fundamental herbs in conventional meds where it is primarily utilized in treating conditions such as expectorant, antitussive, anti-inflammatory, antifungal, antibacterial, antioxidant activity, and utilized in homeopathy both externally and internally (Khubeiz *et al*., 2016).

The present research aimed to screen the antibacterial activity of *Thuja orientalis* extract against isolates and assess its potential use in treating infections brought about by these multi-drug resistant clinical isolate.

2. Materials and Methods

2.1. Samples collection

Fourteen *Klebsiella* isolates were obtained from private hospitals and clinical laboratories in Baghdad city. The bacterial isolates were isolated from patients' urine samples with urinary tract infections (UTIs). All samples were subjected to general urine examination, biochemical tests, and cultural identification.

2.2. Bacterial identification

The suspected *Klebsiella* spp. isolates were identified using a number of morphological, physiological, and biochemical tests. Gram staining, motility, methyl red (MR), hydrogen sulfide production, and Voges-Proskauer Test (VP) were among the tests conducted.

Bergey's Manual of Determinative Bacteriology was referenced for all tests (Bergey and John, 1994).

2.3. Antibiotic Susceptibility Testing

The Kirby-Bauer method was applied to determine the susceptibility of *Klebsiella* isolates to different antibiotics. First pure colonies were grown on the nutrient agar medium and then transferred into the brain heart infusion broth, incubated at (37ºC) for 4 hours to standardize, and obtain turbidity equal to the standard of McFarland tube (0.5) which was previously prepared, to be used as turbidity standard. A portion of the bacterial culture was transferred by using a sterile cotton swab, carefully spread on the Mueller-

Hinton agar medium, and left for 10 min. Subsequently, antibiotic susceptibility screening was performed using the disc diffusion method. Antibiotic discs (Mastdiscs, U.K) used include (Amikacin, Cefixime, Ciprofloxacin, Gentamicin, Imepenem, Levofloxacin, Tetracycline, and Trimethoprim) effective against gram-negative bacteria. Disks were placed on the agar medium by sterile forceps and pressed firmly to ensure contact with the agar, after that, the plates were inverted and incubated at 37ºC for 18 hours. The test was done as Vandepitte *et al.*, (2003) described.

Inhibition zones that developed around the discs were measured by millimeters (mm) by using a metric ruler mentioned by CLSI, (2020). Isolates were interpreted as either susceptible, intermediate, or resistant to a particular drug by comparison with standard inhibition zones as illustrated in Table-1.

Table 1: Standard inhibition zones (CLSI, 2020)

Id	Antibiotics	Conc. µg/disk	Diameter of inhibition zone by (mm)		
			Susceptible	Intermediate	Resistant
1	Amikacin	10	≥17	15-16	≤14
2	Cefixime	5	≥19	16-18	≤15
3	Ciprofloxacin	10	≥26	22-25	≤21
4	Gentamicin	10	≥15	13-14	≤12
5	Imepenem	10	≥23	20-22	≤19
6	Levofloxacin	5	≥21	17-20	≤16
7	Tetracycline	10	≥15	12-14	≤11
8	Trimethoprim	10	≥16	11-15	≤10

2.4. Preparation of plant-extract

The plant extract was obtained from a local market. The stock solution of the plant extract was diluted by nutrient broth to make serial dilutions by dissolving 1 ml of the plant extract in 1 ml of nutrient broth, and three-fold serial dilutions were prepared from it to reach the final concentration of 25 µg\ml. Four concentrations (stock (200), 100, 50, and 25µg\ml) of the plant extract were tested against *Klebsiella* isolates in Muller Hinton agar by making wells in the plates. The plates were streaked with *Klebsiella* isolates and 50µl of plant extract was put in the wells, then plates were incubated for 18 hours at (37ºC) in order to test the plant extract effect against the growth of isolates.

3. Result and Discussion

3.1. Identification of bacteria

All of the possible isolates that produced colonies like those of *Klebsiella* spp. were sub-cultured in order to evaluate their physiological, morphological, and biochemical characteristics. All isolates of bacteria tested were facultative anaerobes, Gram-negative, and negative for the Indole test.

Isolates could ferment sucrose and glucose but could not produce H2S in the Triple Sugar Iron (TSI) test, were non-motile, negative in the MR test but positive in the VP test and lactose fermenters appeared as pink colonies on MacCkonkey agar Figure-1.

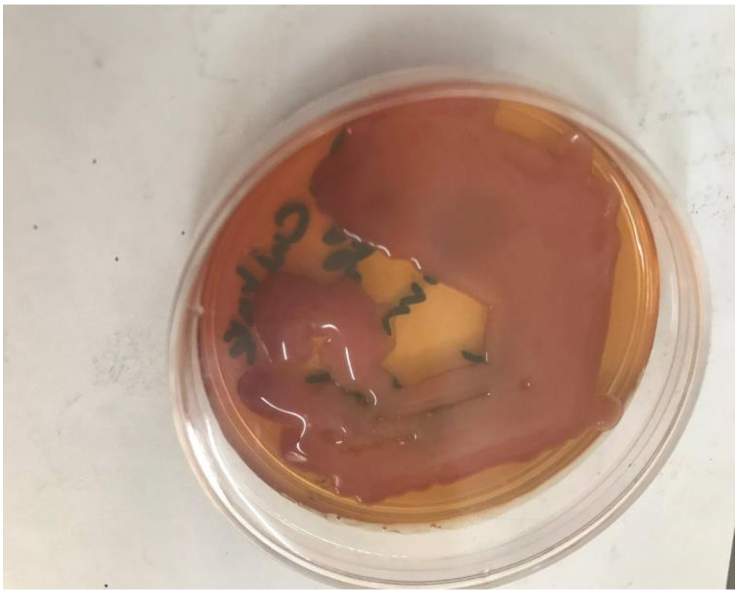

Figure 1: ***Klebsiella*** **on MacCkonkey agar**

3.2.Evaluation the antibiotic susceptibility of bacterial isolates

Susceptibility of *Klebsiella* isolates to different antibiotics was as shown in Figure-2. The disc diffusion test was performed as a quick screening method to indicate the susceptibility of isolates to antibiotics. Results of antibiotic susceptibility of our isolates are summarized in Table-2. According to the results of the Kirby-Bauer method, the highest percentage of resistance was against Cefixime and Trimethoprim (100%), while the lowest bacterial resistance was against Ciprofloxacin, Levofloxacin, and Imepenem (28.57%) as shown in Figure-3.

Figure 2: Antibiotic susceptibility test shows the resistance of ***Klebsiella*** **against different antibiotics**

Table 2: Results of antibiotic susceptibility test for *Klebsiella* against different antibiotics

No.	Antibiotics	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Amikacin	R	R	I	R	R	I	I	R	R	I	R	R	I	I
2	Cefixime	R	R	R	R	R	R	R	R	R	R	R	R	R	R
3	Ciprofloxacin	S	R	I	S	R	S	S	S	R	I	S	R	S	S
4	Gentamicin	R	R	S	R	R	S	S	R	R	S	R	R	S	S
5	Imepenem	I	R	S	I	R	S	S	I	R	S	I	R	S	S
6	Trimethoprim	R	R	R	R	R	R	R	R	R	R	R	R	R	R
7	Tetracycline	R	R	R	R	R	R	I	R	R	R	R	R	R	I
8	levofloxacin	S	R	I	S	R	S	S	S	R	I	S	R	S	S

S: Susceptible **I: Intermediate** **R: Resistant**

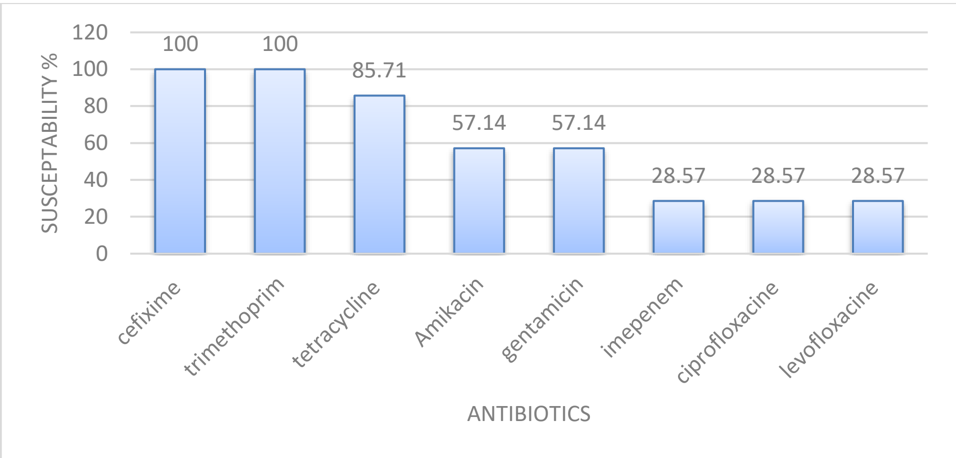

Figure 3: Resistance percentage of *Klebsiella* spp. against Amikacin, Cefixime, Ciprofloxacin, Gentamicin, Imepenem, Levofloxacin, Tetracycline, and Trimethoprim

The results showed that Cefixime and Trimethoprim were the less effective antibiotics against Klebsiella spp. with a 0.0% effect, this was identical to the reported results of (Akinpelu et al., 2020), as they found (100%) of Klebsiella isolates were resistant to cefixime. Our results differed from (Özgen and Eyüpoğlu, 2020) results, as they reported that (48.78%) of *Klebsiella* isolates were resistant to Trimethoprim.

The resistance of *Klebsiella* toward Tetracycline was (85.71%), this was nearly close to that of (Shahwany *et al*., 2016), as they found that 100% of isolates were Tetracycline resistant.

While the resistance against Amikacin and Gentamicin was (57.14%), these results were different from the results of (Özgen and Eyüpoğlu, 2020), they found that the bacterial resistance to Amikacin was (8.15%), but similar to the results of (Sharmeen *et al*., 2012), they reported that Gentamicin resistance was 55%.

Finally, Imepenem, Levofloxacin, and Ciprofloxacin showed the lowest resistance (28.57%). These results were different from the results of (Özgen and Eyüpoğlu, 2020), they found that the bacterial resistance toward Imepenem was (12.77%), but similar to Levofloxacin resistance (29%) and close to Ciprofloxacin resistance 33.46%.

3.3. The impact of plant extracts on bacterial growth

The effect of plant extract on the growth of *Klebsiella* was tested by making wells in the Muller Hinton agar plate, and then *Klebsiella* were streaked on the agar plates, and the plant extract was put in the wells. Different concentrations of the plant extract were tested (200, 100, 50, and 25 µg\ml). Only the concentration of 200 µg\ml was effective against the *Klebsiella* isolates, it showed a growth inhibition zone, indicating that the plant extract inhibited the growth of *Klebsiella* (figure 4).

Figure 4: Effect of plant extracts on *Klebsiella* growth

The results of the presented study showed similarities with other results of previous Iraqi studies that were conducted to determine the antimicrobial activity of *T. orientalis*, and it was effective in inhibiting microbes against some pathogenic microorganisms including *Klebsiella* (Kamona, 2011).

Two studies were done in Turkey, showed a moderate effect of *T. orientalis* extract (Digrak *et al*., 2002 and Oskay *et al*., 2009).

Other studies in India and Nigeria indicated similar results of the antibacterial potential of *T. orientalis* extract, it was quite effective against *Klebsiella* (Bissa *et al*., 2008; Joginder *et al*., 2013; Ololade *et al*., 2014 and Olonisakin *et al*., 2017).

Conclusions

The results of antibiotic susceptibility showed a higher resistance of *Klebsiella* toward different antibiotics and the plant extract exhibit an inhibition effect against *Klebsiella* growth. This justifies the increased traditional use of plants, and therefore it is considered a better alternative therapy for the treatment of *Klebsiella* infection as the antibiotic resistance of bacteria increases yearly.

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حساسية بكتريا ***Klebsiella* spp.** للمستخلص الخام من نبات العفص ***Thuja orientalis***

عمار جواد كاظم، ضحى بدر محمود وسليمان داود سليمان
قسم علوم الحياة/ كلية الفارابي الجامعة/ بغداد - العراق

الخلاصة

أجريت الدراسة الحالية للتحقق من الفعالية المضادة للبكتيريا لنبات العفص *Thuja orientalis* ضد أربعة عشر عزلة من بكتريا *Klebsiella* من مرضى مصابين بعدوى المسالك البولية. تم إجراء اختبار الحساسية للمضادات الحيوية ضد ثمانية مضادات حيوية لتحديد المقاومة البكتيرية. كانت أعلى مقاومة جرثومية ضد Cefixime وTrimethoprim (100٪) بينما أقل مقاومة كانت ضد Ciprofloxacin ، Levofloxacin و Imepenem (28.57٪). وأخيرا تم اختبار تأثير مستخلص نبات العفص Thuja oriantalis وقد أظهرت النتائج أن هذا المستخلص النباتي له تأثير مثبط ملحوظ ضد جميع عزلات *Klebsiella*.