Synergistic Effect of Some Natural Substances in Combination with Antibiotics on MDR *Klebsiella* isolates

Mohammed Shaker Mahmoud Alani[1], Laith Musleh Najeeb Al Nuaimi[2]

[1]Bacteriologist/ Al-Amiriya General Hospital / Ministry of Health / Al-Anbar/ Iraq, [2]Assist prof./Department of Biology/ College of Science / University of Anbar Al-Anbar /Iraq

Abstract

This study included the collection of 210 samples of Mid-stream urine samples of the patients who were confirmed to be infected with UTI by conducting a general urine examination and the patients of AL-Amriya General Hospital, Ramadi Teaching Hospital and Fallujah Teaching Hospital for the period between 2017 and January 2018. The results of the diagnosis showed a seven isolations of 17.5% of the total of 40 bacterial isolates. These isolations were diagnosed in aerobic conditions according to the microscopic examination and Culture characteristics on blood agar base and MacConkey agar as well as the biochemical tests, Diagnosis was confirmed using the modern diagnostic system VITEK2.

The synergistic activity of the natural substances used (sesame oil, olive oil, and royal Jelly) was studied against *Klebsiella* isolates by interfering with some of the antibiotics by disk diffusion method (Gentamicin, Amikacin, Ceftriaxone, Trimethoprim-Sulfamethoxazol, Nitrofurantoin, Amoxicillin/Clavulanic acid, Ciprofloxacin), and the synergic efficacy of natural materials was studied by adding 10 ul of sterile pasteurized natural substance directly to the antibiotic disk separately. The results showed increased efficacy of some antibiotics, especially Ciprofloxacin, Ceftriaxone and Amoxicillin/Clavulanic acid. so it can be concluded that sesame oil and royal Jelly have synergistic effectiveness in increasing the effectiveness of some antibiotics against *Klebsiella*.

Key Words: *Klebsiella spp., Natural substance, Antibiotic resistance, Synergism*

Introduction

The genus *Klebsiella spp* belongs to the enterobacteriaceae. This species includes several types: *K.pneumonia*, which affects all sites of the human body, especially the respiratory tract and urinary tract. The type *K.ozaenea*, affects the nose, as well as the respiratory tract and the urinary tract and blood and for the type (*K.rhinoscleromatis*) it affects the nose, and (*K.oxytoca*) It is similar to the first type in its ability to infect all sites of the human body[(1)].

These bacteria cause many diseases for both human and animal such as pneumonia, urinary tract infection, soft tissue inflammation, sepsis, meningitis, diarrhea [(2)].

Corresponding author:
Mohammed Shaker Mahmoud Alani
mhammed_shaker2003@yahoo.co.uk

The development of bacterial resistance to antibiotics, as well as the emergence of new strains of the most important factors causing the disease, as it constitutes a major concern for society and also for public health [(3)].

The study of plant extracts is important to demonstrate the mechanics of their work and effectiveness, as well as their toxic effect. World Health Organization (WHO) has approved the use of medicinal plants to treat various diseases[(4)]. WHO has shown that 80% of the world's population (mostly developing countries) still rely on conventional medicine for the treatment of common diseases[(5)].The most important components of most vegetable oils are: Carvacrol, Eugenol, Linalool, Thymol. These compounds have been shown to have a wide spectrum of antimicrobial activity, some of which inhibit the growth of bacteria and some is lethal according to the concentration used. Sesame oil is one of the most important oils used because it contains fatty

acids (saturated and unsaturated). Unsaturated fatty acids reduce oxidation. In addition, oil contains flavonoids (Sesamol, Sesamolin, Sesamin), which have anti-oxidant properties,which contributes to keeping sesame oil in its natural properties for a long time without oxidation, and it also stimulates the work of vitamin E[6].

Research has shown that olive oil has an important effectiveness as phenolic olive oil compounds have antimicrobial properties, especially phenol compounds (oleuropein, hydroxytyrosol, tyrosol), which have been shown to have strong antimicrobial activity against several bacterial strains responsible for intestinal inflammation, respiratory tract infections and urinary tract infections[7,8]. Antimicrobial activity against foodborne pathogens such as *Staphylococcus aureus* and *Salmonella enteritidis* has been demonstrated in the laboratory when use high concentrations of oleuropein[9,10] have been used. In addition, oleuropein and hydroxytyrosial have antimicrobial effects against pathogenic bacteria and viruses[11,12].

On the other hand, some studies have shown that royal jelly has an anti-bacterial effect higher than the rest of bee products. The most sensitive organisms recorded are *S. aureus*, followed by *E. coli*[13]. Recently, royalisin, a powerful antimicrobial protein in royal jelly, has demonstrated the selective growth inhibition against gram-positive bacteria (*Lactobacilus helveticus*, *Clostridium*, *Corynebacterium*, *Leucnostoc*, *Stafilococcus*, *Streptococcus*), with effective concentrations less than 1μm[14]. Propolis and albumin in royal jelly have a role in inhibiting bacterial growth. Propolis also has a synergistic effect with antibiotics in the treatment of infections and also reduces resistance of antibacterial walls to bacterial cells[15].

Materials and Methods

Collection of samples

Urine samples were collected according to method[16], in special and sterile container, who have been found to have UTI through primary examination, which includes see of Pus cells and RBCs In the urine under microscope , to confirm the infection of the urinary tract, and then transferred directly to the laboratory under refrigerated conditions for the purpose of culture and diagnosis for the period between October 2017 to January 2018.

Isolation and diagnosis of *Klebsiella* spp.

Diagnosis by simple methods

Bacterial isolates were identified based on both culture characterize, microscopic examination, and biochemical tests, and based on what Goldman and Lorence[17].

- Culture characteristic and microscopic examination

the shape of the colonies on the different culture media , size, height, shape of edges, color, and ability to ferment some of the sugars were observed and made smears for gram stain and examined under a light microscope to observe the shape and arrangement of bacterial cells and also their response to the gram stain.

- Biochemical tests

These tests were conducted according to the way they were[18] and also[19].

Diagnosis in modern methods

The diagnosis was confirmed using the VITEK2 diagnostic kit was used to further confirm the diagnosis of isolates for the species level[20].

Antibiotic sensitivity test

The efficacy of antibiotics (Gentamicin, Amikacin, Ceftriaxone, Trimethoprim-Sulfamethoxazol, Nitrofurantoin, Amoxicillin/Clavulanic acid, Ciprofloxacin) was tested against bacteria by following the disc diffusion method, depending on the method [21], then the bacteria were considered resistant if the inhibition diameter reached as indicated by the indicator In the standard specifications mentioned in (CLSI 2013).

Testing of Interference between Natural and Antimicrobial Materials

The interference test between the antibiotics under study with the natural substances (sesame oil, royal Jelly and olive oil) was performed for bacterial isolates, depending on the method [21], and then put 10μl of sterile sesame oil in pasteurization, and then incubated the dishes at a temperature of 37°C for a period of 18-24 hours and the diameter of the inhibition zone of the antibiotic with sesame oil was measured. and the same treatment was repeated with olive oil as well as royal jelly separately.

The Statistical analysis

The statistical analysis of the statistical package of

social sciences-Version-22 (SPSS).

Results

The results showed the isolation and diagnosis of *Klebsiella spp*. With a rate of (17.5%) of the number of isolates by 7 isolates, which are Gram negative bacteria, the *Klebsiella spp*. isolates were distinguished Which was isolated by the fact that its colonies are large in size and with mucosal shape due to they contain capsule, They are pink on the MacConkey medium for their ability to ferment the lactose sugar and are non-moving bacteria, positive for the Urease and Catalase tests as well as for the vogas- Proskauer, , And for the test of the indol, methyl red and the oxidase, they are negative and non produce hydrogen sulfide.

Klebsiella isolates showed resistance to (Ciprofloxacin, Trimethoprim-Sulfamethoxazole, Amoxicillin / Clavulanic acid, Ceftriaxone) were 100% and the resistance ratio of (Gentamicin, Amikacin and Nitrofurantion) was 85.7% for each antibiotics.

The results of the statistical analysis showed significant differences at the level of probability ($P \leq 0.05$). The results showed that the overlap of natural substances (sesame oil, olive oil, royal Jelly) with the antibiotics under study has a special role in increasing the sensitivity of antibiotics.

The results of the interaction of the antibiotics with the natural substances used showed significant differences in all antibiotics discs. The results of the Ciprofloxacin interaction showed significant differences, especially the increase in synergy with the royal Jelly. The rate of inhibitory diameter of the antibiotic disc was increased after its synergy with the royal Jelly compared to the standard of (4 mm) to 7.28 mm. Ciprofloxacin with both sesame oil and olive oil was increased for inhibition compared with the standard (5.14 mm and 4.85 mm) respectively, From 6.14 mm to 9.1 4 mm) compared to standard when synergistic with royal Jelly, as shown in the table 1.

Table 1: interaction of the antibiotics with the natural substances

P value	Synergistic antibiotic with natural substances			Control (antibiotic only)	Antibiotics
	Royal Jelly	Olive oil	Sesame oil		
0.002*	7.28	4.85	5.14	4	Ciprofloxacin
0.029*	12.14	13	12.57	12.57	Amikacin
0.043*	9.14	8	6	6.14	Augmentin
0.006*	2	2.71	2.85	1.71	Trimethoprim-Sulfamethoxazole
0.006	11.42	14.42	13.57	13.42	Nitrofurantion
0.000*	5.85	4.57	3.14	3.71	Ceftriaxone
0.006*	10.28	10.57	10.42	10.28	Gentamicin

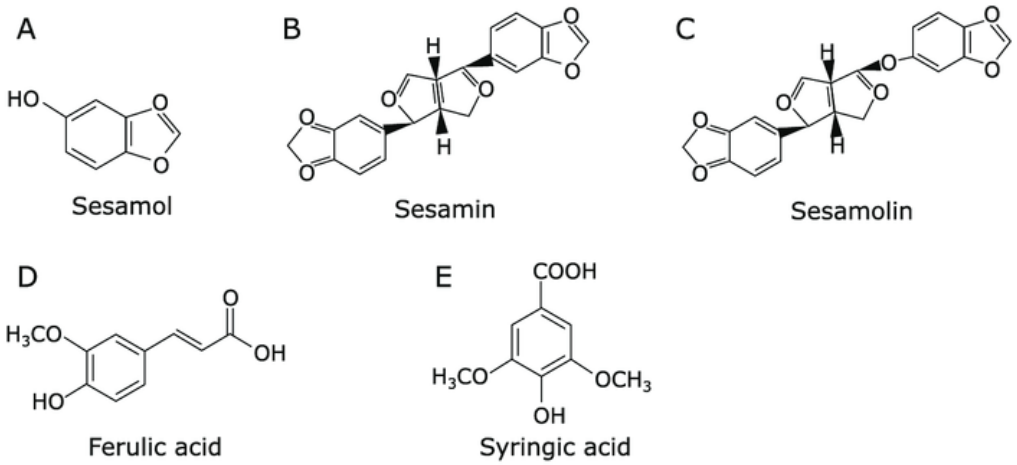
* Significant difference at a level below (0.05)

The results showed that there is a significant difference in the sensitivity of the isolates to the antibiotics alone, on the one hand, and the change of sensitivity in the use of antibiotics with natural substances on the other, due to the synergistic act between the natural substances and antibiotic.

The results of this study agree with what he found[22], which showed that the synergistic action affects the resistant and sensitive bacteria. The synergistic effect has a significant impact on the resistant and sensitive species and in percentages that may vary depending on the species. The synergy of natural substances and antibiotics reduces antibiotic resistance and also cooperates with antibiotics in the killing of bacteria [23] as confirmed in their study.

The results of the present study are consistent with what is found [24] in increasing the sensitivity of bacterial isolates to antibiotics mixed with honey, indicating the synergistic action between them.

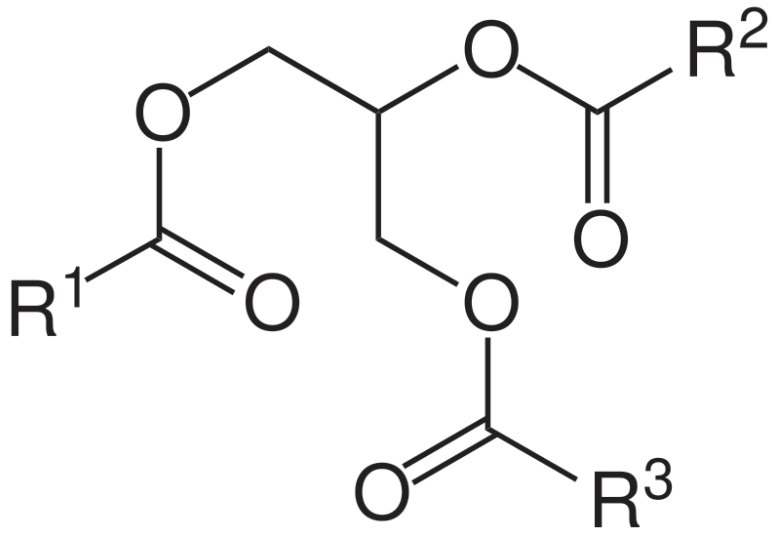
And the current study is consistent with[25] what found sesame oil shows different therapeutic characters , which indicates its antibacterial properties on Enterobacteriaceae .



Sesame oil contains phenolic groups capable of binding or depositing water soluble proteins. This disrupts the cell function and causes the cell to stop functioning by inhibiting enzymes, destroying the plasma membrane[26].

It can be said that the use of this extract will be a natural substance that provides the body with important compounds as well as the inhibitory effect of pathogenic Klebsiella bacteria when used by patients and this was the main purpose of using this extract against bacteria[27].

Our results are in concordance with those reported by[28] and[29], who observed higher activity in olive oils . The authors attributed this activity of olive oil to the phenolic compounds; the dialdehidic form of decarboxymethyl oleuropein and ligstroside aglycons, hydroxytyrosol, and tyro-sol were the phenolic compounds.



Phenolic products consisting of polyphenol, flavonoids, and tannic acid have been reported to have satisfactory antibacterial activity against food-borne pathogens[30].

Khan[31] has shown that many phytochemicals have an inhibitory effect on a wide range of microorganisms that have been used as drugs such as alkaloids, soapins, flavonoids and polyphenols.

The mechanism of action of these compounds is not clear, but there are studies indicating that these compounds enter the cell and interfere with the process of cellular metabolism, while other studies indicated that phenols (Carvacrol and Eugenol), Disturb the cell membrane and thus reach the effective site in the Enzyme[32].

Fatty acids and monoglycerides found in olives have been found to have a wide range of killer activity of microbes against bacteria and yeasts[33].

As indicated by[34], who indicated in his results that the inhibitory effect is most likely associated to lipid-containing molecules present in ether soluble fraction of royal jelly. The weaker antibacterial activity in ether-non-soluble fraction of royal jelly may be attributed to royalisin which is a portion of the ether-non-soluble fraction of royal jelly[35].

Studies on raw royal jelly have shown that because of its antimicrobial properties it can inhibit the growth of several types of microbes, including MRSA. In addition, the synergistic effect of mixing royal jelly with other products, such as honey, significantly improves antibacterial activity against dangerous pathogens such as *Staph*. *aureus*,*P*. *aeruginosa* [36,37].

Conclusions

Natural substances under study have a significant effect on increasing the effectiveness of antibiotics against *Klebsiella* spp. Royal jelly is most effective in increasing the efficacy of antibiotics, especially the antibiotic Ciprofloxacin.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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