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Determination of the minimum inhibitor concentration of *Syzygium aromaticum* extract on the growth of *Pseudomonas aeruginosa* isolated from burn infection

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ABSTRACT

The current study aimed to investigate the effectiveness of aqueous Clove extract and detect its minimum inhibitory concentration .This work was conducted at Azadi Teaching Hospital from a total of 56 samples taken from patients having burn infection using cotton swabs, and samples were cultivated in MacConkey and Nutrient agar and incubated inside the incubator for 24 hours. After incubation, samples were diagnosed by the Api20E system. Where 43 isolates were obtained from *P. aeruginosa*, 9 isolates from *E. coli*, 4 isolates from *Klebsiella* and 3 isolates of *Staph. aureus.*

The cold aqueous extraction method of *Syzygium aromaticum* was used and the minimum inhibitory concentration (MIC) was measured after culturing the bacteria in a nutrient broth medium containing known concentrations of the extract. And the susceptibility of the bacteria to a group of common antibiotics has been determined.

The results showed that 72.8% of the burn infection were caused by *P. aeruginosa*, and that the (MIC) value ranged between (0.01-0.1 mg / ml). And that the bacteria showed multi resistance to antibiotics, and high sensitivity to Nitrofurantoin.

1- Introduction

A burn is a type of the skin injury due to heat , electricity, cold, chemicals, radiation, friction. [1] Most burns are caused by heat from hot solids, liquids, or fire. [2].

Burns that affect superficial layers are known as first-degree . It appears red and the pain lasts about 3 days. [3] But injury extended to the lower layers of the skin, it is second-degree burn. Blisters are present very painful. Recovery may take up to eight weeks and scarring.

In a complete burn, the injury extends to all skin layers of. Usually there is no pain and the burned area is stiff. Usually healing does not happen on its own . The burn is often black and often results in the loss of the burnt portion. [4]. *P. aeruginosa* is a Gram-negative bacterium, rod-positive for citrate, catalase and oxidase tests, is of great medical importance and is a multidrug-resistant pathogen, possessing advanced mechanisms of antibiotic resistance.

And its association with serious diseases such as hospital-acquired infections such as pneumonia associated with a ventilator and various sepsis syndromes. [5] The organism is opportunistic and generally affects immune compromised persons but can also infect immune compromised persons as in hot tub folliculitis [6].

Pseudomonas aeruginosa infection may be difficult due to its natural antibiotic resistance. And when more advanced antibiotic drug regimens are needed, harmful effects can be produced. [7,8]

Cloves are aromatic flower buds of the *Syzygium aromaticum* that is widely cultivated in Indonesia, and is used as a spice. Cloves are available throughout the year due to the different harvest seasons in different countries. [9] Evergreen plant 8–12 meters (26–39 feet) high, and has long been used in traditional medicine. And clove containing eugenol is effective for pain like dental pain. [10] [11] Due to the bioactive chemicals of cloves, it can be used as an ant repellent. [12]The smell of cloves comes from eugenol [13].

2- Material and methods

2-1 Collection of bacterial samples

56 swabs were collected from people with wound infections who were lying in the Azadi Teaching Hospital, Burns Department. The swabs were implanted on the center of MacConkey agar and incubated at 37Co for 24 hours. After incubation the isolates were initially diagnosed based on the phenotypic characteristics of the colonies on the culture media, including the size, edge, height and color of the colonies. Then the cells were stained with Gram stain, and the phenotypic characteristics of the cells were observed under a combined optical microscope, including the shape, size of the cells, the method of grouping and the result of the interaction of the Cram stain. Then the diagnosis was confirmed by the Api20E system. As shown in Figure (1).

Fig. 1: Diagnosis of *P. aeruginosa* using the Api20E system

2-2 Collection of plant samples

The Clove flower buds were collected and washed with distilled water to remove the suspended soil. Then the samples were dried Within 24 hours at room temperature, and the dried samples were ground with an electric grinder and kept in sealed plastic bags to avoid contamination and moisture until use.

2-3 Preparion of plant extracts

In this study, cold aqueous extraction method was used. 40g of powder is mixed with 160ml of distilled water and placed in the refrigerator for 24 hours for the purpose of soaking. Then it was filtered with a microfiltration unit to prevent the passage of germs and non-mashed vegetable parts, and put it in the oven at 40°C until the water has completely evaporated to obtain a powder. The storage solution was prepared by taking (1 g) of the extracted powder and dissolving it with distilled water and then completing the volume to 10 ml to obtain a concentration of 100 (mg / ml)[14].

2-4 Bacterial suspension

Prepared by taking a volume of pure colonies and planted in tubes containing of Nutrient Broth, and mixed well to ensure the spread of bacteria in the nutrient medium, then the degree of growth in the liquid medium was measured with a standard McFarland tube. By doing a number of dilutions up to a concentration of (1.5 X 108 Cell / ml) [14].

2-5 Extract dilution preparation

1- A number of tubes were taken at the rate of 10 tubes, 9 ml of sterile Nutrient Broth were placed in the first tube of the ten tubes, and 5 ml of the nutrient broth were placed in each of the nine tubes.

2- Complete the volume to 10 ml in the first tube by added 1ml of extract and mixed well using a Vortex electric mixer until it was dispersed and mixed with the nourishing broth well. Thus, we diluted the extract ten times [15].

3- Take 5 ml from the first tube containing 10 ml of the disinfectant and nourishing broth and put in the second tube from the ten tubes containing 5 ml of the nutritious broth and mix well to homogenize the solution well.

4- This process was repeated above several times until reaching tube No. 10 and 5 ml was taken from it and neglected to be complete and double alleviation, and the homogeneity of the solution with the disinfectant was ensured completely in all relieving.

5- After completing the dilution preparation process, 0.1 ml of the previously prepared bacterial suspension was added to each of the ten prepared dilutions above and mixed well using a mixer device to ensure the homogeneity of the bacterial suspension with the disinfectant solution.

6- The tubes incubated at 37 ° C for 24 hours, the extent of the tubes' turbidity was observed, as well as the tubes in which no growth or turbidity appeared, as the last tube in which no growth or turbidity appeared, the MIC returned, after which the tube was found to have growth. The MIC of the extract was determined to determine the effectiveness of the extract by inhibiting or killing the bacteria. [14, 15]

2-6 Antibiotic Susceptibility Test

Anti-sensitivity test was performed using the standard (Kirby - Bauer method) as follows

1- The bacterial suspension was prepared by taking (4-5) young colonies of (16-24 hours) growing on the agar plate and sown in 5 mL of physiological solution with good shaking to homogenize the solution.

2- The density of the bacterial suspension was compared with the standard MacFarland turbidity constant (0.5 MacFarland turbidity standard) by adding an amount of suspension or physiological solution to give an equivalent turbidity (1.5 × 108 Cell / ml).

3- Spread the bacterial suspension on the surface of the pre-prepared Muller-Hinton agar medium by

using a sterile cotton swab by dipping the swab into the suspension tube after shaking well and rotating the swab on top of the tube to remove the excess solution. The surface of the sticks were inoculated with the swab in three directions, taking into account the wiping of the sides of the plate at the end, and the caps were left to dry for (5-10) minutes at room temperature.

4- The antibiotic discs were placed on the surface of the cultivated plate using sterile forceps, taking into account the sterilization of the forceps with flame after taking each tablet.

5- The dishes were incubated in the incubator at a temperature of 37 ° C for a period of 24 hours, and after the end of the incubation period, the diameters of the inhibition areas were recorded in millimeters around the disks and the results were compared with the standard specifications. [16, 17].

Result

The results of the current study showed that most of the burn infections were caused by *P. aeruginosa* with a rate of 72.8% but 15.2% *by E. coli*, 6.8% by *Klebsiella* and 5.2% by *Staph. aureus* as 43 , 9, 4, 3 isolates respectively diagnosed out of a total of 56 cases. As shown in Figure (2).

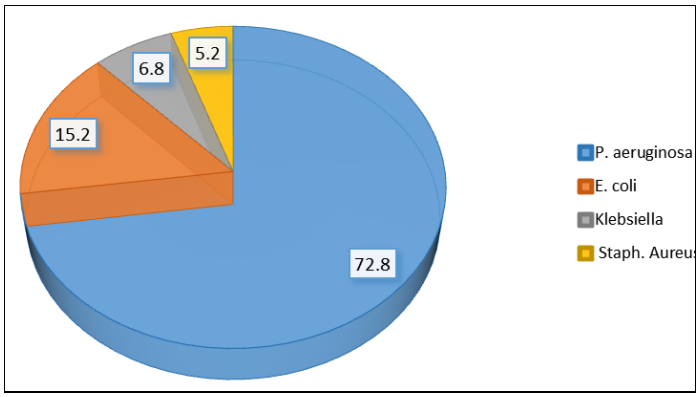

Fig. 2: shows the percentage of infection

And showed the effectiveness of clove plant extract against the growth of *P. aeruginosa* bacteria, as the minimum inhibitor concentrations of clove plant extract were between (0.01-0.1 mg / ml), by (0.01mg / ml) for (53.5%) of isolates and (0.1mg / ml) for (46.5%) isolates. Figure (3).

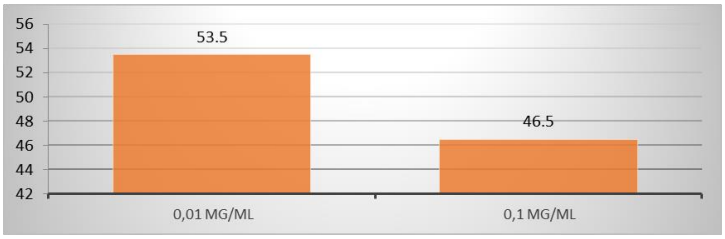

Fig. 3: shows the range of (MIC) against the growth of *P. aeruginosa*

The results of the current study showed multiple resistance of *P. aeruginosa* to antibiotics, as all isolates were resistant to the following antibiotics (Erythromycin, oxacillin, trimethoprim, ampicillin, tetracycline and clindamycin), While all isolates showed high sensitivity to Nitrofurantoin and intermediate sensitivity to the following antibiotics (Ogmentin, chloramphenicol, amikacin and meropenem) As shown in Figure (4).

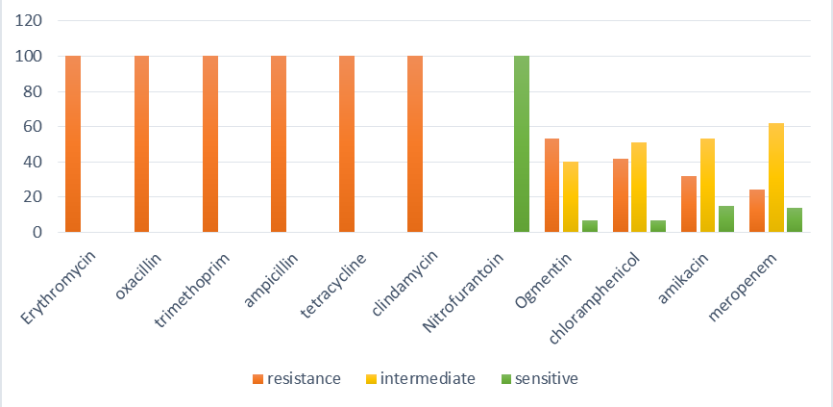

Fig. 4: shows Sensitivity of *P. aeruginosa* to antibiotics

Discussion

The burn is an open wound to the outside, so it is vulnerable to contamination with germs in the environment, especially opportunistic species, as *P. aeruginosa* is one of the most important types of bacteria that cause infections acquired in hospitals and it is one of the common types that cause burn inflammation and its danger lies in its resistance to many antibiotics [8].

This study is the approach of a Pakistani study conducted in 2014 on the types of bacteria that cause burn infections, as the results showed that *P. aeruginosa* and *S. aureus* are among the most common types of wound infection [15]. The extract of the clove plant has shown its effectiveness against the growth of bacteria because it contains substances that are anti-bacterial growth. The components of the essential oil include vanillin, acetyl eugenol, kartgulic acid, beta-caryophylline and tannins [10]. And gallutanic acid, methyl salicylate (a pain reliever), ramnitin, flavonoids eugenin, kaempferol, and eugenitin, triterpenoids such as cambesterol, stigmasterol, and many more sesquiterpene [13].

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تحديد التركيز المثبط الادنى (MIC) لمستخلص *Syzygium aromaticum* على نمو بكتريا *P. aeruginosa* المعزولة من الحروق الملتهبة

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الملخص

تهدف الدراسة الحالية إلى تحديد فعالية مستخلص القرنفل المائي والكشف عن تراكيز المثبط الادنى ، وقد تم إجراء هذا العمل في مستشفى آزادي التعليمي من إجمالي 56 عينة مأخوذة من مرضى مصابين بالحروق باستخدام مسحات قطنية ، وتم زراعة العينات في وسط MacConkey agar و Nutrient agar وتحضينها داخل الحاضنة لمدة 24 ساعة. بعد الحضن تم تشخيص العينات بواسطة نظام Api20E. حيث تم الحصول على 43 عزلة من *P. aeruginosa* , 9 عزلات من *E. coli* , 4 عزلات من *Klebsiella* و 3 عزلات من *Staph. aureus*.

تم استخدام طريقة الاستخلاص المائي البارد لـ *Syzygium aromaticum* وتم قياس تراكيز المثبط الادنى (MIC) بعد زراعة البكتيريا في وسط مرق مغذي يحتوي على تراكيز معروفة من المستخلص. وكذلك تم تحديد مدى حساسية البكتيريا لمجموعة من المضادات الحيوية الشائعة.

أظهرت النتائج أن 72.8٪ من الإصابة بالحرق سببها *P. aeruginosa* وأن قيمة MIC تراوحت بين (0.1−0.01 ml / mg). وأن البكتيريا أظهرت مقاومة متعددة للمضادات الحيوية ، وحساسية عالية لمضاد Nitrofurantoin.