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Effect Of Aqueous Extract And Fresh Solution Of Arabic Gum On *P. Aeruginosa* Bacteria Isolated From Burns Infections

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Summary

*Pseudomonas aeruginosa*is an opportunistic bacteria cause many nosocomial infections.It has many virulence factors help bacteria to infected patients and causing diseases, such as biofilm formation, secretion systems enzymes and toxins.

In current study 50 isolates of *P. aeruginosa* isolated and identified of seventy five samples were collected from patients suffering from burn infections. *P. aeruginosa* was found more prevalence between females 66% % compared with 34% of mails. Most bacterial isolates were found resistance to most used antibiotics. Also result showed that *P. aeruginosa* isolates were biofilm formation in 50% strong biofilm production and the other 50% week biofilm production. The present results showed that the effect of fresh solution of Arabic gum better than water extract of Arabic gum and the concentrations of (75-100) mg/ml is the (MIC) concentration to inhibit growth both strong and week*P. aeruginosa* biofilm production.

Introduction

*Pseudomonas aeruginosa*is an opportunistic bacteria cause many nosocomial infections particularly in intensive care units. Its cause lung infection for patients under artificial respirators, wounds infection and blood infection due to used intravenous devices (Cohen *et al*., 2017). *P. aeruginosa*known to be responsible of burns infections, urinary tract infections, otitis media, and skin infections, also cause severe infections of AIDS patients (Fournier *et al*., 2016).

*P. aeruginosa*has many virulence factors help bacteria to infected patients and cause diseases, such as biofilm formation, secretion systems enzymes and toxins. The ability of this bacteria to form biofilms considered one of the most important virulence factor. The biofilm is aggregation of cells surrounded by extracellur matrix produced by the bacteria, protected bacteria from the antibiotics effected, patients' immunsystem and environment effected (Cassin and Tseng.,2019). The biofilms asset bacteria to produce diseases and increased bacterial resistance to antibiotics. It's difficult treated bacteria that produce biofilm as a results of produce some factors such as β-lactam and carbapenem group of antibiotics and acquired antibiotics resistance (Schaible*et al*.,2019). Increased bacterial resistance to antibiotics led to search for alternative methods to defends these bacteria including *P. aeruginosa*. Plant extracts consider a good sources of drugs such as Arabic gum.

The Arabic gum is natural polysaccharides produced by the *Acacia senegal*and Acacia seyaltres (Azeez, 2005). Gum Arabic is commonly used in the pharmaceutical and food industriesas emulsifier and stabilizer as suspending agent soluble drugs (Lelon*et al*.,2010). The Arabic gum

works as food preserver and in cosmetics' productions which contains oils and water surfaces. Because it's composed of a mixture of natural products of hydrophilic carbohydrates and an emulsion of hydrophobic protein components that absorbs on the surface of oil droplets, while the hydrophilic carbohydrate component prevents flocculation, incorporation of molecules and voids in food additives (Lelon*et al*., 2010 ; Yael *et al*., 2006).

*P. aeruginosa*mutidrug resistance bacteria to several type of antibiotics and responsible to nosocomule infections, the current study aims to find alternative methods such as using Arabic gum solution to treat burn infections.

Material and Methods

Samples collection

Seventy five samples were collected from patients resident at al-Hussain hospital in Kerbala province suffering from burns infection. Samples were collected randomly using sterile swaps from period of December 2020 to February 2021. Samples were collected from both genders and different ages, and sent immediately to the hospital lab for primary isolation and identification.

Identification of bacterial isolates

The bacterial isolates were primary identified according the morphology and some chemical tests according to (Collee et al 1996) Then the VITEK- 2 was used to confirm the primary identification of isolated bacteria and determinant the antibiotics sensitivity tests of these isolates by using VITEK-2 Compact system at Al-Huga private hospital)Mondelli*et al* ., 2012).

Biofilm formation

Bacterial isolates of *P. aeruginosa* were tested for biofilm formation using tubes method according to Christensen *et al*., (1985). In prife, loop full of tested bacterial isolates were added to a 10ml of sterile nutrient broth, incubated for 18 t0 24 hr., then the culture was removed and the tubes were washed several time with naturalized phosphate buffer after that the solution of crastyl violate was added to the tubes after air drying. Then the results were read after the dye was removed.

Preparation of plants extracts

Preparation of Arabic gum extract

The aqoueus extract was prepare according to Amra*et al*., (2006), 50 g of Arabic gum powder was dissolved in 500 ml of distilled water (V:W – 10:1). Then left at room temperature for 24 hr. after that, the solution was filtrated using several layers of medical gauze to removed unsolved material. The solution was centrifuged for 3000c/m, then filter sterile using millipore filter paper No.1cm. finally the solution drayed at incubator at 40 C° and kept at dark bottle until using.

Preparation of fresh solution of Arabic gum

A stock of fresh solution of Arabic gum was prepared by dissolved 50 gram of Arabic gum powder in 200 ml of Distilled water to get 0.25g/ml concentration of solution. After that, the stock solution was filtered sterilized and kept until use. Concentration of (25, 50, 75, and 100) mg/ml were preparation from stock solution.

Sensitivity test of aqoueus extract and fresh of Arabic gum

Well diffusion assay was used to tested *P. aeruginosa* isolates to the water extract and fresh Arabic gum according to (Sengul*et al*., 2009).

The taste bacterial isolates were tasted against several concentrations of fresh and water extracted of Arabic gum including (25, 50, 75, and 100) mg/ml.

A wells of 6 mm were done on nutrient agar plates were already seeded with *P. aeruginosa* isolates using cork borer. 50µ of water extracts and fresh solution of Arabic gum of each concentration were added to the well. After one hr. the plates were incubated at 37 c° for 24 hr. after incubation period the results were read by measuring the inhibition zone around each well and compered with biofilm formation of each isolates.

Results and Discussion

Samples were transfer to the lab for primary diagnoses after were collected from burn patients. Samples were culture on culture media and differential media. After incubation period isolates were submit to the morphology, biochemical and VITEK-2 for farther diagnoses to species. 50 (66.6%) of samples were positive of *P. aeruginosa*. This result agrees with result of study by (Jain and Singh, 2007), they found 48.8 of their samples positive to *P. aeruginosa*. Wile, other study by (Sousa *et. al.,* 2018) 75% of their burn patients positive to P. aeruginosa. we concluded that this bacteria common infected burn patients.

Sex and gender

Current study includes 75 samples were collected randomly from both gender 38 male and 37 female and different age stages between 2 -42 years old. Only 50 sample gave positive results of *P. aeruginosa*. The results shown that females more than males suffering from burn and infected with *P.*

aeruginosa. The females were 33 (66%) while mail were 17 (34%) as shown at figure (1-4). In addition, the results show that *P. aeruginosa*infected all ages, however the age (21-30) are the most infected with this bacteria.

Table 1-4 distribution of patients infected with P. aeruginosa according to six and genders

Age group/ Years	No. of males	No. of females	Total patients
1 - 10	3	3	6
11 - 20	1	1	2
21 - 30	6	13	19
31 -40	6	13	19
41 -50	1	3	4
Total	17	33	50

These results not match with result of (Qader*et al*., 2020), they found that *P. aeruginosa*common in male more than female in city of Arbil 57.65 and 42.4% respectively. Also in Dahuk found this bacterial more prevails in mail compared with female 46% and 36% respectively.

Study the sensitivity and MIC of antibiotics

P. aeruginosa isolates were subjected to sensitivity tested against 14 most known used antibiotics according to Protocol (CLSI 2016). These antibiotics include Ticarcillin ,Ticarcillin/Clavulanic Acid , Piperacillin, Ceftazidime, Cefepime, Imipenem, Meropenem, Amikacin, Gentamicin, Tobramycin, Ciprofloxacin, Pefloxacin, Minocycline, Trimethopim /Sulfamethoxazole. The results shown that most *P. aeruginosa*isolates resistant to Ceftrazidime, while sensitive to Imipenem as shown at table (3-4). The results agreed to results of study by (Tam et al., 2009) their found *P. aeruginosa*high resistant to most used antibiotics. The current

results and other results referred to dangerous of burns infected with *P. aeruginosa*, particularly the *P. aeruginosa* have spontaneous resistance in addition to acquired resistance (Khamenehet *al*., 2016). Also *P. aeruginosa*produce biofilm which also protected bacteria from antibiotics effect (Gellatly and Hancock,2013; Kadar *et al*., 2010). Because it's hard to eliminate the infection caused by this bacterium it preferred to using mixer of different antibiotics such as mixture of Beta-lactams, wide spectrum antibiotics or search for alternatives methods to treat these infections.

Table (3-4) resistance of *P. aeruginosa* to antibiotics

Antibiotics	MIC	Total	R	%	S	%
Ticarcillin	≥ 128	50	19	38	31	62
Cefepime	≥ 64	50	22	44	28	56
Ceftazidime	≥ 64	50	31	62	19	38
Piperacillin	≥ 128	50	24	48	26	52
Ticarcillin/Clavulanic Acid	≥ 128	50	22	44	28	56
Tobramycin	≥ 16	50	15	30	35	70
Gentamicin	≥ 16	50	17	34	33	66
Amikacin	≥ 64	50	25	50	25	50
Meropenem	≥ 16	50	18	36	32	64
Imipenem	≥ 16	50	13	26	37	74
Trimethopim/Sulfamethoxazole	≥ 320	50	26	52	24	48
Minocycline	8	50	23	46	27	54
Pefloxacin	≥ 16	50	14	28	36	72
Ciprofloxacin	≥ 4	50	20	40	30	60

Study biofilm production by *P. aeruginosa*

The tubes method of biofilm formation was used to confirm the ability of *P. aeruginosa* isolates in present study. The results showed all isolates were produced biofilm with different concentration, 50% of isolates were high antibiotics' resistance production strong biofilm. While, the other 50% less resistance to antibiotics and non or week biofilm production as showed in at table (4-4). Therefore, the results confirm the role of biofilm in resistant to antibiotics. This results agreed with results (O'Toole *et al*., 2000; Ekrami and Kalantar.,2007) they found that resistant *P. aeruginosa*bacteria isolated from burn patients produce high biofilm. Also the results agree with results of Hadi (2007) their found that biofilms producing *P. aeruginosa* isolates cause severe infection and strong resistance of antibiotics.

Table (4-4) the relationship between the antibiotics resistance and biofilm production of *P. aeruginosa*

No. of isolates sensitive to antibiotics and non or week biofilm production	No. of isolates resistance to antibiotics and high biofilm production	Total No. of isolates
25	25	50

Effect of water extract and fresh solution of Arabic gum on *P. aeruginosa* isolates

The ability of water extracts and fresh solution of Arabic gum toward six isolates of *P. aeruginosa*were chosen in currant study using well diffusion assay. The results showed that fresh solution gave a good results of inhibition the chosen isolates, while water extracts give no effect on chosen isolates as shown at table (4-5).

Table (4-5) the comparison of effect of water extract and fresh solution of Arabic gum against six chosen *P. aeruginosa* isolates

Diameter of inhibition zone of fresh solution of Arabic gum	Diameter of inhibition zone of water extract of Arabic gum	No. of isolate
18ml	4ml	1
15ml	5ml	2
12ml	3ml	3
14ml	5ml	4
13ml	6ml	5
15ml	4ml	6

Estimated the inhibition ability of fresh solution of Arabic gum against *P. aeruginosa* isolates

different concentrations of fresh solution of Arabic gum including (25, 50, 75, and 100) mg/ml were used to estimate the best concentration (MIC). The results showed that 75 – 100 give the highest inhibition zone on both high and low biofilm formation as showed at figure (3-4). In addition to effect of fresh solution of Arabic gum on ability of bacteria to form biofilm with increased of concentration.

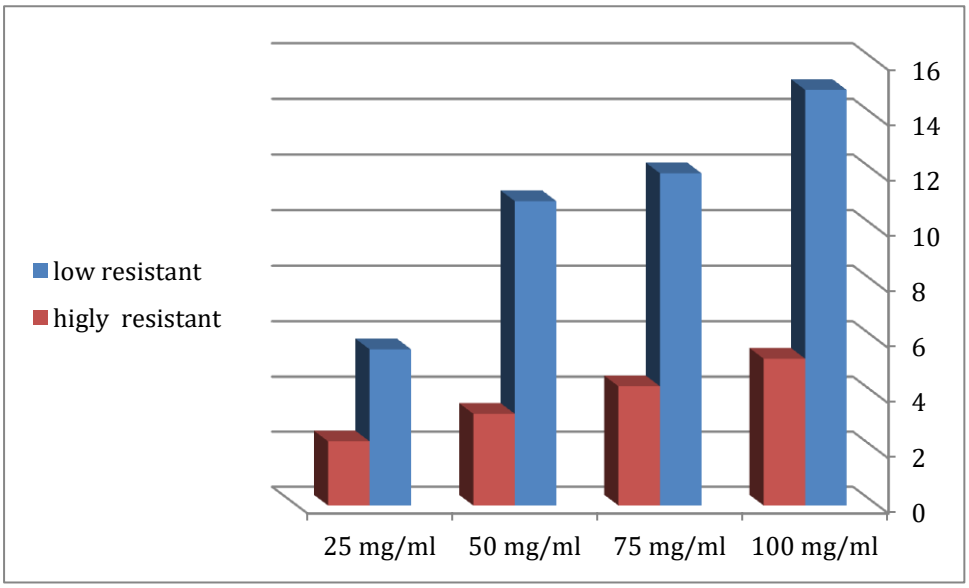

Figure (3-4) effect of different concentration of fresh solution of Arabic gum against producing biofilm *P. aeruginsa* isolates

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