Study of virulence factors and inhibitory effect of salvia on identified gram positive and gram negative isolated from soil

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Abstract

Soil is considered one of the most suitable environmental for microbial growth . In this study and 130 bacterial isolates were from soil in musian city the first most common ratio was ***Escherichia .coli*** 30 (23.07) % and less of occurrence ratio was ***Citrobacter freundii*** 1(0.76) % also study some virulence factors like heamolysin the high ratio was ***Enterobacter spp*** 10(4)% and low ratio in ***Citrobacter freundii*** 1(1)% , protease was 9(27)% and ***Actinomycetes spp*** and ***Erwinia spp*** 0% finally in bacitracin was 8(32)% ***E.coli*** . Aqueous suspensions of the dried **Allium sativum** (Liliaceae) bulbs extract was screened for its antimicrobial activity using the agar-well diffusion method. The highest zone of inhibition was estimated with the highest concentration of aqueous suspension *in* ***Bacillus cereus*** was (17 mm) followed by lower inhibition zone in ***Enteroabacter cloacae*** was (5mm). It is tested against Gram-positive bacteria and gram-negative bacteria .The suspensions were tested at concentrations of 1, 10, 100 and 1000 µg/ml when using garlic extract as antimicrobial substances, salvia as inhibition factor, and inhabited zone of saliva in ***Micrococcus spp*** was 10 mm. Also inhibited adhesion bacterial on epithelial cell by saliva was ***E.coli*** group B from 68.5±30.3 to 40.2±18.1 and in group B ***Staphylococcus aureus*** inhibited bacteria on epithelial cell from 50.4±1.4 to 30.3±9.3 , conclusion soil contain many bacteria have some virulence factor and use saliva as inhibited adhesion factors .

Key words: Soil bacteria ,Virulence factors , saliva ,saliva bacteria , Saliva adhesion factor garlic extract .

Introduction:

Soil is considered one of the most suitable environment for microbial growth that the microorganisms which have been isolated from the soil of having leading in this area [1]. Bacteria found in soil can be rods (bacillus)cocci (spherical)and spirillum(spirals) of which bacillus are more numerous than others .They are one the major groups of soil bacteria and widely disturbed [2].May species such as ***Streptomyces spp*** ,***Bacillus spp*** and Penicillium have been studied continuously for their ability to produced antibiotics insoluble form and that these antibiotics have been found to be cheaper and more effective these microorganisms are preferable for commercial production, these microorganisms are preferable for commercial production the target is to produce antibiotics such as polymyxin and bacitracin from bacillus [3] .It was reported the members of the species bacillus generally produced polypeptide type bacitracin and that these antibiotics generally affect gram-positive bacteria [4] . Discovering virulence factors is important in understanding bacterial pathogenesis and their interactions with the host which also serve as novel targets in drugs and vaccine development [5].Bacterial virulence factors can divided in to several groups on the basis of the mechanism of virulence and function. These are membrane proteins, which play role in adhesion ,colonization and invasions promote adherence to host cell surfaces are responsible for resistance to antibiotics and promote intracellular communication [6] Human whole saliva which contains a variety of antimicrobial factors is bathing the teeth and oral mucosal surfaces .These antimicrobial salivary systems contain both non immunoglobulin agents and secretory antibodies [7, 8] . The level of IgA were collected with reduction in the number of bacterial receded after infection as well as reduction in disease [9]. However positive findings have higher of amounts of lysozyme and agglutinins and IgA [10,11]. Garlic (allium sativum) has traditional dietary and medicinal applications as an anti-infective agent [2-3].

Some studies showed that saliva contains many antimicrobial substances . B-defensins which are cationic peptides with broad spectrum antimicrobial activity are produced by human salivary glands and oral mucosal surfaces and most abundant in

tissue of associated inflammation human B-defensins play an important role in the innate defenses against oral microorganisms [5]. The aims of study were to isolated and identify of some soil bacteria and use saliva to inhibit theses bacteria . Also Study the virulence factors of isolates like heamolysin , protease ,bacitracin and bacterial adhesion on epithelial cells and inhibited adhesion by saliva further more study included herbal extract like garlic for evaluation antimicrobial substance.

Materials and Methods:

- **Materials:**

Soil samples were collected from musian city .Use gram iodine stain ,gram´s crystal violet stain ,gram ´s safranin solution , nigrosine stain solution ,nutrient broth ,and use biochemical test .

- **Dilution of sample :**

One gram of soil sample was weighted and 99 ml from distilled water added 1 ml from the sample was taken out and add to 9 ml of distilled water and incubated in 37C° for 24 hour and repeated the isolation bacteria from soil by taken 1 gram of soil for 50 sample in Missan city .

- **Isolation of microorganism :**

After culture of soil sample diluted on ager plate by streaking methods on ager plate sealed and kept in 37C for 24 hour in incubator for growth, purification of bacteria was done doing by anther spread plate technique and
Incubation and using gram stain , sample was observed under microscope and characterized based on [12]. Identification of isolates by biochemical test based on bergey´s manual.

Preparation of Inoculum about 24 hour broth culture of the test bacteria isolate was suspended into sterile nutrient broth. It was standardized according to National Committee for Clinical Laboratory Standards [13] by gradually adding normal saline to compare their turbidity to McFarland standard of 0.5 which is approximately 1.0×10^6 cfu/ml and in the heamolysin use the method of[14] and protease [15] and bacitracin [13].

2- Garlic (Allium sativum): used in the present study was purchased from the local market. Bulbs were peeled and washed from the foreign particles. A Fifty grams of the bulbs were squeezed. The extraction was filtered using Whatman no. 1 filter

paper. Four concentrations of each extract (1, 10, 100, and 1000 mg/ml). Concentrations were resuspended in sterile distilled water (aqueous) [16].

3- Bacterial adhesion test :

Take the mid-stream urine MSU from normal healthy female and centrifuge in 3000\RPM for 5min and wished the sedmented contain 75 epithelial cell this is used for testing .Mix 0.5ml from suspension bacteria in age 24hr in brain heart infusion ager with 0.5ml from epithelial cell for all testing bacteria incubated at 37C for 60 min and staining all slides by gram stain then examine all slides under oil immersion and recorded it and use saliva to inhibition bacterial adhesion by [17]. Human saliva samples were taken from healthy persons. The bacterial isolates were identified according to bergey´ s manual of systematic bacteriology. Blood agar culture medium was used for cultivation of pathogenic bacterial isolates and purified [18] .Nutrient agar used for spreading soil bacteria and making hole contain saliva.

Results:

Soil has many microorganisms like bacteria .The isolation process is a procedure of isolation mixture of colonies to single colony. In table (1) show the type of isolates bacteria and percentage of each bacteria the high isolate was ***Escherichia .coli*** 30(23.07) % and lower ratio was ***Citrobacter freundii*** 1(0.76) %. Also in table (2) show the some virulence factors of isolates bacteria from soil like heamolysin protease ,adhesion and bacitracin . In heamolysin the high percentage was (11)22% ,the lower percentage of heamolysin was ***Actinomycetes spp*** and ***Erwinia spp***, ***Micrococcus spp*** and ***Citrobacter freundii*** was 1(2)% and in protease the high ratio was ***E.coli*** 9(27)% and low ratio in ***Actinomycetes spp*** and ***Erwinia spp*** was 0% in bacitracin high percentage ratio in ***E.coli*** 8(32)% and low ratio in ***Klebsiella ozancnae*** , ***Bacillus spp*** , ***Micrococcus spp*** and ***Actionomycetes*** spp 0%.In table (3) show the inhibition zone of garlic against isolates and show In table (4) and table (5) show adhesion of bacteria on epithelial cells but when use of saliva for inhibition adhesion effect more on group B ***E.coli*** **68.5 ± 30.3** inhibit to **40.2 ± 18.1** and in group B ***Staphylococcus aureus*** inhibited from **50.4 ± 11.4** to **30.3 ± 9.3.** In figure (3) and figure (4) show the adhesion bacteria and inhibition adhesion bacteria by saliva on ***E.coli*** .

Table (1): Distribution of Bacterial isolates from soil

Type of bacteria	Number	%
E. coli	30	23.07
Pseudomonas aeroginosa	21	16.15
Staphylococcus aureus	20	15.38
Streptococcus suis	15	11.53
Enteroabacter cloacae	10	7.69
Klebsiella ozaenae	8	6.15
Bacillus cereus	7	5.38
Proteus mirabilis	5	3.85
Micrococcus spp	5	3.85
Actinomycetes spp	4	3.07
Erwinia spp	4	3.07
Citrobacter freundii	1	0.76
Total	130	100%

Table (2): The virulence factor of identified isolates from soil .

Types of bacteria	NO.	Heamolysin	%	Protease	%	Bacitracin	%
Escherichia. coli	30	5	10	9	27	8	32
Pseudomonas aerogenose `	21	2	4	5	15	3	12
Staphylococcus aureus	20	4	8	7	21	2	8
Streptococcus suis	15	8	16	1	3	1	4
Enteroabacter cloacae	10	11	22	2	6	3	12
Klebsiella ozaenae	8	9	18	3	9	0	0
Bacillus cereus	7	2	4	3	9	0	0
Proteus mirabilis	5	7	14	2	6	3	12
Micrococcus spp	5	1	2	1	3	0	0
Actinomycetes spp	4	0	0	0	0	0	0
Erwinia spp	4	1	2	0	0	1	4
Citrobacter freandii	1	1	2	1	3	4	16
Total	49	51	102	34		25	100

Table (3): Garlic extract inhibition zone against soil bacteria

Type of bacteria	Garlic Inhibition zone
Escherichia. coli	12 mm
Pseudomonas areogenosae	14 mm

Staphylococcus aureus	**11 mm**
Streptococcus suis	**11 mm**
Enteroabacter cloacae	**5 mm**
Klebsiella ozancnae	**10mm**
Bacillus cereus	**17 mm**
Proteus mirabilis	**11 mm**
Micrococcus spp	**10mm**
Actinomycites spp	**13 mm**
Erwinia spp	**11mm**
Citrobacter freandii	**13 mm**

Table (4): Saliva inhibition zone to isolates of bacteria from soil

Type of bacteria	**Saliva Inhibition zone**
Escherichia. coli	**9mm**
Pseudomonas areogenosae	**7mm**
Staphylococcus auras	**9mm**
Streptococcus suis	**5mm**
Enterobacter cloacae	**3mm**

Klebsiella ozancnae	**0**
Bacillus cereus	**16 mm**
Proteus mirabilis	**8mm**
Micrococcus spp	**10mm**
Actinomycites spp	**0**
Erwina spp	**0**
Citrobacter freandii	**3mm**

Table (5): Adhesion epithelial cell and inhibition adhesion by saliva

Groups of bacteria	**Adhesion Mean ±SD**	**Inhibition by Saliva Mean ±SD**
Group A *Escherichia. coli*	40.4 ± 20.4	30.1 ± 12.1
Group B *Escherichia. coli*	68.5 ± 30.3	40.2 ± 18.1
Group C *Escherichia. coli*	54.3 ± 41.1	35.8 ± 20.6
Group A *Staphylococcus aureas*	30.9 ± 12.3	20.1 ± 9.8
Group B *Staphylococcus aureas*	50.4 ± 11.4	30.3 ± 9.3
Group C *Staphylococcus aureas*	33.4 ± 12.1	20.9 ± 8.1

Discussion:

In this paper show the isolation of pathogenic bacteria from soil and study some virulence factors in most common bacteria were ***E.coli*** and ***Staphylococcus aureus***, ***Pseudomonas aerogenosae*** and others in table (1) and figure (1) show the high ratio of isolates was ***E.coli*** 30(23,07)% this study disagree with [19] they were obtain the high isolates was ***Bacillus cereus*** 9(50)% the low ratio was ***E.coli*** 1(5.5) % from soil .Also in table (2) and figure (2) show the some virulence factors the high ratio in heamolysin was 10(11)% in ***Enteroabacter cloacae*** , these result is differ with [20] that study heamolysin enzyme as virulence factors in ***Staphylococcus aureus*** show that 53(58.9) % secreted heamolysin also differ with [21] show that ***E.coli*** contain exotoxin alpha heamolysin as virulence factor and the ratio of heamolysin was 50 %, protease 9(27) % was ***E.coli*** [22] that show protease enzyme is very important in pathogenesis of ***Staphylococcus aureus*** and bacitracin 8(32)% was ***E.coli*** these result was differ with [22] that show about (86)% from ***Pseudomonas aerogenosae*** that produce bacitracin inhibitory to the above indicators. In addition salivary contain antibacterial effects through the generation of reactive nitrogen intermediated including nitricoxide and nitrosonitrosyl species against ***E.coli*** and ***Candida spp***.[12].The inhibition zone of effect salvia were presented in this paper show in table (4)and table (5) show saliva inhibited zone in ***Micrococcus spp*** (10mm) act as antiseptic because it contain lysozyme an d prevent the growth of bacteria these study differ with [19] that show the saliva inhibition zone was in ***Staphylococcus aureus*** sample 1,2,3 (9mm ,10mm ,17mm) .In this study also show the adherent isolates bacteria ***E.coli*** and ***Staphylococcus aureus*** on epithelial cells and inhibited adhesion by invasive ***E.coli*** **68.5 ± 30.3** inhibit to **40.2 ± 18.1** and in group B ***Staphylococcus aureus*** inhibited from **50.4 ± 11.4** to **30.3 ± 9.3** these study is new search and no study about inhibited epithelial by saliva and show in figure (3) and figure (4). According to herbal extract against bacteria and show ***Bacillus cereus*** the high ratio was 17mm and low ratio in ***Enterobacter cloacae*** 5mm.This results were similar from data reported by [23] who observed that garlic extracts inhibited the growth of Staphylococcus aureus.

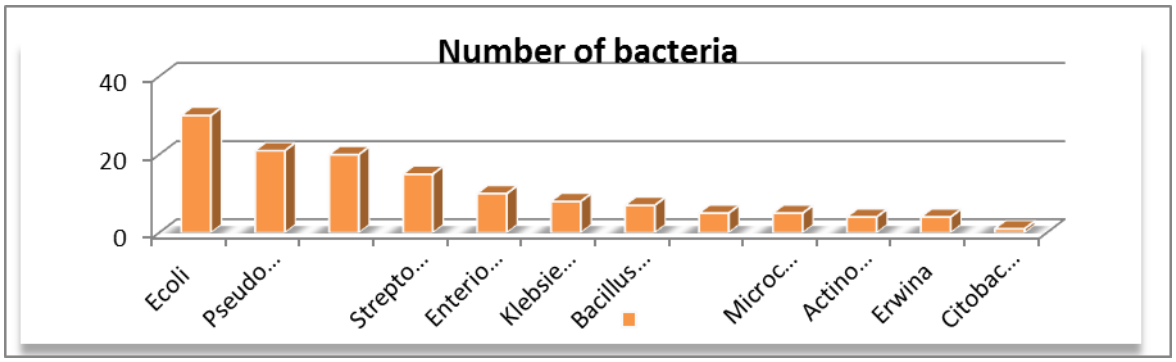

Figure (1): The percentage of isolates bacteria from soil

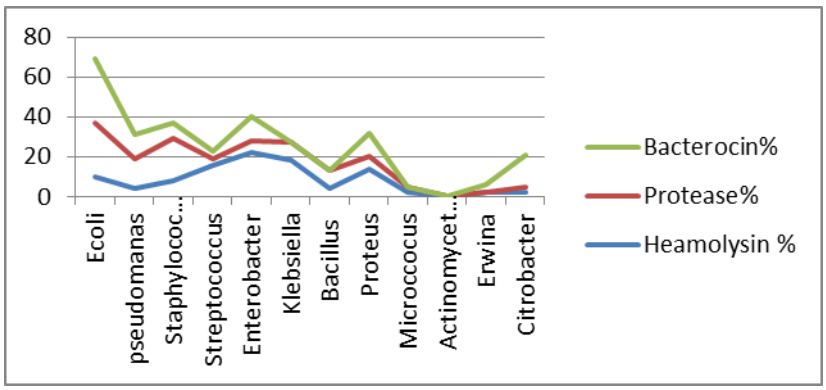

Figure (2): Virulence factors of isolates from soil

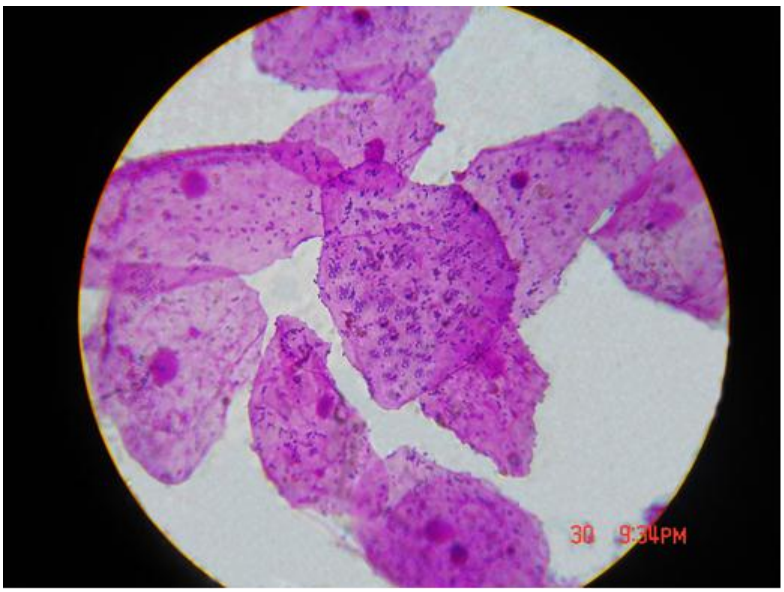

Figure (3): Adhesion of **Staphylococcus aureus** on epithelial Cell

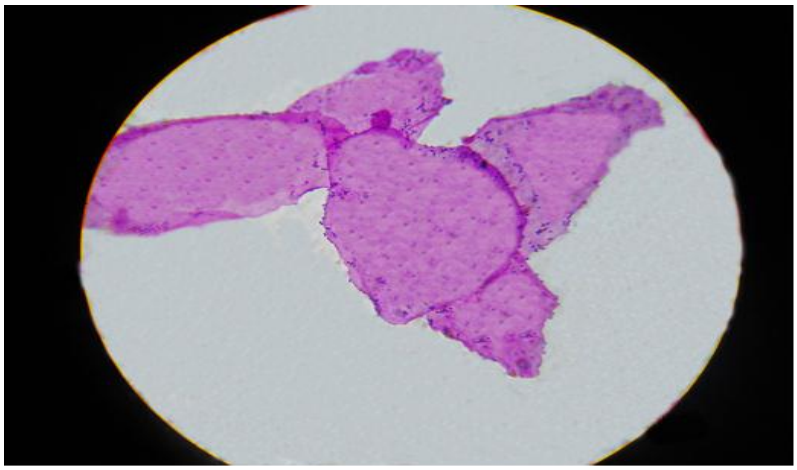

Figure (4): Inhibition adhesion ***Staphylococcus aureus*** by saliva

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دراسة بعض عوامل الضراوة وتثبيط عامل الالتصاق البكتيري باللعاب لبكتريا الموجبة والسالبة لصبغه غرام المعزولة من التربة

الخلاصة

تعد التربة المحيط الملائم لنموا نواع مختلفة من الاحياء المجهرية . حيث تضمن هذا البحث عزل 50 عينه من التربة في محافظه ميسان وكانت النتائج المسجلة هي اعلى نسبه هي في بكتريا 23.07% الاشيريشيه القولونية واقل نسبه في بكتريا الستروبكتر فريندلي بسبه (1% وتضمنت الدراسة أيضا دراسة بعض عوامل الضراوة البكتريا ومنها الهيمولويسين وهو الأنزيم المحلل لكريات الدم الحمر حيث سجلت بكتريا الانتيروبكتر 10(4)%واقل نسبه هي بكتريا الستروبكتر 1(1)% وعامل البروتييز وهو العامل المحلل للبروتين في جدار الخليه الحيه حيث كانت الاشيريشيا القولونيه 9(27)% واقل نسبه في بكتريا الاكتينومايسيت وبكتريا الاروينيا (0)% وكذلك دراسة أيضا عامل البكتريوسين حيث سجلت بكتريا الاشيريشيا القولونيه 8(32)%. وتم دراسه أيضا المستخلص النباتي المائي للثوم باعتباره مضاد للنمو البكتريا واختبار خاصيه التثبيط للبكتريا وسجلت بكتريا الباسلاس سيريس اعلى نسبه 17 ملم واقل نسبه بكتريا انتيروبكتر كلوسي 5 ملم ز وعند استخدام اللعاب كعامل تثبيط فكانت اعلى حلقه تثبيط في بكتريا المايكوكوكس اما في حاله تثبيط الالتصاق البكتيري على الخلايا الطلائية باستخدام اللعاب فكانت المجموعة ب للاشيريشيا القولونية 68و5 ا± 30و3 الى 18و1 ± 40و2 أما في المجموعة ب البكتريا العنقودية فسجلت نسبه تثبيط الالتصاق بللعاب البكتيري بلعاب 50و4 ±11و4 الى 30و3 ± 9و3 . وأخيرا ان التربة تحتوي مجموعه من البكتريا تمتلك عوامل ضراوة وقد تم استخدام اللعاب كعامل مثبط للبكتريا الملتصقة على الخلايا البكتيرية لأنه يحتوي على مواد مناعيه مثبطه .