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Molecular Detection of *Pseudomonas aeruginosa* and Study the Effect of Fresh Garlic juice on Some Virulence Factors of this Bacteria.

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ABSTRACT

The present study included the collection of 169 samples from various clinical and environmental sources for investigation of existence of *Pseudomonas aeruginosa* in those sources, the clinical samples collected from some hospitals in Baghdad city and included 126 samples of wound, burns, cystic fibrosis, ear infections and urinary tract infections (35, 52,4, 22 and 13) samples respectively, while environmental samples were distributed by 25 samples from soil, and 18 samples from wastewater and some swamps. Samples were identified based on the morphological and microscopical characteristics of the colonies when they were culturing in a number of culture media as well as biochemical tests. The molecular detection was used to confirm diagnostic test for the isolates based on the *16SrRNA* gene, as a detection gene, which has specific sequences for DNA of *P.aeruginosa* bacteria.The results of molecular detection showed that 51 isolates belong to target bacteria were distributed as 20 of burns, 9 isolates of wounds, 7 isolates of ear infection, 4 isolates of cystic fibrosis, 2 isolates of urinary tract infection, 5 isolates of soil and 4 isolates of water. The results of phenotypic detection of some virulence factors showed that 49 isolates with percent (95%) produced beta-hemolysis, while 46 isolates (90%) showed variance in the strength of pycocyanin production with a absorbance ranging from (0 to 0.52) at 690 nm, and 47 (92%) of isolates were producing protease enzyme, 44 (86%) of the isolates showed a differences in their ability to biofilm formation. As a result of the increased resistance of *P.aeruginosa* bacteria towards antibiotics, it is became a problem in the treatment of the infectious diseases caused by this bacteria therefore, the current study focused on the use of fresh garlic extract towards of some virulence factors because it being easy and commonly used. The results of the statistical analysis showed a significant difference when P-value (<0.05) in the production of some virulence factors, the isolates lost their ability to form biofilm, and decreased their ability to produce protease enzyme, and pyocyanin pigment, while did not affect the production of hemolycin..

1 INTRODUCTION

Pseudomonas aeruginosa is Gram negative bacteria, aerobic, rod, and motile, belongs to Pseudomonadaceae family, since the revisionist taxonomy based on the conserved macromolecules (e.g. 16s ribosomal RNA), the family includes only members of the genus *Pseudomonas* which are divided into eight groups and *Pseudomonas aeruginosa* is one type of this group(1).

P. aeruginosa is one of the opportunistic human pathogen that preferentially infects patients with cancer or AIDS, immunocompromised patients by surgery, cytotoxic drugs or burn wounds, people with cystic fibrosis, eye, ear and urinary tract infections (2). *P. aeruginosa can* produce any of the opportunistic extra-intestinal infections caused by members of the Enterobacteriaceae and may progress to bacteremia (3). Septicemia and endocarditis may occur in patients who are debilitated due to concomitant infection, malignancy or immunosuppressive therapy (4). In some cases of *P. aeruginosa* bacteremia, cutaneous papules develop that progressto black, necrotic ulcers (5). *P. aeruginosais* also one of the most common causes of infection in environmentally contaminated wounds , eg:

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osteomyelitis after compound fractures or nail puncture and body wounds (3). The success of *P. aeruginosa* in diverse environments is attributed to its impressive arsenal of virulence factors , which include multiple cell-associated factors such as alginat, lipopolysaccharide , flagella and pili, and secreted virulence factors, including toxins, elastases, alkalin protease, hemolysin, pyocyanin, as well as small molecules that include phenazines , rhamnolipid, and biofilm formation (6).

Alkalin protease it is an extracellular protease that is produced by *P. aeruginosa* and it plays an important role during acute infection, however details of their actions are sometimes unclear (7). Alkaline proteases are referring to proteolytic enzymes which work optimally in alkaline pH(8). These extracellular proteases is important and enablethe cell to absorb and utilize hydrolytic products, at the same time, these extracellular proteases have also been commercially exploited to assist protein degradation in various industrial processes (9).

Hemolysin is produced by several bacterial strains (Gram positive and Negative) like *Salmonella enteric, Streptococcus pyogenes ,Staphylococcus aureus ,Vibrio vulnificus ,Sibirio parahemolyticus ,E.coli ,Proteus mirabilis ,Proteus vulgaris ,Pseudomonas aeruginosa P. aeruginosa* produce two types of hemolysin: the first is called phospholipase C (PLC), which is thermally labile and has a effective anti-emulsification,and the other is called Rhamnolipid hemolysin ,which is thermally stable(10). Biofilm is a community of microorganisms attached to substrate surface and submerged into extracellular slimy matrix (11). Genetic diversity of organisms that form the biofilm and variety of environmental conditions where it emerges prove that biofilm is an ancient ubiquitous life form of microorganisms (12). The formation of biofilms contributes to the high resistance of *P. aeruginosa* to antibiotics making the treatment of biofilm infections more difficult (13). In addition, bacteria in biofilm were demonstrated to show elevated resistance to the host immune system clearance (14).). Factors which explain the high antimicrobial resistance of biofilms include decreased oxygen and nutrient decreased diffusion of antibiotics through the biofilm matrix,decreased growth rates and metabolism (15). Pyocyanin is the major pigment produced by *P.aeruginosa* and has shown to contribute to its pathogenicity (16). The presence of pyocyanin is easy to detect due to its blue color that turns stationary phase cultures of *P.aeruginosa* green, and is commonly found to stain infected tissues, pus, or dressings One important function of the pigment is to assist in iron uptake from the environment, i.e. host tissue, Due to its redox-active properties, pyocyanin can cause reduction and release of iron from transferrin, a protein that transports iron in our bodies. Hence this crucial mineral can be accumulated by the bacteria(17).

More advanced approaches to more identification have been developed, including polymerase chain reaction (PCR), restriction fragment length polymorphism (RFLP), and DNA sequencing (18). Molecular tools such as *16SrRNA* gene sequencing provide trusty results although it might have problems to assign at specie level (Fernandez-Olmos *et al*., 2012). Selective amplification of *Pseudomonas* 16SrRNA gene by PCR has been used to detect the differentiate of *Pseudomonas* species (19).

Since ancient times, crude herbal extracts of aromatic plants have been in use for different purposes such as food, drugs and perfumery, Plant-derived drugs remain an important resource, especially in developing countries, to combat serious diseases. Approximately, 60% - 80% of the world's population still relies on traditional medicines for the treatment of common illnesses (20). Man are using natural products of animals, plants and microbial sources for thousands of years either in the pure forms or crude extracts, Bioactive compounds from these diverse sources have been isolated and characterized worldwide. Systematic screening of plant materials represent an all-important effort to find some new bioactive compounds with the needed therapeutic potential to fight against pathogenic microorganisms, particularly with respect to those are hospital based.(21). Phytoplants have been good alternatives to synthetic chemical antimicrobial agents and antibiotics, because of the serious side effects, antimicrobial resistance and the emergence of previously uncommon infections which have been increasing due to inappropriate or widespread overuse of antimicrobials (22).

Garlic (*Allium sativum* Linn.) was seriously investigated over the years, It has been used for centuries to fight infections , The early Egyptians used it to treat diarrhea, the ancient Greeks used it to treat intestinal and extra-intestinal diseases, while the ancient Japanese and Chinese used it to treat headache, flu, sore throat and fever (23). The antimicrobial activity of garlic was first described by Louis Pasteur and it was used as an antiseptic to prevent gas gangrene during World War II (24). Garlic is widely used in culinary and medicine (25). Therapeutic effect of garlic is possible because of its oil and water soluble organosulfur, Thiosulfinates (eg. Allicin) play an important role in the antibiotic activity of garlic 26). Pure allicin is a volatile molecule that is poorly miscible inaqueous solutions and which has the typical odor of freshly crushed garlic, amino acid that is present in large quantities in garlic cloves and which they named alliin (27). Alliin was found to be the stable precursor that is converted to allicin by the action of an enzyme termed alliinase which is also present in the cloves , Allicin is produced catalytically when garlic cloves are crushed and the enzyme allinase of the bundle sheath cells mixes with its substrate, alliin, which is released from mesophyll cells, Allicin exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA synthesis

although DNA and protein syntheses are also partially inhibited, suggesting that RNA is the primary target of allicin action (28).

With the rise in bacterial resistance to antibiotics, there is considerable interest in the development of other classes of antimicrobials for the control of infection ,so that the study goal to the use Fresh Garlic juice as natural antagonist against some virulence factors of *P.aeruginosa.*

2 METHODS

2.1 Collections of samples

In this study, 126 samples were collected from clinical state including both sexes with different ages, who suffered from; urinary tract infections, wounds, burns, ear infection from different teaching hospitals in Baghdad city. These samples were taken by sterile cotton swabs and put in sterile tubes containing normal saline, then transferred to Graduate Laboratory at the college of Science, Anbar University for culturing .then swab was suspended in nutrient broth and incubated for 24 hrs at 37°C. UTI samples were collected by sterile container directly, and stored in a cool place until transported to laboratory, Another 43 samples were collected from different environmental sites (soil and water).

2.2 Morphological diagnosis

Primary diagnostic tests based on the morphological characteristic of bacterial growth on MacConkey agar , Blood agar , Pseuedomonas agar ,and cetrimide agar were studied including, colony shape, texture, color , edges **,** and ability to lactose ferment.

2.3 Microscopic and Biochemical tests dignosis

The diagnosis was done according to Collee *et al.*, 1996 (29).

2.4 Haemolysin production

Plate haemolysis test was done for the detection of beta haemolysin the bacteria was inoculated on 5% blood agar and incubated overnight at 37 °C. Haemolysin production was detected by the presence of a zone of complete lysis of the erythrocytes around the colony(30).

2.5 Pyocyanin Production

Pyocyanin production determinate according to Parsons *et al.*, 2007 (31)**.**

2.6 Protease production

Protease production determinate according Parsons *et al.*, 2007 (31).

2.7 Biofilm Production

Biofilm Production determinate according to Rewatkar, 2013 (32).

2.8 DNA extraction method:

The bacterial DNA extracted according to Genomic DNA mini Kit which provides by Geneaid company.

2.8.1 DNA quality evaluation

The extracted DNA wconcentration and purity was estimated by Nano drop equipment.

2.8.2 Methods of PCR for Detection of *16SrRNA* Gene

Primers were supplied by the Bioneer Company as a lyophilized product of different picomol concentrations .Primers solution final concentration of (10) Pmol/µl were prepared separately by dissolving 10µl of stock solution for each primer and added to 90µl free nuclease distilled water unionic, mixed well and kept in-20 ºC ,they mixed by vortex to homogenize before use (33).

2.8.4 Preparation PCR mixture

The mixture 25µl. composed of Green master mix (bioneer) , primer solution , deionized water and template DNA with following volume :

PCR program for *16SrRNA* gene detection:

PCR was used for detecting of *Pseudomonas aeruginosa* .The PCR tubes containing the mixture were transferred to preheated thermocycler and started the program as in the following table(1-3).

Table (1-1): Sequence of PCR primer and molecular size of PCR product

Gene		Sequence of forward and reverse Primer(5´- 3´)	Product bp
16SrDNA	F	GGGGGATCTTCG GACCTCA	956 bp
	R	TCCTTAGAGTGC CCACCCG	

Table: (1.2) PCR mixture used in the current study.

No.	Contents of reaction mixture	Stock concentration Pmol/ µl	Volume of reaction mixture for a single tube(µl)
1	Green master mix		5 µl
2	Forward primers	10 picomol	1µl
3	Reverse primers	10 picomol	1µl
4	DNA template		3µl
5	Nuclease free water		15µl
Total volume			25 µl

Table(1-3):PCR amplification program for *16SrRNA* gene detection

step	Temperature (˚C)	Time	No.of cycle
Initial denaturation	**95**	**3 min**	**1**
Denaturation	**94**	**45 sec**	**32**
Annealing	**58**	**45sec**	
extension	**72**	**45sec**	
Final extension	**72**	**7min**	**1**
Hold temperature	**4**	**3min**	**-**

Five microleter of the product was subjected to electrophoresis, while the remain product was saved at -20◦C for other experiment.

2.9 Preparation of Garlic Extract

2.91 Preparation Crude Juice of Garlic:

Fresh garlic was collected from local markets of Baghdad city. Garlic bulbs were cleaned with tape water to remove any adhering soil on their surfaces followed washing with distilled water again. Ten g of garlic were taken after removal of outer skin surfaces then cut into small pieces. The small pieces were blended with 25 ml sterile distilled water by sterile warring blender for 5minute, then it was used directly through experimental of virulence factors detection(34).

2.9.2 Determination of minimum inhibitor concentration (MIC)

Determination of minimum inhibitor concentration according to (43).

2.9.3 Growing of isolates in MIC to Detect Effect of Extract against some Virulence Factors.

Amount of100 µl of MIC extracts was added to tubes contain (10 ml of nutrient broth + 100 µl of bacterial growth), then incubated for 24 hours at 37°C. to be used as a growth cultures to detect some virulence factors after treatment with natural oils, in the same methods as previously mentioned.

2.9.4 Inhibition of Biofilm Production

Amount of 100 µl of MIC extracts was transferred into wells of a sterile 96-well polystyrene microtiter plate, then100µL of the overnight growth cultures was added to each well, with control wells containing broth + extract concentration (extract control). All the microtiter plates were incubated for 24 hours at 37°C (22), to follow the same steps of work as mentioned in **1.7** paragraph.

2.10 Statistical analysis

The statistical analysis was conducted based on Statistical package of social sciences –Version-22 (SPSS) at a probability level less than 0.05($P<0.05$) for study of extract MIC impact on towards virulence factors of *P.aeruginosa* bacteria.

3 RESULTS & DISCUSSION

3.1 Isolation and Identification of *Pseudomonas aeruginosa*

A total of 169 samples were collected**,** initially these samples were cultured on MacConkey and Blood agar to distinguishes Gram positive bacteria and Gram negative bacteria. The result revealed that some of these sample grown on blood agar only that mean it's Gram positive bacteria, while the others wrer grown on both media, which mean those sample are Gram negative bacteria.

The samples that demonstrated growth on MacCkonky agar cultured on Pseudomonas agar as a selective medium for *Pseudomonas* genus, and incubated at 42°C for being an important diagnostic characteristic for *P.aeruginosa* bacteria than other species of *Pseudomonas* genus, these samples re-cultured on cetrimide agar to confirm diagnostic for isolates. The results of the biochemical tests showed (table 3-1) all the isolates were positive for oxidase and also positive for catalase. IMViC tests results showed that all isolates were negative for indol, methyl red (MR) and Voges-Proskauer (VP) and were positive for Citrate as a sole carbon source.

3.2 Molecular Identification test:

Chromosomal DNA Extraction and Purity:

The extraction of chromosomal DNA done according to **2.8** paragraph, and the results of gel electrophoresis showed presence of DNA bands on agaros gel in same level for all isolates.Then, the concentration and purity of DNA extracted measured by Nano drop equipment, where the results showed that the range of concentration (51.6-170.2) ng/ul. While the range of purity (1.76-2.23).

Polymerase Chain Reaction (PCR) For Detect *16SrRNA* Gene.

The result of gel electrophoresis for amplification PCR product showed that presence of bands in same level for all isolates, that means the primers of 16SrRNA bind specifically to complement sequence within DNA template, which may indicate that 16SrRNA gene is a detection gene for *P.aeruginosa* ,and the molecular weight of these bands compared with DNA leader 100bp, where the results showed similarity of molecular weight of these bands (956bp) with molecular weight of 16SrRNA gene. On the other hands, 16SrRNA genes considered one of the basic criteria in the classification because of its regions were highly constant and unable to change over time, also contain areas of highly covariance among types of bacteria so that provides a specific sequence to each type, which means this gene may play important role in diagnosis (35). When the

diagnostic methods ended, were obtained of 51 isolates of *P.aeruginosa* bacteria distributed as table (3-1).

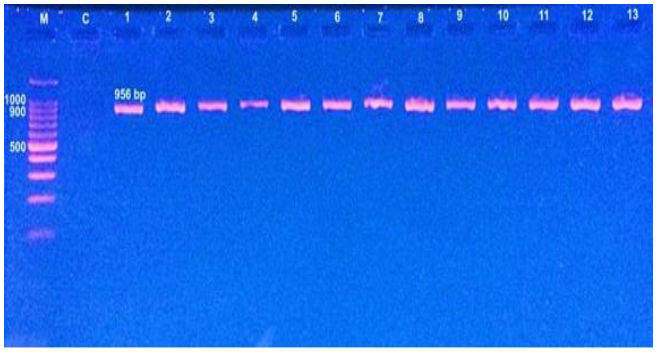

Figure (1): (Agarose gel electrophoresis (1.2%agarose, 7 V/cm2for 90min) of 16SrRNA gene (956bp amplicon). Lane M 100bp DNA ladder, Lane C represent negative control, Lanes1-13 represent bands of P.aeruginosa isolates).

Table (3-1):Prevalence of *P.aeruginosa* in Clinical and environmental samples.

type of sample	Number of samples	Number of isolates	Percentage
Burn	52	20	38.4%
Wound	35	9	26.4%
Ear	22	7	33.3%
Cystic fibrosis	4	4	100%
Urine	13	2	15.3%
Soil	25	5	20%
Water	18	4	22.2%
Total	169	51	

The results of current the study showed that highest percentage of clinical isolates belong to burns 39.2% (20/51) followed by wounds 17.6% (9/51). These results agree with Alkaabi, 2012 (36) who found that *P.*aeruginosa is one of the more bacterial species that cause burns and wounds infection in hospitals. While other infections percentage represent 13.7% (7/51) from ear, 3.9% (2/51) from UTI infection of total clinical isolates and 9.8% (5/51) from soil, 7.8% (4/51) from water of total environmental isolates.

3.3 Hemolysin production

The results of hemolysin production showed that 96% (49/51) of *P.aeruginosa* isolates were able to produce hemolysin (beta-hemolysis) on blood agar media, while 2/51(3.9%) of isolates demonstrated unablility to hemolysis .These results agree with Khalil ,2015 (37) who found that 96% of *P.aeruginosa* isolates produce beta-hemolysis. Hemolysin causes tissue damage and releasing of host nutrients such as iron, where get of iron improve ability of bacteria to growth and reproduction within host cell.

3.4.Protease production

The results of protease production (table 3-3) showed that 92% (47/51) of *P. aeruginosa* isolates were able to produce protease in skimmed milk media (1%) by seen zone inhibition around the colony ,but varying degrees ranged from 20 to 6mm. this variance in zone inhibition may be due to types of isolates and culture condition, while 4/51(7.8%) of isolates didn't demonstrated any zone inhibition around their colony, these results agree with Onal 2015(38) who found that most of clinical isolates represent 93% and were able to produced protease. increasing the effectiveness of protease production is increasing after 24-48hrs.of incubation that means effectiveness of this enzyme increasing through growth phase especially in the lag phase up to the stationary phase when essential nutrients lack. Protease considered one of the important virulence factors it analyzes tissue proteins such as elastin and collagen, and helps bacteria to invade infected tissues, especially in people with burns, also acts to protect of bacteria from body defenses (38).

3.5 Pyocyanin Production

pyocyanin pigment estimated by measure of absorbance in ELISA reader equipment at 690nm. The results show that 90% (46/51)of isolates have the ability to produce pyocanin through 3 days of incubation but in varying degrees (figure 3-3) ,where the highest absorption value recorded for P1 isolate reached to 0.52, this result agree with Parsons *et al* (31) who observed that 92% of isolates produced this pigment. The rate of pyocyanin production increases over time, this explains the state of competition among bacterial cells on nutrients whereas the pigment consider is an antagonist.

Table (3-2) ability of *P.aeruginosa* isolates to produce of pyocyanin.

No. of isolates	Absorbance at 690nm	No. of isolates	Absorbance at 690nm	No. of isolates	Absorbance at 690nm
P1	0.52	P18	0.23	P35	0.20
P2	0.26	P19	0.50	P36	0.22
P3	0.23	P20	0	P37	0.19
P4	0.11	P21	0.41	P38	0.15
P5	0.17	P22	0.44	P39	0.22
P6	0.11	P23	0.33	P40	0.13
P7	0.45	P24	0.32	P41	0.15
P8	0.16	P25	0.34	P42	0.11
P9	0.32	P26	0.18	P43	0.08
P10	0.30	P27	0.28	P44	0.07
P11	0	P28	0.10	P45	0.09
P12	0.35	P29	0.13	P46	0
P13	0.20	P30	0.11	P47	0.24
P14	0.22	P31	0	P48	0.22
P15	0.28	P32	0	P49	0.14
P16	0.35	P33	0.10	P50	0.16
P17	0.21	P34	0.32	P51	0.12

3.6 Detection of biofilm formation

The results(table 3-3) showed that 86.2% (44/51) of isolates produce biofilm, but varying degrees with compare to negative control. The isolates capable of forming the biofilm ranged in intensity among high, medium and weak adherent.This results agree with (39) who found that 91% of isolates produced biofilm by (MTB) method. This high productivity of biofilm formation may be back to sensitivity of (MTB) method to measure the few quantities formed, and considered important method in studying the early stages of biofilm formation because it uses constant conditions and it can be effective in studying many of virulence factors to form of biofilm such as pilli and flagella, also it can be used this method with different type of bactria (40).

From other hand Heydari, 2014 indicated that the variation in the ability of isolates to form biofilm results from its association with its ability to produce ß-Lactamase, where the isolates produced of multiple types of enzyme are produce a strong biofilm, compared with isolates that produce one type of enzyme that is weak, while the isolates that do not produce this enzyme do not form biofilm(39).

Table (3-3) ability of *P.aeruginosa* isolates to biofilm formation.

No. of isolates	Absorbance at 630nm	No. of isolates	Absorbance at 630nm	No. of isolates	Absorbance at 630nm
P1	0.44	P18	0.24	P35	0
P2	0.21	P19	0.18	P36	0.05
P3	0.33	P20	0	P37	0.17
P4	0.24	P21	0.06	P38	0
P5	0.36	P22	0.42	P39	0.25
P6	0.25	P23	0.55	P40	0.09
P7	0.26	P24	0.12	P41	0.12
P8	0.11	P25	0.16	P42	0
P9	0.13	P26	0.30	P43	0.32
P10	0.08	P27	0.18	P44	0.33
P11	0	P28	0.27	P45	0.15
P12	0.14	P29	0.18	P46	0.34
P13	0.09	P30	0.30	P47	0.28
P14	0.22	P31	0.26	P48	0.05
P15	0	P32	0	P49	0.15
P16	0.07	P33	0.13	P50	0.19
P17	0.11	P34	0.62	P51	0.11

Natural Antagonist

With the rise in bacterial resistance to many class of antibiotics, there is great interest in the development of other classes of antimicrobials for the control of infection caused by bacteria, also the bacteria that able to grow as biofilm were protected from host defenses and are often resistant to antibiotics. So that through the present study the garlic extract used as antagonist against some of virulence factors and biofilm formation, this is because garlic has many properties as an antimicrobial, as well as for easy availability.

Determination of minimum inhibitor concentration (MIC) of Garlic extract.

Resazurin methods used to determination of MIC, this color method is based on reduction of resazurin pigment, it is used to check the sensitivity of the drug, antibiotics and plant extracts. This method is simple and gives quick results and is efficient, resazurin is a blue pigment commonly used as a chemical indicator, where the retention of the blue color of the pigment in wells indicates non-growth that's mean the extract inhibition of the bacterial growth due to non-reduction of pigment by bacteria, while turning the pigment color to pink color indicates a bacterial growth due to reduction of Resazurin to resorufin, with comparison of positive and negative control.The result showed that the concentration 1/8 was the MIC for Crude juice of garlic, this concentration was chosen for other experiment against some virulence factors of *P.aeruginosa*.

Effect of Garlic Extract on Production of some Virulence Factors and Biofilm Formation.

After determining the minimum inhibitory concentration(MIC) of garlic extract, used this MIC to inhibit production of some virulence factors, which are produced by *P.*aeruginosa. The results showed that garlic extract inhibits or reduces the production of protease, where the zone inhibition after using the garlic extract was 6mm, while before used of the extract was 20mm. So the garlic did not affected on hemolysin production.

The top 10 isolates of the biofilm formation were selected to explain effect of garlic extract on biofilm formation, where the results of statistical analysis indicated there are significant differences at the level of significant when P-value (<0.05) in biofilm formation after used the garlic extract, so that there were no significant differences between the values of the biofilm formation when using the extract and between the control values (table 3-7) , this results agree with (22) who found that garlic extracts inhibition of planktonic bacteria.

The efficiency of the fresh juice of the garlic is due to the cutting of garlic leads to the breaking of the Parenchyma tissue of garlic, leading to the release of Allinase enzyme, which is act to convert of allin amino acid to allicin which disrupts cell metabolism by oxidation and association with SH group of proteins or amino acids,The phenolic compounds within the garlic content act to precipitate of proteins within bacterial cells and disrupt the functions of the necessary enzymes, also alkaloids have antimicrobial effect by binding with DNA of bacteria, then prevent growth (41).

Bacterial attachment is the initial step for biofilm for mation, so that previous study pointed that allicin may be has important role in preventing adherence of cells to form communities within biofilm(42).

Table (3-4) the difference between the values of the biofilm formation before and after use of garlic extract.

No. of isolates	Biofilm value without Garlic	Biofilm value with Garlic	Control
P1	0.44	0.08	0.06
P2	0.62	0.11	0.06
P3	0.55	0.13	0.06
P4	0.42	0.07	0.06
P5	0.35	0.06	0.06
P6	0.33	0.07	0.06
P7	0.30	0.03	0.06
P8	0.34	0.03	0.06
P9	0.32	0.04	0.06
P10	0.33	0.04	0.06

Conclusions

1- Detection by *16SrRNA* gene is very simple and rapid technique compare with other conventional method**.**

2- The use of fresh garlic extract is more effective against virulence factors and leads to the loss of bacteria ability to form biofilm.

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التشخيص الجزيئي لبكتيريا الزوائف الزنجارية ودراسة تأثير مستخلص الثوم الطازج على بعض عومل الضراوة لهذه البكتيريا.

حسن فلاح لاحج مثنى حامد حسن لمى عامر ياسر

تضمنت الدراسة الحالية جمع 169 عينة من مصادر سريرية وبيئية متنوعه لغرض التحري عن وجود بكتيريا *P.aeruginosa* في تلك المصادر ، حيث جمعت العينات السريرية من بعض مستشفيات بغداد وتضمنت 126 عينة من اخماج الحروق والجروح والتهاب الاذن والتهاب المجاري البولية والتليف الكيسي بواقع (52 , 35 , 22 , 13, 4) عينة على التوالي، اما العينات البيئية فقد توزعت بواقع 25 عينة من التربة و18 عينة من مياه الصرف الصحي وبعض المستنقعات. شُخصت العينات بالاعتماد على صفات المستعمرات المظهرية والمجهرية عند تنميتها في عدد من الاوساط الزرعية فضلا عن الفحوصات البايوكيميائية. تم استخدام التشخيص الجزيئي كتشخيص تأكيدي للعزلات على المستوى الجيني بالاعتماد على الجين التشخيصي *16SrRNA*والذي يحمل تتابعات خاصة ببكتيريا *P.aeruginosa* اظهرت نتائج التشخيص النهائي ان 51 عزلة تعود للبكتيريا المستهدفة توزعت بواقع 20 من حالات الحروق و9 عزلات للجروح و7 عزلات لألتهاب الاذن و4 عزلات للتليف الكيسي وعزلتين تعود لألتهاب المجاري البولية و5 عزلات من التربة و 4 عزلات من المياه. وبينت نتائج الكشف عن بعض عوامل الضراوة بصورة مظهرية ان 49/51 عزلة وبنسبة (95%) كانت منتجة لأنزيم الهيمولايسين (beta-hemolysis) ، في حين كانت 46/51 عزلة بنسبة (90%) كانت منتجة لصبغة البايوسيانين بشده متباينة وبامتصاصية تراوحت بين صفر و0.52 عند طول موجي 690 نانومتر ، بينما كانت47/51 عزلة منتجة لأنزيم البروتييز الحال للبروتين وبنسبة (92%) ،كما أظهرت العزلات تبايناً في قابليتها على تكوين الغشاء الحيوي حيث كانت العزلات المكونة للغشاء 44 عزلة (86%). ونتيجة لأزدياد مقاومة بكتيريا *P.aeruginosa* للمضادات الحيوية اصبحت مشكلة تحيل دون معالجة الاخماج المتسببة عنها ، لذلك ركزت الدراسة الحالية على استخدام مستخلص الثوم الطازج تجاه بعض عوامل الضراوة كونه سهل الاستخدام وشائع الاستعمال ، حيث بينت نتائج التحليل الاحصائي وجود فرق معنوي في انتاج بعض عوامل الضراوة حيث فقدت العزلات قابليتها على تكوين الغشاء الحيوي ،كما انخفضت قابليتها على انتاج انزيم البروتييز، في حين لم يتأثر انتاج الهيمولايسين.