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EFFECT OF GARLIC AND MOUTHWASH ON SOME BACTERIA ISOLATED FROM THE GINGIVITIS AND THEIR RELATIONSHIP WITH SOME ANTIBIOTICS IN DIYALA PROVINCE, IRAQ

Rana Salah Al-zubaidi*

Middle Technical Univ.

***Corresponding Author: Rana Salah Al-Zubaidi**

Middle Technical Univ.

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ABSTRACT

The study included 14 isolates of some bacteria isolated from Gingivitis (7 isolates of *E. coli* and 7 of *Pseudomons aeruginosa*), which were obtained from external laboratories from January 2025 to march 2025 for the purpose of detecting the effect of some antibiotics (meropenem, amikacin, gentamycin and ciprofloxacin), as well as to detect the effect of Garlic crude juice and mouthwash on these bacteria, the results showed that the effect uneven between antibiotics to garlic and mouthwash, which was, in general, higher against *E. coli* than *Pseudomonas*.

GARLIC, MOUTHWASH, *E. COLI*, *PSEUDOMONAS AERUGINOSA*

1: INTRODUCTION

Since the birth, the human mouth has endured large numbers of beneficial and harmful microbes for oral and dental health whether. Various factors in the oral environment contribute to the multiplication and diversity of microbes, particularly the extent to which natural teeth are normal Hygiene and maintenance of dental prostheses, the safety and vitality of gum tissue and ligaments around the teeth, as well as an important factor related to the quality of food (Nisengard and Newman, 1994).

The saliva in the mouth precipitates protein-sugary compounds forming thin, transparent layers called elbows Which helps the oral microbes adhere to these layers and then multiply and fertilize the enamels (enamel surfaces) In large quantities and thin organic layers known as microbial dental plaque on the surfaces of the teeth, above and below edge of the gums. It is scientifically proven that if the teeth are not cleaned well or permanently every day if the plaques continue to be cleaned which is characterized by redness, swelling of the gums and ease of bleeding and may develop into the disease of Gingivitis microbial, it leads to gingivitis

Age-related inflammation / dental support tissue However, microbial plaque can be removed and only tissue tissue surrounding the tooth can be protected with good daily care and regular professional care (Darout and Skaug, 2004).

1-1: Objectives of the study

1- Know the effect of antibiotics on the bacteria isolated from the gums.

2- Compare the effect of crude garlic juice with mouthwash and which is more effective in eliminating oral bacteria**.**

2: Literature review

2-1 Gingivitis bacteria

Gingivitis is a common cause of mouth disease, which is due to poor oral hygiene, tooth brushing or poor tooth fillings. All of these causes a number of pathogens. Gum disease often begins when accumulation or accumulation of food residues in the oral cavity. Between gums and teeth If these accumulated residues are not disposed of, they will ferment into an environment suitable for the growth and reproduction of germ.

The using of antibiotics has an important role in the treatment of many infections and bacterial infections, including gingivitis, but the use of long periods and randomly lead to the emergence of bacterial strains resistant to these antibiotics (Brooks *et al*., 1999).

2-2 *E. coli*

E. coli is the head of a large bacterial family, *Enterobacteriaceae* or so called the enteric group; this bacterium was first discovered in human colon in 1885 by a German bacteriologist Escherich it was first called *Bacillus coli* (Kenneth, 2002).

E. coli is found in the intestines as part of the normal flora in the intestines, but when leaves its natural position, many of them have the ability to cause the

disease by having various virulence factors such as Exotoxin, which have two types: Stable Toxin, Heat Labile Toxin, and Haemolysin which helps bacteria to breaks down the cells and increases the presence of iron and thus significantly improves bacterial growth, these bacteria constitute 85% of nosocomial infections. It has flagella on its surface that interact with urinary epithelial cells, this helps bacteria to attach more with the bladder cells, as well as it is one of the most common types of urinary tract infections, especially among pregnant women, with a prevalence of 90% (Jawetz *et al*., 1998). This is a very common cause of erythema and bladder infection in women and is the leading cause of kidney injury events.

2-2-1 General characteristics of *E.coli*

E.coli is facultative anaerobic Gram–negative, nonspore forming rod, motile by peritrichous flagella (Kenneth, 2005), most strains of *E.coli* can grow on simple laboratory media containing glucose as the main carbon source. Most strain recovered in the clinical laboratory ferment lactose and thus grows smooth, glossy, pink colonies on MacConkey agar (Collee et al., 1991).

It gives positive reaction in indole, catalase and methyl red while negative tests in oxidase, urease, H_2S production, Vogas Proskure and citrate utilization, it has a distinctive greenish metallic sheen when grown on Eosin Methylen Blue agar (EMB) (Kenneth, 2002).

2-3 *Pseudomonas aeruginosa*

This type of bacteria is resistant to chemicals (sterilizers and disinfectants) and physical factors (temperature, humidity, dehydration), P. aeruginosa are characterized by their natural resistance to many antibiotics because they have different resistance mechanisms that have caused the failure of antibiotics to eliminate this type of bacteria, which called on specialists to search for new sources of antimicrobial agents used as alternatives to traditional antibiotics (Fraimow and Abrutyn, 1995).

P. aeruginosa are widespread in human environment as they are found in soil, water and plants, also they are an acquired bacteria in hospitals (Nosocomial infections) (Atlas, 1995).

The widespread use of antibiotics has led to the emergence of resistant strains. This resistance is due to the transmission of resistance genes through bacterial plasmids, which helps to exacerbate and overcome the resistance of antibiotic resistance (Obritsch *et al*., 2004).

2-3-1 : General characteristics of *P. aeruginosa*

It's a Gram-negative, bacilli, motile, obligate aerobic and produce a distinctive aroma resembling fermented grapes, and some strains produce haemolysin. They are smooth circular colonies of green color on culture medium. Some strais produce pyocyanin dye which is spread in the agar, some of their strains don't produces this dye, but produces a fluorescent dye called pyoverdin, which is green on the agar, as well as a dark red dye called pyorubin, also produce a dark red dye called pyomelanin. These bacteria grow well at 37-42 ° C and their growth at 42° C make them differentiate from other species. It is positive for oxidase, lactose fermentation and carbohydrates, but most of their strains oxidize glucose (Meyers and Klastersky, 1984).

2-4 : Garlic plant

Garlic (*Alluim sativum Linn*.) is one of those plants that was greatly used over the years. It has been used for centuries to combat infectious diseases, 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 (Kock and Lawsen, 1996). Garlic (*Alluim sativum Linn.*) exhibits a broad spectrum activity against both Gram-positive and Gram-negative bacteria and it can be used for formulation of newer spectrum antimicrobial substances (Abubakar, 2009).

From the published researches articles, it's clear that the raw juice of garlic was effective against many common pathogenic bacteria and against that have become resistant to antibiotic (Ariga and Seki, 2006).

Therapeutic effect of garlic is possible because of its oil- and water-soluble organosulfur compounds. Thiosulfinates (eg. Allicin) play an important role in the antibiotic activity of garlic. Feldberg and his group (1988) showed that 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.

2-5 : Mouthwash

Medical mouthwash is a successful solution for treatment of gingivitis, toothache and plaque formation, because it contains effective chemical compounds such as thymol, eucalyptol, menthol, fluoride, enzymes, calcium and sodium benzoate, which provide the refreshing smell of mouthwash. It is also used as a killer of bacteria, and should be used after washing with toothbrush at least an hour because the toothpaste contains a compound of Sodium Lauryl Sulfate, which inhibits the effectiveness of some components of mouthwash (Cochran and Sylvia, 2009).

3: MATERIALS & METHODS

3-1 : Plant study & Mouthwash

A quantity of garlic was collected from local market and freshly squeezed and used to prepare the fresh juice. Mouthwash (Oral-1) purchased from the pharmacy.

3-2 Bacterial isolates
7 isolates of *E. coli* and 7 isolates of *P. aeruginosa* were obtained from gingivitis diseased people from an external laboratory from January 2025 to March 2025, which was recultured on macConkey agar, and nutrient agar for primary detection.

3-3 Crude juice of garlic preparation
Mixing 10 g of chopped garlic cloves with 25 ml of sterile distilled water and mixing in an electric mixer and used directly in the test (Reuter, *et al.,* 1996).

3-4 Identification of bacteria
3-4-1 Detection of lactose fermentation
MacConkey agar plates were inoculated with single isolated colony of *E. coli*. Plates were incubated at 37˚C for 24-48 hrs for detection of lactose fermentation.

3-4-2 Detection of Pyocyanin production
Nutrient agar plates were inoculated with single isolated colony of *P. aeruginosa*. Plates were incubated at 37˚C for 24-48hrsfor detection of Pyocyanin production (Forbes *et al.,* 2007).

3-4-3 Preparation of bacterial suspension
The bacterial suspension prepared by direct colony suspension method. In which a small volume of sterile water was poured inside a test tube and mixed with some colonies of the test organisms (Isu and Onyeagba, 2002)**.**

3-4-4 Antibiotic susceptibility test
Bacterial suspension was cultured on muller-hinton agar medium using a cotton swab in all directions, the dishes were left 5 minutes then the antibiotics were placed using sterile forceps with 4 discs per one dish using disc diffusion method. Dishes incubate at 37 ° C for 18 - 24 hours. The results were read by measuring the diameters of the inhibition zones around each disc and for the knowledge of resistance and sensitivity, the measured diameters were compared with the standard diameters of these antibiotics (CLSI 2007).

3-2-4-6: The inhibitory activity of crude juice 3-2-4-6-1: Inhibitory activity of discs
A discs of the filter paper with 5 mm diameter were prepared by using paper punch and then immersed in 100% of crude garlic juice, then placed on the surface of the cultured dishes and incubated at 37 ° C for 18-24 hours. The diameters of the inhibition zones around the discs were measured and compared with the diameters of inhibition zones of antibiotics.

3-2-4-6-2: Inhibitory activity of wells
A wells were made in petri plates (in agar) by using sterile cork borer, 50 µl of 100% crude garlic juice was added in the well, plates were incubated at 37 ° C for 18-24 hours. The diametersof the inhibition zones around the well were measured and compared with the diameters of inhibition zones of antibiotics (Ameen *et al*., 2015).

3-2-4-7: The inhibitory activity of mouthwash 3-2-4-7-1: Inhibitory activity of discs
A discs of the filter paper with 5 mm diameter were prepared by using paper punch and then immersed in 100% of mouthwash, then placed on the surface of the cultured dishes and incubated at 37 ° C for 18-24 hours. The diameters of the inhibition zones around the discs were measured and compared with the diameters of inhibition zones of antibiotics.
3-2-4-7-2: Inhibitory activity of wells
A wells were made in petri plates (in agar) by using sterile cork borer, 50 µl of 100% mouthwash was added in the well, plates were incubated at 37 ° C for 18-24 hours. The diameters of the inhibition zones around the well were measured and compared with the diameters of inhibition zones of antibiotics (Ameen *et al*., 2015).

4: Results & Discussion 4-1: Bacterial isolates
7 bacterial isolations of *E. coli* & 7 bacterial isolations of *Pseudomonas aeruginosa* were obtained from external laboratories.

4-2 : Cultural characteristics
4-2-1 : Cultural characteristics of *E. coli*
Because of lactose sugar existence in macConkey agar, fermentation of this sugar was detected by changing the color of all colonies and culture medium to pink, this corresponds to what (Al-Sarage, 2004) noticed.

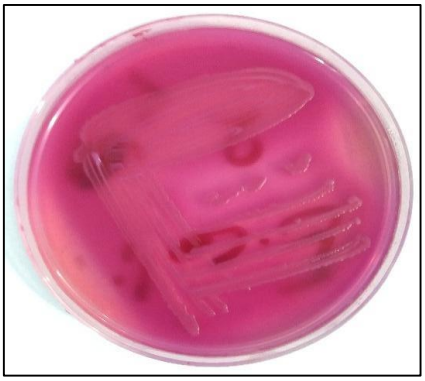

Figure (1): *E. coli* on macConkey agar with pink color colonies.

4-2-2 Cultural characteristics of *Pseudomonas aeruginosa*

Figure (2): *P.* aeruginosa on nutrient agar with production green dye.

Pyocyanin production was detected by production of green dye on agar, (Meyers and Klastersky , 1984).

4-3 Antibiotics effect on bacteria

4-3-1 Antibiotics effect against *E. coli*

Table (1) indicates the effect of antibiotics on *E. coli*. The sensitivity of bacteria to antibiotics was varied towards 3 antibiotics, the percentage of this sensitivity of bacteria to meropenem was 28.5%, and 14.2% for amikacin and gentamicin every one alone, 42.8% of isolates were moderately affected by amikacin, while all bacterial isolates were resistant to ciprofloxacin.

Table (1): Inhibition zones dimeters of antibiotics effect against *E. coli* isolations.

Isolation num.	Inhibition zones dimeters in (mm)			
	MEM	AK	CX	CN
1	38 mm	15 mm	0	0
2	0	20 mm	0	9 mm
3	28 mm	15 mm	0	0
4	0	0	0	15 mm
5	0	15 mm	9 mm	0
6	10 mm	6 mm	0	0
7	0	0	9 mm	0

These results didn't agree with (Al-Zubaidy and Al-Zuhairi, 2017) which all of their *E.coli* isolates were sensitive to meropenem in 100% and ciprofloxacin in 90%.

4-3-2 Antibiotics effect against *P. aeruginosa*

Table (2) indicates the effect of antibiotics against *P. aeruginosa*. In general, meropenem was the only influencer in its inhibitory effect on the bacteria with diameters ranging from 28mm to 31mm and the ratio of sensitivity was 42.8%, some isolations were intermediate influenced by amikacin with ratio 28.5%, gentamicin and ciprofloxacin were the least efficient as they did not give any inhibitory effect on any bacterial isolates.

Table (2): Inhibition zones dimeters of antibiotics effect against *P. aeruginosa*

Isolation num.	Inhibition zones dimeters in (mm)			
	MEM	AK	CX	CN
1	0	0	0	8 mm
2	0	0	0	8 mm
3	0	0	0	9 mm
4	28 mm	13 mm	0	0
5	27 mm	16 mm	0	10 mm
6	31 mm	15 mm	0	12 mm
7	13 mm	9 mm	0	6 mm

The resistance to antibiotics may be due to the production of a bacterial enzyme to resist the antibiotics (Singh *et al*., 2000). Also random use of antibiotics or acquisition of bacteria for genetic factors that carry the multiple resistance of antibiotics by conjugation as in the transmission of plasmids (Manzoor *et al*., 2005).

4-4 Garlic's & Mouthwash effect on bacteria 4-4-1: Well's & discs effect against *E. coli*

By the table below, information pointed that the effect of garlic's crude juice against some *E. coli* isolations (42.8%) was less than antibiotics, while one of the isolates did not affected by garlic juice ompletely and this was identical to the effect of antibiotics also. Other isolates (42.8%) were effected by garlic juice and this effect was higher than antibiotics effect, garlic effect was closer with meropenem sometimes. All the effect was returned to crude juice in wells because saturated discs with garlic juice did not appear any effect of the bacterial isolates. Mouthwash effect was higher than garlic and antibiotics against some isolates and less than them against the other isolates.

Table (3): Inhibition zones dimeters of Garlic's crude juice & Mouthwash effect against *E. coli.*

Isolation num.	Inhibition zone dimeters of Garlic juice		Inhibition zone dimeters of mouthwash	
	Well's effect	Disc's effect	Well's effect	Disc's effect
1	20 mm	0	40 mm	25 mm
2	0	0	15 mm	0
3	11	0	20 mm	0
4	18 mm	0	0	0
5	18 mm	0	0	0
6	0	0	0	0
7	15 mm	0	35 mm	25mm

4-4-2: Garlic's crude juice effect & discs effect against *P. aeruginosa*

Table (4) described that the effect was alternating between garlic juice and antibiotics, garlic discs didn't effect anymore. Mouthwash effect also was alternating with antibiotics and garlic effect. The discs here did not affect the bacteria at all, unlike the *E. coli* which some of their isolates were affected by the discs and the other part by the wells filled with mouthwash.

Table (4): Inhibition zones dimeters of Garlic's crude juice & Mouthwash effect against *P. aeruginosa.*

Isolation num.	Inhibition zone dimeters of Garlic juice		Inhibition zone dimeters of mouthwash	
	Well's effect	Discs effect	Well's effect	Well's effect
1	10 mm	0	0	0
2	12 mm	0	20 mm	0
3	0	0	15 mm	0
4	16 mm	0	0	0
5	0	0	0	0
6	14 mm	0	0	0
7	12 mm	0	0	0

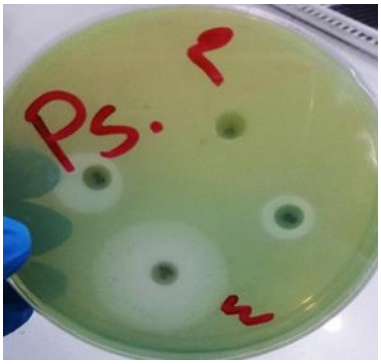

Figure (3): Inhibition zones dimeters of antibiotics, Garlic's crude juice and mouthwash effect against *P. aeruginosa.*

The sensitivity of *E. coli* towards mouthwash was higher than or somewhat similar to its sensitivity to garlic juice. This may be due to the fact that bacteria affected by sodium fluoride contained in the composition of mouthwash as well as by some compounds in the composition of garlic plant as organosulfur and phenolic compounds involved in the antimicrobial activity in garlic. Most of *P. aeruginosa* isolates did not show sensitivity to mouthwash, this may be due to their high resistance mechanisms, while most of them were sensitive or moderately sensitive to garlic juice, as well as garlic containing some ingredients such as Allicin, that acts by partially inhibiting DNA and protein synthesis and also totally inhibiting RNA synthesis as a primary target (Eja, *et al*., 2007). The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids (Griffiths, *et al*. 2007). Organosulfur compounds and phenolic compounds have been reported to be involved in the garlic antimicrobial activity (Jombo, *et al.*, 2011; Aboaba, and Efuwape, 2001).

CONCLUSIONS

- Some of bacteria was effected by antibiotics, the other by garlic or mouthwash.
- Garlic effect on *E. coli* was closer to antibiotics effect, while mouthwash effect was little higher than garlic and antibiotics.
- Saturated discs with garlic juice did not affect any more on *E. coli.*
- Saturated discs with mouthwash did not affect anymore on *P. aeruginosa*, as well, garlic discs.
- *P. aeruginosa* resistance was higher than E. coli resistance.
- No antibiotic was affect on *P. aeruginosa*, except meropenem.

Recommendations

- Studying the effect of Garlic extract in different concentrations on *E. coli* & *P. aeruginosa*.
- Studying of more & various types of antibiotics against *E. coli* & *P. aeruginosa*.
- Mixing of mouthwash with Garlic and knowing their synergy effect on the bacteria.

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