Inhibition of bacterial adhesion for Ecoli by ciprofloxacin from patients suffering gingivitis

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Abstract

Gingivitis:is an inflammatory destructive disease mainly caused by microbial plaques. This study was aied isolate the pathogenic bacteria from gingival of 50 patients with oral gingivitis and 24 isolate by using. enriched and specific media used for isolation of bacteria. Identification of isolated bacteria performed by gram staining and biochemical tests. Twenty four isolate were gram negative and gram positive .The most frequent gram negative isolates were *E coli* 5(20.8%), *Streptococcus spp* 4(16.6%),*Klebseilla spp* 3(12.5%) ,*Protus valgaris*, *Pseudomonas spp*,*Klebseilla oxytoca, Enterobacter cloacae*,*Escherichia alkalgance* were 2(8.5%), and the last *Protus mirabilis* and *Citrobacter spp* were 1(4.1)% . Antibiotics bacterial resistant was most common in gram negative than in gram positive .The high bacterial antibiotics resistant was *Ecoli* Ampicillin, , Cephalexin, Ciprofloxacin and Ceftriaxone (100)% while the low bacterial resistant to antibiotics was *Citrobacter spp* . Bacterial gingivitis adhesion on epithelial cell and inhibition adhesion by sub MIC, used the plate test double dilution agar method for MIC & sub MIC ciprofloxacin antibiotic. The results of present study indicate that a wide range of pathogenic bacteria are responsible for destructions of gingival among patients. The diagnosis and treatment of the patients is necessary according to the public health care systems .The aim of study was isolate and identification the more pathogenic bacteria and the bacterial antibiotics resistant antibiotics and study inhibition adhesion virulence factor by sub MIC ciprofloxacin .

Key words: Gingivitis, bacterial gingivitis ,Ecoli, adhesion

Introduction:

Gingivitis: Is the most common form of oral diseases, which is often caused by poor oral hygiene. It can lead to the destruction of bone and loss of teeth [1].Gram-positive and gram-negative bacilli or cocci have been isolated from dental plaque and gingivitis [2].Gingivitis is a chronic infection and is caused by accumulation of bacteria in the gingival crevices causing an inflammatory reaction [3-4].The most common form of gingivitis is a response to bacterial adherent to tooth surfaces, and called (a plaque-induced gingivitis) [5]. The plaque accumulates in the small gaps between the teeth, in the gingival

grooves and in areas known as (plaque traps locations) that serve to accumulate and maintain plaque. Although these accumulations may be tiny, the bacteria in them produce chemical materials such as degrative enzymes, and toxins, like lipopolysaccharide otherwise known as endotoxin or lipoteichoic acid that promote an inflammatory response in the gum tissue. This inflammation can cause an enlargement of the gingival and subsequent formation[6]. The gram positive bacteria proliferate and the number of gram-negative bacteria increases in the first two days of plaque formation during gingivitis. After 2-4 days, fusobacteria and filamentous bacteria are added to the previous population[7]. Many people have gingivitis to a varying degree. It usually develops during puberty or early adulthood due to hormonal changes and may persist or recur frequently, depending on the health of your teeth and gums [8]. Adhesion of bacteria to epithelial cells plays an important role in the initiation and pathogenesis of infection, particularly in the epithelial cell [9].

Material and methods:

Culture isolates:

A total of 50 patients with plaque-induced gingivitis.Specimens obtained from infectious areas of upper and lower gingival from patients gingivitis from September 2010 to May 2011. The samples of gingivitis taken from Hay Al-Husain Specialist central and sent to laboratory of microbiological investigations to make analysis.Use blood agar base (Oxoid Company) in which 7 mL of blood sheep was added to prepare blood Agar.MacConkey agar from (Oxoid Company) ,nutrient broth (Oxoid).Mueller-hinton agar (Biolife company), was used for the sensitivity of the bacteria against antibiotics and using standard strain *Ecoli* (ATCC 25922).

The sensitivity test: The method of Bauer *et al.* (1966) was used for the purpose of sensitivity of bacteria for the antibiotics by discs diffusion techniques.Isolation of bacteria was done by streaking procedure on (blood agar , MacConkey agar, nutrient agar in order to identify gram negative and positive isolates . The sensitivity test for bacterial isolates to 8 antibiotics was Ampicillin , Cephalexin, Ciprofloxacin , Imipenem ,Tetracycline, Trimethoprim ,Cefotasime and Amoxicillin. Discs diffusion tests performed according to (Bauer *et al*; 1966) on Muller - Hinton agar.The inhibition zone diameter was measured and recorded according to [9]. These results comprised with standard strain of (*E coli* ATCC 25922).In bacterial adhesion used Sub MIC ciprofloxacin powder ,urinary epithelial cell and phosphate buffer solution according to [10].

Results:

A total of 40 patients studied from December 2011 to April 2012, were reported that (24) isolates were gram negative and gram positive . The most frequent isolates were *Ecoli* 5(20.8%), *Streptococcus spp* 4(16.6%),*Klebseilla*

spp 3(12.5%) , *Protus valgaris*, *Pseudomonas spp*,*Klebseilla oxytoca Enterobacter cloacae*,*Escherichia alkalgance* were2(8.5%),and the last *Protus mirabilis* and *Citrobacter spp* were1(4.1)% shows in table(1). Antibiotics resistance were most common in gram negative than in gram positive by using antimicrobial Sensitivity test in table(2) the high resistant bacterial was *Ecoli* Ampicillin, , Cephalexin, Ciprofloxacin and Ceftriaxone **(**100**)%** and the low bacterial resistant to antibiotics was *Citrobacter spp* for all antibiotics .Table (3) shows bacterial gingivitis adhesion on epithelial cell and inhibition adhesion by sub MIC ,used the plate test (Double dilution agar method) for MIC &sub MIC ciprofloxacin antibiotic in this search [11].

Table (1): Distribution of bacterial gingivitis

Type of bacteria	No.	
E coli	5	5(20.8%)
Streptococcus spp	4	4(16.6%)
Klebseilla spp	3	3(12.5%)
Protus valgaris	2	2(8.5%)
Pseudomonas spp	2	2(8.5%)
Klebseilla oxytoca	2	2(8.5%)
Enterobacter cloacae	2	2(8.5%)
Escherichia alkalgance	2	2(8.5%)
Protus mirabilis	1	1(4.1%)
Citrobacter spp	1	1(4.1%)
Total	24	

Table (2): The value of resistance bacterial gingivitis antimicrobial agent

Type of Bacteria	SXT*	I*	Cip*	AM*	CL*	CN*	P*	TE*
E coli	40%	20%	60%	100%	100%	100%	60%	20%
Streptococcus spp	100%	75%	75%	100%	100%	100%	75%	75%
Klebseilla spp	100%	0	0	100%	100%	100%	100%	33%
Protus valgaris	50%	50%	100%	50%	50%	50%	50%	50%
Pseudomonas spp	100%	100%	100%	100%	100%	100%	100	100%
Klebseilla oxytoca	50%	0	50%	100%	100%	50%	50%	100%
Enterobacter cloacae	50%	100%	50%	100%	100%	50%	50%	50%
Escherichia alkalgance	100%	100%	100%	100%	100%	100%	100%	100%
Protus mirabilis	0	0	0	0	100%	0	0	0
Citrobacter spp	0	0	0	0	0	0	0	0
Total								

P*Penicillin,*Imipenem,TE*Tetracycline, AM*Ampicillin, , CL* Cephalexin, , CIP*Ciprofloxacin , SXT*Trimethoprim ,. * This value mean the clear zone of resistant of bacteria to antibiotics

Table (3): The value of bacterial gingivitis adhesion on epithelial cell and inhibition adhesion by MIC ciprofloxacin

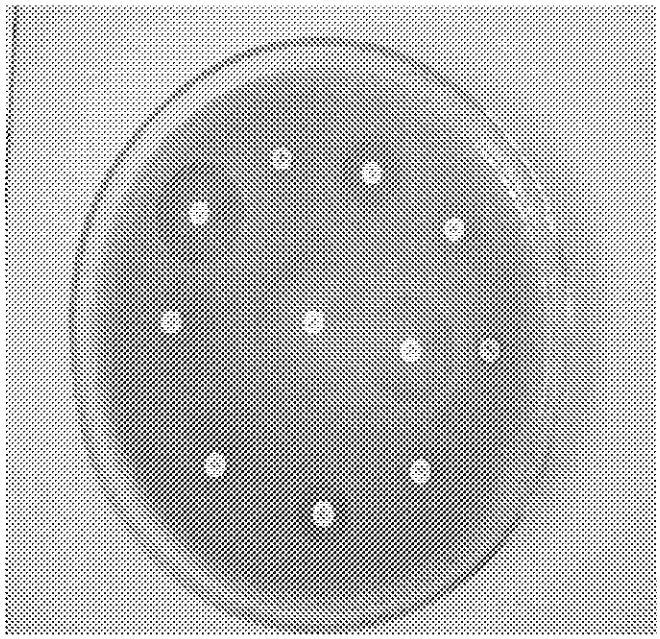

Figure (1): Sensitivity test to antibiotics in cases of ***Ecoli***

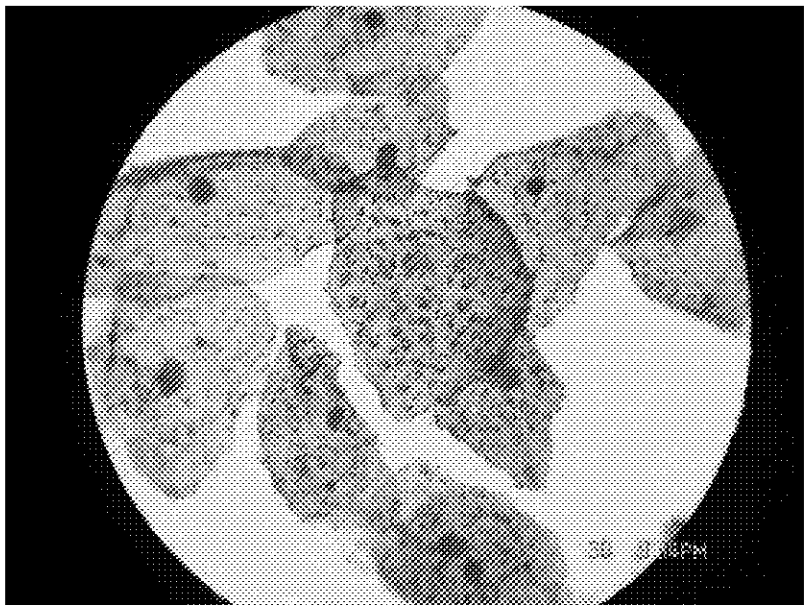

Figure (2): Adhesion ***Ecoli*** to epithelial cell

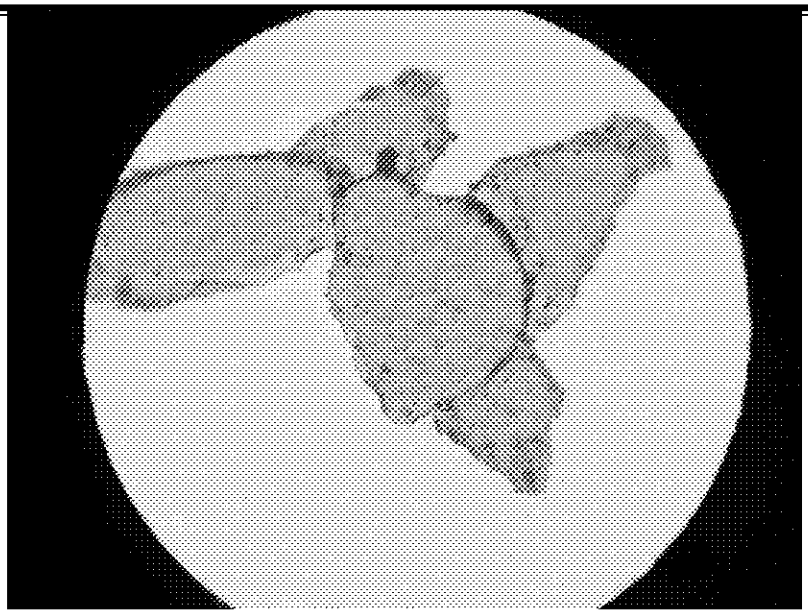

Figure (3) : The inhibition adhesion ***Ecoli*** by Ciprofloxacin.

Discussion:

The high bacterial causing gingivitis was *Ecoli* 5(20.8%)this study disagree with other worker [12-13] shows the high ratio of bacterial gingivitis was *Staphylococcus spp* .The oral cavity is colonized by natural microflora, which is relatively stable in individuals and the composition of which is the result of a long-term relationship between the microorganisms and the host. This balance can easily be disrupted by the action of numerous external and internal factors. Microorganisms play an important role in the development of many pathological states in the oral cavity This study disagree with [13] shows the high ratio of bacterial gingivitis was actinobacillus spp ,porphyromonas gingivalis ,capnocytophage sputigena and eikenella corrodence.also this study disagree with [14] who emphasized the importance of gram positive cocci in the initiation of dental plaque formation leading to the gingivitis .The study of [2] was the high pathogenic bacteria causing gingivitis was 6 species of streptococci than 3 species of staphylococcus therefore this study disagree with our search . According to the bacterial resistant to antibiotic in this study *E.coli* was bacterial resistant to Ampicillin ,Cephalexin ,ciprofloxacin and cetriaxone (100%) this disagree with [15] that uses metronidozal then clindaycin .The oral cavity is colonized by natural microflora occurrence of periodontal pathogens in patients treated with fixed orthodontic appliances bacterial adhesion virulence factor on epithelial cell is very necessary due to the first step of pathogenesis is adhesion than penetration and reproduction . Table 3 and figure (2,3) show bacterial adhesion was 150 ,100,80,70,50,this number decreased with sub MIC ciprofloxacin as 100, 90, 80, 60, 50, 20 and inhibition adhesion bacteria this study agree with[16] shows the number of *Ecoli* adhesion in oral buccal cavity is degreased in number.

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تثبيط عامل الالتصاق لبكتريا الاشريشيه القولونيه بالتركيز المثبط الأدنى للمضاد الحيوي السبروفلاكساسين لمرضى التهاب اللثه

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الخلاصه

التهاب اللثة من الإمراض الشائعة الانتشاربسبب الميكروبات الموجودة في المادة البيضاء المتراكمة والملتصقة .علما ان سبب التهاب اللثه هي البكتريا الموجبه والسالبه لصبغه غرام الكرويه و العصويه وتم عزل24 عزله بكيريه من 50مريض يعانون من اعراض التهاب اللثه وقد استعملت الاوساط الزرعيه الغنيه والمتخصصه وصبغه غرام وبعض الفحوصات البايوكميائيه في العزل .ان الاربع والعشرين عزله كانت للبكتريا الموجبه والسالبه لصبغه غرام .علما ان اعلى نسبه بكتريا سجلتها البكتريا السالبه لصبغه غرام وكانت النسب كالاتي :

E coli 5(20.8%) *Streptococcus spp 4*(16.6%), *Klebseilla spp* 3(12.5%) , *Klebseilla, Pseudomonas spp,Protus valgaris, oxytoca, Enterobacter cloacae,Escherichia alkalgance* were 2(8.5%),*Protus mirabilis* and *Citrobacter spp* 1(4.1) %.

سجلت البكتريا القولونيه اعلى نسبه لمقاومتها للمضادات الحيوية .

الاتيه ،البنسلين والسيفالوسبورين والسبروفلوكساسين والسيفالوتراكزون 100% بينما سجلت الستروبكتر اقل نسبه بكتريا مقاومه للمضادات الحيوية المستعملة.في هده الدراسة تم تثبيط التصاق البكتريا القولونيه بالخلايا الطلائيه بالتركيز المثبط الأدنى لمضاد السبروفلاكساسين وباستعمال طريقه تخفيف الإطباق المضاعفة للأوساط الصلبة. ان هدف الدراسة هو كشف المدى الواسع من البكتريا المرضية المسؤوله عن التهاب اللثة حيث يعتبر التشخيص والعلاج ضروري للصحة ألعامه وكذلك عزل وتشخيص أكثر الأنواع البكتريالمرضية ومقاومه بكتريا التهاب اللثة لبعض المضادات الحيوية ودراسة التصاق البكتريا بالخلايا الطلائيه للثة وتثبيط التصاقها بالتركيز المثبط الأدنى للمضاد السبروفلاكساسين.