

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 aimed to isolate the pathogenic bacteria from gingival of 20 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* (20,8%), *Streptococcus spp* (16,6%), *Klebseilla spp* (12,5%), *Protus valgaris*, *Pseudomonas spp*, *Klebseilla oxytoca*, *Enterobacter cloacae*, *Escherichia alkaligance* were (8,5%), and the last *Protus mirabilis* and *Citrobacter spp* were (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 degradative 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 with 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 5 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 *E. coli* (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, Cefotaxime 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 *E. coli* 60(20,8%), *Streptococcus spp* 4(16,6%), *Klebsiella*

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spp ٣(١٢,٥%) , *Protus valgaris*, *Pseudomonas spp*, *Klebseilla oxytoca* *Enterobacter cloacae*, *Escherichia alkalgance* were ٢(٨,٥%), and the last *Protus mirabilis* and *Citrobacter spp* were ١(٤,١)% shows in table(١). Antibiotics resistance were most common in gram negative than in gram positive by using antimicrobial Sensitivity test in table(٢) the high resistant bacterial was *E. coli* Ampicillin, , Cephalexin, Ciprofloxacin and Ceftriaxone (١٠٠)% and the low bacterial resistant to antibiotics was *Citrobacter spp* for all antibiotics .Table (٣) 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 [١١].

Table (١): Distribution of bacterial gingivitis

Type of bacteria	No.	
<i>E. coli</i>	٥	٥(٢٠,٨%)
<i>Streptococcus spp</i>	٤	٤(١٦,٦%)
<i>Klebseilla spp</i>	٣	٣(١٢,٥%)
<i>Protus valgaris</i>	٢	٢(٨,٥%)
<i>Pseudomonas spp</i>	٢	٢(٨,٥%)
<i>Klebseilla oxytoca</i>	٢	٢(٨,٥%)
<i>Enterobacter cloacae</i>	٢	٢(٨,٥%)
<i>Escherichia alkalgance</i>	٢	٢(٨,٥%)
<i>Protus mirabilis</i>	١	١(٤,١%)
<i>Citrobacter spp</i>	١	١(٤,١%)
Total	٢٤	

Table (٢): The value of resistance bacterial gingivitis antimicrobial agent

Type of Bacteria	SXT*	I*	Cip*	AM*	CL*	CN*	P*	TE*
<i>E. coli</i>	٤٠%	٢٠%	٦٠%	١٠٠%	١٠٠%	١٠٠%	٦٠%	٢٠%
<i>Streptococcus spp</i>	١٠٠%	٧٥%	٧٥%	١٠٠%	١٠٠%	١٠٠%	٧٥%	٧٥%
<i>Klebseilla spp</i>	١٠٠%	.	.	١٠٠%	١٠٠%	١٠٠%	١٠٠%	٣٣%
<i>Protus valgaris</i>	٥٠%	٥٠%	١٠٠%	٥٠%	٥٠%	٥٠%	٥٠%	٥٠%
<i>Pseudomonas spp</i>	١٠٠%	١٠٠%	١٠٠%	١٠٠%	١٠٠%	١٠٠%	١٠٠%	١٠٠%
<i>Klebseilla oxytoca</i>	٥٠%	.	٥٠%	١٠٠%	١٠٠%	٥٠%	٥٠%	١٠٠%
<i>Enterobacter cloacae</i>	٥٠%	١٠٠%	٥٠%	١٠٠%	١٠٠%	٥٠%	٥٠%	٥٠%
<i>Escherichia alkalgance</i>	١٠٠%	١٠٠%	١٠٠%	١٠٠%	١٠٠%	١٠٠%	١٠٠%	١٠٠%
<i>Protus mirabilis</i>	١٠٠%	.	.	.
<i>Citrobacter spp</i>
Total								

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

Table (٢): The value of bacterial gingivitis adhesion on epithelial cell and inhibition of adhesion by MIC ciprofloxacin



Figure (١): Sensitivity test to antibiotics in cases of *E. coli*



Figure (٢): Adhesion of *E. coli* to epithelial cell

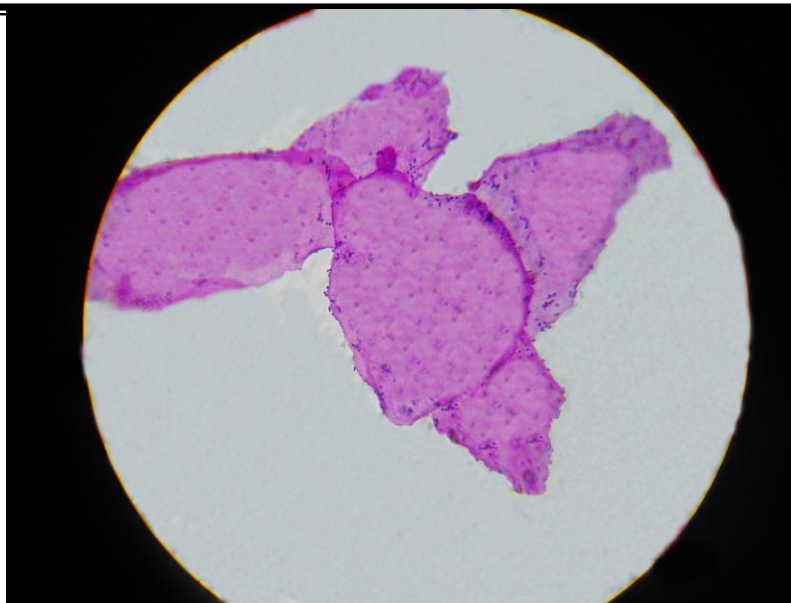


Figure (٣) : The inhibition adhesion *E. coli* by Ciprofloxacin.

Discussion:

The high bacterial causing gingivitis was *E. coli* (٢٠,٨%) this study disagree with other worker [١٢-١٣] 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 [١٣] shows the high ratio of bacterial gingivitis was *actinobacillus spp*, *porphyromonas gingivalis*, *capnocytophage sputigena* and *eikenella corrodence*. also this study disagree with [١٤] who emphasized the importance of gram positive cocci in the initiation of dental plaque formation leading to the gingivitis. The study of [٢] was the high pathogenic bacteria causing gingivitis was ٦ species of streptococci than ٣ 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 ceftriaxone (١٠٠%) this disagree with [١٥] that uses metronidazole then clindamycin. 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 ٣ and figure (٢,٣) show bacterial adhesion was ١٥٠, ١٠٠, ٨٠, ٧٠, ٥٠, this number decreased with sub MIC ciprofloxacin as ١٠٠, ٩٠, ٨٠, ٦٠, ٥٠, ٢٠ and inhibition adhesion bacteria this study agree with [١٦] shows the number of *E. coli* adhesion in oral buccal cavity is decreased in number.

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تثبيط عامل الالتصاق لبكتريا الاشريشية القولونية بالتركيز المثبط الأدنى للمضاد الحيوي

السبروفلاكساسين لمرضى التهاب اللثة

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الخلاصة

التهاب اللثة من الأمراض الشائعة الانتشار بسبب الميكروبات الموجودة في المادة البيضاء المتراكمة والملتصقة. علما ان سبب التهاب اللثة هي البكتريا الموجبة والسالبة لصبغة غرام الكرويه و العصويه وتم عزل ٢٤ عزله بكيريه من ٥٠ مريض يعانون من اعراض التهاب اللثة وقد استعملت الاوساط الزرعيه الغنيه والمتخصصه وصبغه غرام وبعض الفحوصات البايوكيميائيه في العزل. ان الاربع والعشرين عزله كانت للبكتريا الموجبه والسالبه لصبغه غرام. علما ان اعلى نسبه بكتريا سجلتها البكتريا السالبه لصبغه غرام وكانت النسب كالآتي :

E. coli ٥(٢٠,٨%), *Streptococcus spp* ٤(١٦,٦%), *Klebseilla spp* ٣(١٢,٥%), *Klebseilla*, *Pseudomonas spp*, *Protus vulgaris*, *oxytoca*, *Enterobacter cloacae*, *Escherichia alkalgance* were ٢(٨,٥%), *Protus mirabilis* and *Citrobacter spp* ١(٤,١) %.

سجلت البكتريا القولونية اعلى نسبه لمقاومتها للمضادات الحيوية

الاتيه , البنسلين والسيفالوسبورين والسبروفلوكساسين والسيفالوتراكرون ١٠٠% بينما سجلت الستروبيكترا اقل نسبه بكتريا مقاومه للمضادات الحيوية المستعملة. في هذه الدراسة تم تثبيط التصاق البكتريا القولونية بالخلايا الطلائيه بالتركيز المثبط الأدنى لمضاد السبروفلاكساسين وباستعمال طريقه تخفيف الإطباق المضاعفة للأوساط الصلبة. ان هدف الدراسة هو كشف المدى الواسع من البكتريا المرضية المسؤولة عن التهاب اللثة حيث يعتبر التشخيص والعلاج ضروري للصحة العامة وكذلك عزل وتشخيص أكثر الأنواع البكتريا المرضية ومقاومه بكتريا التهاب اللثة لبعض المضادات الحيوية ودراسة التصاق البكتريا بالخلايا الطلائيه للتهاب وتثبيط التصاقها بالتركيز المثبط الأدنى للمضاد السبروفلاكساسين.