Antibacterial Pattern of Lactic Acid Bacteria on *Streptococcus Mutans* Isolates from Dental Caries

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Abstract

Several approaches have been taken in the prevention of dental caries; the main approach is to render enamel surface more resistance to attack of acidogenic bacteria. In this study one hundred dental plague samples with 20-30 mg of plagues were collected. These patient's samples were collected during March to May 2014 from Baghdad dental clinics. Culturing of dental plagues was inoculated on different selective, enrichment and diagnostic media to be obtain pure culture of *Strep. mutans*. *Streptococcus mutans* is considered the main cause of destruction of dental tissues, with the incidence 13%. The inhibitory effect of *Lactobacillus gasseri* against Streptococcus *mutan*s isolates was studied by well-diffusion method on solid media. The results showed that the *Lactobacillus gasseri* has a highly effects of inhibition zone on the *Strep. mutans* growth (92.30%) and the inhibition diameters zone were more than 12 mm. Also this effect compared with other antibacterial drugs, which were showed that the isolates had different responses of resistance to Ampicillin (AM) 5(38.4%), Azithromcin (AZM) and Lincomycin (LN) 2(15.4%) for each, Gentamycin (CN) 4(30.7%), so that the Cefotaxim (CTX) antibacterial recorded lowest resistance 1(7.6). Hence, this study reveals the potential of *Lactobacillus gasseri* as an alternative and complementary treatment medicine for dental caries by inhibiting the growth of *Streptococcus mutans*. Also these results may provide a basis for alternative therapies to prevent or treatment of dental caries.

Key words: Dental caries, Lactic acid bacteria, *Streptococcus mutans*

الخلاصة:

استخدمت طرق عديدة للوقاية من تسوس الاسنان ولكن الطريقة الافضل هي جعل سطوح الاسنان أكثر مقاومة لالتصاق البكتيريا المرضية وايقاف نشاطها. جمعت في الدراسة الحالية مائة عينة من مرضى مصابين بتسوس الاسنان وبواقع (30–20) ملغم. وجمعت عينات المرضى للفترة من آذار إلى أيار 2014 من عيادات طب الأسنان في بغداد. تم استزراع العينات في اوساط زرعية انتقائية، إغنائية ، إختيارية وتشخيصية مختلفة للحصول على مستعمرات نقية من بكتيريا ***Streptococcus mutans*** والتي تعتبر المسبب الرئيسي لإصابة انسجة الأسنان واحداث التسوس.

أشارت نتائج العزل والتشخيص الى وجود بكتريا ***Streptococcus mutans*** بنسبة (13%). وعند دراسة تأثير بكتريا حامض اللبنيك (***Lactobacillus gasseri***) على نشاط وفعالية بكتريا ***Streptococcus mutans*** لوحظ وجود فعالية عالية ضد بكتريا ***Streptococcus mutans***وبنسبة (92.30%) وظهور منطقة تثبيط للنمو على البكتيريا بأقطار المنطقة كانت أكثر من (12mm). وعند مقارنة نتائج فعالية بكتريا حامض اللبنيك مع العقاقير المضادة للبكتيريا المختارة في الدراسة ، أوضحت النتائج ظهور تباين في إستجابة عزلات بكتريا تسوس الاسنان لهذه المضادات حيث أظهرت العزلات مقاومة لل Ampicillin بنسبة (38.4%)، Azithromcin وال Lincomycin بنسبة (15.4%) ، Gentamycin (30.7%) . بينما كانت العزلات حساسة بنسبة عالية (92.3%) لمضاد Cefotaxim . تكشف هذه الدراسة إمكانيات وفعالية بكتريا حامض اللبنيك كأدوية علاج البديلة والتكميلية لتسوس الأسنان عن طريق تثبيط نمو او تكاثر بكتريا ***Streptococcus mutans***. كذلك نستنتج من هذه النتائج إمكانية توفير أساسا للعلاجات البديلة في منع أو علاج تسوس الأسنان.

Introduction

Dental caries is a localized, transmissible, pathological infectious process that ends up in the destruction of hard dental tissue [1]. Dental plaque is an adherent deposit of bacteria and their products, which forms as a white greenish or even yellow film on teeth surfaces [2]. So that the dental plaque accumulates naturally at stagnant or retentive sites formed after one to two days with no oral hygiene, it is a biofilm composed of a complex microbial community [3]. Dental caries is one of the most common human infectious diseases, which also it's the most common and costly diseases in the world [4].

In order to decrease the prevalence of caries, an improved understanding of the role of the microorganisms in dental diseases must be needed [5, 6]. The formation of a biofilm overlying tooth structure is essential for the initiation and progression of caries [7].

Streptococcus mutans are gram-positive cocci shaped bacteria. These facultative anaerobes bacterium are commonly found in the human oral cavity, and is a significantly contributor of tooth decay [8].

Streptococcus mutans is considered as a major pathogen of dental caries due to its ability to adhere and accumulate to the tooth surface [8, 9]. Additionally it is a cariogenic microorganism that breaks down sugar for energy and produces an acidic environment, which demineralizes the superficial structure of the tooth. The result of the conversion disintegrates the coating of the tooth then later dissolves the Calcium molecule creating a hole [10].

Principle identification or diagnosis of mutans streptococci is usually made from the characteristic morphology of its colonies on 5% sucrose containing culture media [11].

Lactic acid bacteria (LAB) are group of bacterium which are associated with several health benefits related to the maintenance of gut homeostasis and the immune system, also it have the potential for improving human health through prophylactic and therapeutic applications, that are terme as probiotics [12]. Probiotics may have antimicrobia, anticarcinogenic, antidiarrheal, anti-allergenic and antioxidant activities [13].

In recent decades, much attention has been paid to the health-promoting (probiotic) properties of *lactobacilli*, as it has been claimed that when administered in adequate amounts, they confer a health benefit on the host [14].

Certain LAB have the capacity to occupy mucosal niches of humans, including the oral cavity, gastrointestinal tract, and vagina. Among commensal, LAB are species of the acidophilus complex, which have proven to be a substantial reservoir for microorganisms with probiotic attributes. Specifically, *Lactobacillus gasseri* is an autochthonous microorganism which has been evaluated for probiotic activity based on the availability of genome sequence and species-specific adaptation to the human mucosa [15].

The aim of this study is to evaluate antibacterial pattern (the inhibitory effects) of lactic acid bacteria on *Streptococcus mutans* (as pathogenic agents) isolates obtained from patients infected with dental caries by agar-well diffusion method, also the study compare these antibacterial effects with some antibiotics drugs that common used in dental diseases.

Materials and Methods

1. Patients and specimen collection

One hundred dental plague samples were collected from patients whom were attended to the Baghdad dental clinic, with symptoms of dental infections. These patients were aged from 20-30 years, and all samples were collected during March to May 2014. All patients had not taken any antibiotics within the previous 5 to10 days before attending the clinic. Dental plagues were taken by a specialist dentist from infected teeth through sterile dental curettes; with small quantity of hard plague about 20-30 mg.

Plagues samples for culture were placed

in sterile tubes containing 5 ml of nutrient broth, until being examined in the laboratory [16].

Seventy micro liter of nutrient broth were immediately inoculated on nutrient agar plates, blood agar plates and MacConkey agar plates (These media from Himedia Labs, Mumbai, India). The plates were then incubated aerobically and anaerobically at 37^{o}C for 24 hrs [17]. Suspected colonies as streptococcus bacterium were selected and plated on mitis salivarius bacitracin (MSB) agar (Difco Laboratories, Detroit, MI, USA) at 37^{o}C for 24 hrs [18]. All media that used for isolation and diagnosis were taken from companies and prepared according to the recommendation of these companies.

2. Bacterial diagnosis

Bacterial diagnosis as *Strep. mutans* was done according to Collee *et al.* [19] and through macroscopic findings of single colony which was taken from pure bacterial culture that depended morphology, appearance, texture, and other phenotypic nature. Microscopic examination of selected colony was investigated after gram stain to observe specific features of these isolates. Biochemical tests of oxidase, catalase, coagulase, vancomycin susceptibility, and motility test were done to obtain the final diagnosis.

3. Bacterial suspension

The isolates of lactic acid bacteria *lactobacillus gasseri* have been obtained from the Laboratory of Biological Department, College of Sciences, AL-Mustanseryia University. Purification of the culture was confirmed by Gram staining. Pure colonies were again cultured on specific media (MRS broth from Himedia Labs, Mumbai, India). After obtaining pure culture, biochemical tests were performed for final identification and diagnosis. Also the diagnosis were confirmed by motility test, acidification of sugar (sugar tests), Growth at 4 and 6.5% NaCl %, growth on 0.3% methylene blue, temperature tolerance test [20]. *Lactobacillus gasseri* suspension was performed for use in susceptibility testing with 1% concentration from liquid culture of bacteria that contain 1 X10^{8} cells/mL [21].

4. Antibacterial preparations

Agar-well diffusion method was used and the dilutions of antibacterial agents (from Bioanalyse-Turkey) which used were Ampicillin (AM) 10µg, Azithromcin (AZM) 15µg, Lincomycin (LN) 2µg, Gentamycin (CN) 10µg and Cefotaxim (CTX) 30µg, there as the inhibition zones were recorded on Mullor-Hinton agar [22].

5. Statistical analysis

Chi-Square was used to determine statistically significant differences of variables. Mean value and percent were used for the differences between *lactobacillus gasseri* and antibacterial susceptibility of agents. While the percentage values were used for detection of the incidence of bacterial distributions [23].

Results and Discussion:

Results of isolation found that there was only 13 (13%) cases showed positive bacterial culture for *Strep. mutans* with statistical differences ($p<0.001$) to negative bacterial culture (Fig. 1 and Table1). Four (30.7%) dental plagues samples showed mixed growth colony with highly statistical differences ($p<0.001$) than single bacterial growth, 9 samples (69.2%). (Table 1) These results of incidence of *Strep. mutans* were agree with results obtained by Patrícia *et al.,* (2007) from the incidence of dental caries in 42 Brazilian preschool children [28].

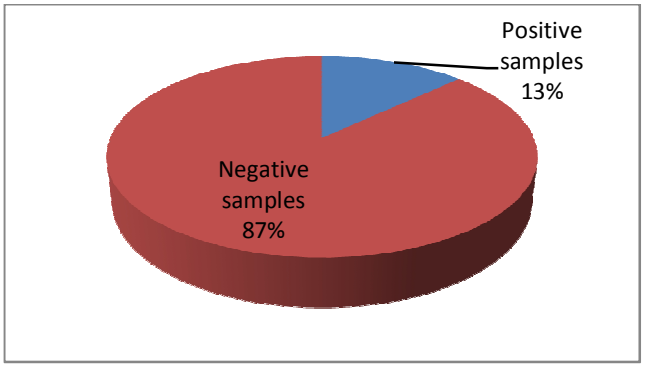

Figure (1): Distribution percentage of samples.

Table (1): Distribution of positive bacterial cultures.

Samples		Cultures	
Positive	**Negative**	**Mixed**	**Single**
(13%)*	(87%)	4(30.7%)*	9(69.2%)
Total: 100 samples		Total: 13 positive cultures	

* Significant differences (P<0.001). Chi-Square=11.68, df. =1.

Lactic acid bacteria are widely used in the production of fermented foods and beverages. Moreover, they are present in large numbers in the normal human and animal gastrointestinal floras. In recent decades, much attention has been paid to the health-promoting (probiotic) properties of lactobacilli, as it has been claimed that when administered in adequate amounts, they confer a health benefit on the host [24].

Several probiotic *lactobacilli* have been shown to survive transit through the human gastrointestinal tract and to maintain a balanced intestinal micro flora [25].

The activity of *lactobacillus gasseri* and the five antibacterial agents (which common used in oral infections) against pathogenic bacterial isolates *Streptococcus mutans* was determined through susceptibility test of agar-well diffusion methods showed that 12 (92.30%) of isolates appeared highly sensitive to *Lactobacillus gasseri* with significant differences (p<0.05). These results (Table -2) explain that many LAB demonstrate antibacterial activity towards a broad range of other bacteria by means of producing several antagonistic compounds, including organic acids, hydrogen peroxide and bacteriocins, which are classified as proteinaceous antimicrobial compounds that kill closely related microbes and have a broad spectrum of activity against Gram-positive bacteria in general, including pathogens [26].

Results of susceptibility tests for *Strep. mutans* isolates to other antibacterial agents showed different responses of sensitivity or resistance, through that the isolates were showed resistance to Ampicillin (AM) 5(38.4%), Azithromcin (AZM) and Lincomycin (LN) 2(15.3%), Gentamycin (CN) 4(30.7%), while for Cefotaxim (CTX) showed highly sensitivity 96 (92.4%). The results of the study were concluded that *Lactobacillus gasseri* (LAB) could be promising agent for targeting dental plaues formation and other cariogenic properties of *Strep. mutans*. Hence it could be a potential antiplaque agent. Furthermore, its property of being nontoxic makes it healthier to be proposed for the preparation of mouth washes and sugar free chewing gums [27].

Table 2: Antibacterials susceptibility profile of *Strep. Mutans* isolates

Isolate* No.	*Lactobacillus* suspension	AM 10µg	AZM 15µg	LN 2µg	CN 10µg	CTX 30µg
1	S**	S	S	S	S	R***
2	S	S	S	S	R	S
3	S	S	S	S	S	S
4	S	S	R	R	S	S
5	S	R	S	S	R	S
6	S	S	S	S	S	S
7	S	S	S	S	S	S
8	S	R	S	S	S	S
9	R	R	S	R	S	S
10	S	S	R	S	R	S
11	S	S	S	S	S	S
12	S	R	S	S	R	S
13	S	R	S	S	S	S

*Significant differences (P<0.05).
*S: Sensitive isolates with zone of inhibition more than 12mm in diameter.
***R: Resistant isolates with zone of inhibition less than 10mm in diameter.

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