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 plaque 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 Streptococcus 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 mutans isolates was studied by well-diffusion method on solid media. The results showed that the Lactobacillus gasseri has a highly effect of inhibition zone on the Streptococcus mutans growth (92.30%) and the inhibition diameters 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%), Azithromycin (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 الأسنان ولكن الطريقة الأفضل هي جعل سطح الأسنان أكثر مقاومة لانحلال البكتيريا المرضية وإيقاف نشاطها. جمعت في الدراسة الحالية عينة من مشاكل مصابين بتسوس الأسنان ووقاع (30-20) ملم. وجمع عينات المرضى للفترة من آذار إلى أيار 2014 من عوائد طب الأسنان في بغداد. تم استغاثة عينات في وسط زراعة إنتراد كلاسية، مختبرية، تشخيصية، واختبارات مختلفة للحصول على مستعمرات سنية من بكتيريا Streptococcus mutans، والتي تعتبر المسبب الرئيسي لإصابة الأسنان وحدوث تسوس. أشارت نتایج الدراسة إلى وجود بكتيريا حامض البشبع (Streptococcus mutans) على نشاط وفعالية بكتريا Lactobacillus gasseri (92.30%) وظهور منطقة تثبيط بزرع على البكتيريا بأطور المنهاج كانت أكثر من (12mm). و عدده Streptococcus mutans بنسبة (13%). وعدده Streptococcus mutans ظهور ودغم فعالية عالية ضد بكتريا Lactobacillus gasseri بنسبة (92.30%) وظهور منطقة تثبيط بزرع على البكتيريا بأطور المنهاج كانت أكثر من (12mm). و عدده Streptococcus mutans بنسبة (15.4%) . بينما كانت العوارض عاية بنسبة عالية (92.3%) لمضادات جنتاميسين (Gentamycin) ، ومضادات Cefotaxim بنسبة (30.7%) . ومضادات Azithromycin بنسبة (38.4%) . ، ومضادات Ampicillin بنسبة (38.4%) . ومضادات Lincomycin بنسبة (38.4%) . تكشف هذه الدراسة إمكانية وفعالية بكتريا حامض البشبع كدواء علاج البذلة والتكملية لتسوس الأسنان عن طريق تثبيط نمو أو دفع بكتريا Streptococcus mutans. كذلك نستنتج من هذه النتایج إمكانية توفير أساس لعلاجات البذلة في معالمة 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 termed as probiotics [12]. Probiotics may have antimicrobia, anticarcinogenic, anti-diarrheal, 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 plaque 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 to 10 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 plaque 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°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°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-Mustanserya 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 X10⁸ 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 (62.9%). (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.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Cultures</th>
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</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
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<tr>
<td>(13%)</td>
<td>(87%)</td>
</tr>
</tbody>
</table>

* 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

<table>
<thead>
<tr>
<th>Isolate* No.</th>
<th>Lactobacillus suspension</th>
<th>AM 10μg</th>
<th>AZM 15μg</th>
<th>LN 2μg</th>
<th>CN 10μg</th>
<th>CTX 30μg</th>
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<tr>
<td>1</td>
<td>S**</td>
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<td>S</td>
<td>S</td>
<td>R***</td>
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<tr>
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<td>S</td>
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<tr>
<td>13</td>
<td>S</td>
<td>R</td>
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*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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References
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