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## Antibacterial activity of Some mouthwash Solutions against *Staphylococcus lentus* Isolated from Mouth Infections

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### Abstract

A total of nine swab samples were collected from inflamed teeth and gingiva of human's oral cavity from a dentist clinic in Baghdad. All specimens were cultured in Mitis Salivarius agar medium and the isolated bacterial pure colonies were then identified by using VITEK2. Three samples were diagnosed and identified as *Staphylococcus lentus*. One of the three isolates which showed a distinctive heavy growth on the media was selected for further analysis in this study. Paper disk diffusion method was used to detect the antibacterial activity of three of mouthwash solutions (Zak, Colgate and Listerine). The results showed that "Colgate" was the most active solution with antibacterial activity compared with the other two solutions.

**Keywords:** *Staphylococcus lentus*; Cetylpyridinium chloride; phenols and sodium fluoride

## الفعالية المضادة للنمو البكتيري لبعض محاليل غسول الفمأتجاه *Staphylococcus lentus* المعزولة من اصابات الفم

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### الخلاصة

جمعت تسعة عينات من افواه اشخاص يعانون من التهابات في الاسنان واللثة من عيادة لطب الاسنان في بغداد. زرعت جميع العينات في وسط Mitis Salivarius agar، ثم شخصت المستعمرات البكتيرية المعزولة باستخدام طريقة VITEK2. ثلاثة نماذج منها شخصت وعرفت على انها *Staphylococcus lentus*، واحدة فقط من هذه النماذج والتي تميزت بنمو كثيف على وسط التتمية اختبرت كعزلة منتخبة في هذه الدراسة. استخدمت طريقة الانتشار في الاقراص الورقية للتحري عن الفعالية التثبيطية لثلاث محاليل لغسول الفم ( ZAK و Colgate و Lesterine ) ضد النمو البكتيري. اظهرت النتائج ان محلول Colgate كان الاكفأ في اعطاء هذه الفعالية مقارنة مع النوعين الاخرين.

### Introduction

The oral cavity houses various habitats for microorganisms which support the growth of microbial communities in the mucosal surfaces (such as those in the lips, cheek, palate and tongue) and the teeth [1]. Dental plaque is the community of microorganisms found on a tooth surface and it is the agent involved in progression of periodontal and gingival diseases [2]. Development of plaque occurs when food residues that contain carbohydrates are left to accumulate on the teeth for long time. These foods stimulate bacteria in the mouth to thrive and form acids. After a period of time, these acids will

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deteriorate the enamel of the tooth and cause decay. Tooth roots under the gum could be also infected by plaque and cause the bone supporting the tooth to breakdown [3]. Dental plaque symptoms give teeth a fuzzy feeling when touched with the tongue. In the advanced stages, it can manifest as a white, grayish or yellowish coating on the teeth [4]. Plaque buildup can be prevented by a number of practices such as brushing the teeth at least twice a day with a particular attention to the space where the teeth and gums meet, using fluoride-containing toothpaste, flossing between teeth at least once a day, using an antibacterial mouth rinse, and consultation by dentist or oral hygienist every six months [5].

Gargling and rinsing the mouth with mouthwashes which are medicated solutions may be recommended for inflammation reduction, infections treatment, pain relief, halitosis reduction, and caries prevention through the local delivering of fluoride. The preventive use of mouthwashes is mainly to control dental caries and to inhibit or reduce plaque associated bacteria. The most effective ingredients are chlorhexidine gluconate, fluoride, phenol, essential oils and cetylpyridinium chloride [6]. *Staphylococcus lentus* is a Gram-positive cocci bacteria which is nonmotile, nonsporing, 0.7-1.2  $\mu\text{m}$  in diameter and occurring singly, in pairs or in tetrads [7]. The cultural characteristics of this bacteria are the S-type and the grayish-white to white or creamy opaque colonies. Some of them are mucoid strains. Colony diameter is about 0.5-5 mm on P agar or 2-7 mm on Tryptic Soy agar. Optimum temperature of growth is between 25-35  $^{\circ}\text{C}$ ; no growth could be seen below 15 or above 45  $^{\circ}\text{C}$ . They grow as facultative anaerobes with weakly anaerobic growth; they do not grow anaerobically in a thioglycolate media, tardily grow in 10 % NaCl medium and poorly grow in 15 % NaCl [8].

*Staphylococcus lentus* is a member of the *Staphylococcus sciuri* family which is a common bacterium in the environment; it can be detected from a diversity of animals (domestic, farm and wild) where it commensally colonizes their skins. It was widely detected from food-producing animals that involve domestic fowl and livestock and from their products. Individuals in contact with animals were also found to be bearers of *S. lentus*. In dairy animals, *S. lentus* was shown to be linked with subclinical mastitis and in infrequent cases passed infections to humans. *S. sciuri* may infect humans and causes severe infections, such as septic shock, bacterial endocarditis, endophthalmitis, pelvic inflammatory diseases, urinary tract infections, incision and wound or abrasion infections [9-11].

The aim of the present study is to detect the actual antibacterial activity of three mouthwash solutions; Listerine, Colgate and Zak against *S. lentus*.

## Materials and Methods

### Solutions (mouthwashes)

Three types of mouthwashes were bought from a pharmacy in Baghdad:

1. Listerine -fresh burst (Italy), as shown in Figure-1.
2. Colgate plax (Brazil), as shown in Figure-2.
3. ZAK (Egypt), as shown in Figure-3.



Figure 1-Listerine label



Figure 2-Colgate label



Figure 3-ZAK label

## Media

1. Mitis salivarius agar (Himedia, india).
2. Muller-Hinton agar (Himedia, india).

All media were prepared according to the manufacturing company. pH was adjusted to be at 7 and sterilization was performed by autoclave (121°C/15 psi) for 15 min. The media were then distributed into Petri dishes. To ensure the sterility, all dishes were incubated for 24 hrs. at 37°C and then stored at 4°C until being used.

## Samples collection, isolation and diagnosis

Sterilized swabs and woody stick were used to collect nine samples from human oral cavity. All specimens were cultured and streaked on Mitis Salivarius agar plates, then incubated at 37°C under aerobic conditions for 18-24 hrs. After incubation, the pure colonies were selected to be diagnosed by VITEK2 (Nature Eye company).

## Mouthwash test

### Mueller-Hinton agar plates preparation

Mueller-Hinton agar was prepared following the manufacturer's instructions. The medium was cooled to a temperature between 45-50°C, poured into the Petri-dishes, and allowed to be scaled on a surface to depth level of about 4mm. After the agar was solidified, the plates were dried for immediate use in about 10-30 min. at 25°C by placing them in the hood in the upright position with the lids tilted.

### Paper Disk Diffusion Method

The Mueller-Hinton plates were cultured by swab; the swab was rubbed all over the surface of the medium for about four times with rotating at an angle of 90° after each application. Finally, the swab

was passed around the edge of the agar surface. The inoculums were left to dry for few minutes at room temperature with a closed lid. Filter papers were cut into small circles (10 mm. in diameter) and sterilized by autoclave for 20 minutes. By using sterile forceps, filter papers were transferred to the plates and submerged with mouthwash solutions (the original concentration and without dilution). The plates were labeled and incubated at 37°C for 18-24 hrs.

### Results and Discussion

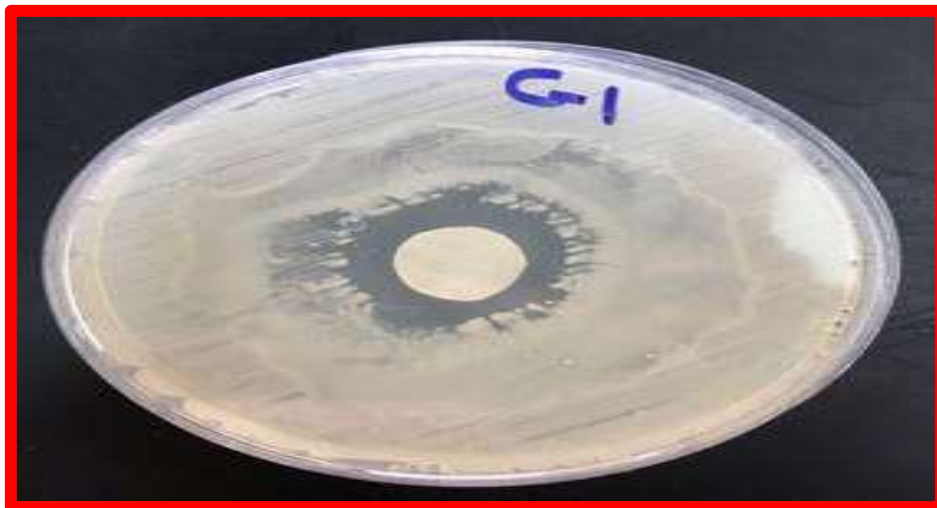
Within nine specimens, only three isolates were identified as *Staphylococcus lentus* (Figure -4). One of these isolates was selected, according to the distinctive heavy growth on media, to be the used isolate in this study (Figure-5). After the end of the incubation period of the selected isolate on Mueller-Hinton agar, the results showed that the mouthwash Colgate was the most active solution which caused an inhibitory zone of about 4 mm. in diameter, followed by ZAK (3.7 mm.), and Listerine (0.5mm.) Figures-(6, 7, 8). The inhibition zones of each plate were measured in millimeters by the aid of a metric ruler.

bioMérieux Customer: System #:		Laboratory Report		Printed Feb 7, 2019 08:15 CST Printed by: LabSuper													
Patient Name: mawj, . Isolate: 150-1 (Approved)				Patient ID: 7b													
Card Type: GP Bar Code: 2420867203564955 Testing Instrument: 000014EEC06B (NATURE EYE) Setup Technologist: Laboratory Supervisor(LabSuper)																	
Bionumber: 430403153753531		Organism Quantity: Selected Organism: <i>Staphylococcus lentus</i>															
Comments:																	
Identification Information		Card: GP	Lot Number: 2420867203	Expires: Apr 11, 2020 13:00 CDT	Analysis Time: 2.13 hours												
Organism Origin		VITEK 2															
Selected Organism		91% Probability <i>Staphylococcus lentus</i> Bionumber: 430403153753531 Confidence: Good identification															
SRF Organism																	
Analysis Organisms and Tests to Separate:																	
Analysis Messages:																	
Contraindicating Typical Biopattern(s) <i>Staphylococcus lentus</i> NOVO(20),AlaA(1),URE(10).																	
Biochemical Details																	
2	AMY	-	4	PIPLC	-	5	dXYL	+	8	ADH1	+	9	BGAL	+	11	AGLU	-
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	+	26	PyrA	+	27	BGUR	-
28	AlaA	+	29	TyrA	-	30	dSOR	-	31	URE	+	32	POLYB	-	37	dGAL	+
38	dRIB	+	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	-	52	dMAN	+	53	dMNE	+	54	MBdG	+	56	PUL	-
57	dRAF	+	58	O129R	-	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Figure 4-VITEK 2 diagnosis of *Staphylococcus lentus*



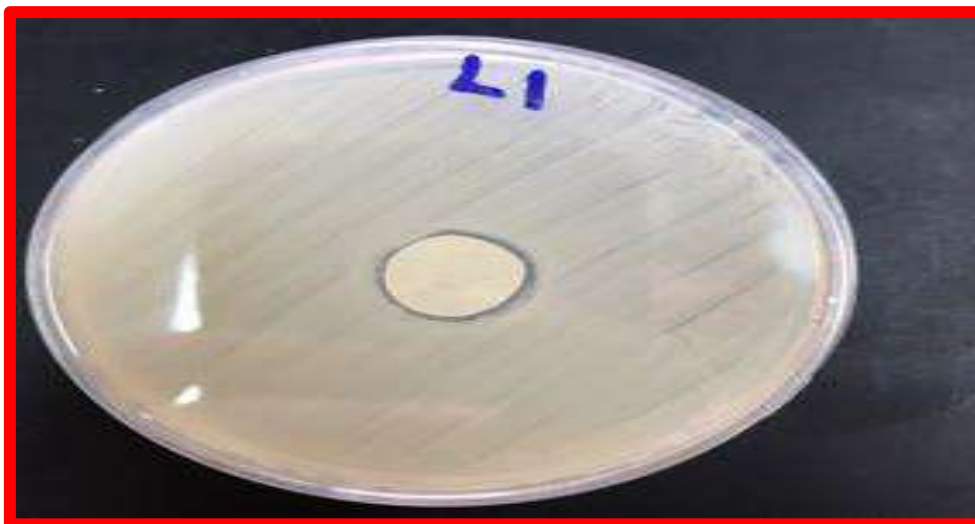
**Figure 5-***Staphylococcus lentus* growth on Mitis Salivarius agar



**Figure 6-** The inhibition zone of Colgate against *Staphylococcus lentus* growth on Muller-Hinton agar



**Figure 7-** The inhibition zone of ZAK against *Staphylococcus lentus* growth on Muller-Hinton agar



**Figure 8-** The inhibition zone of Listerine against *Staphylococcus lentus* growth on Muller-Hinton agar

The differences in the effects of mouthwash solutions may be due to the differences in the chemical compositions. Colgate solution is composed of sodium fluoride 0.05%, cetylpyridinium chloride and phenols conjugated with essential oils (eucalyptol, thymol, menthol and methyl salicylate in up to 26% alcohol). ZAK solution components include chlorhexidine gluconate and phenols conjugated with essential oils (eucalyptol, thymol, menthol and methyl salicylate in up to 26% alcohol). Listerine solution consists of only phenols conjugated with essential oils (eucalyptol, thymol, menthol and methyl salicylate in up to 26% alcohol).

#### **Mechanisms of action of active ingredients**

1. Chlorhexidine gluconate: It is a form of cationic bis-guanide which has a broad spectrum of antimicrobial activities against large extent of vegetative bacteria (Gram-positive and Gram-negative), some fungi and some viruses. The action mode on bacteria depends on the binding of this molecules (positively-charged) to a locus of another molecules (negatively-charged) which is located on the cell wall. This conjugation leads to destabilization of the cell wall and intervenes with osmosis. At this point the cell wall will be damaged and permits chlorhexidine to penetrate into the cell and attacks the cytoplasmic membrane, leading to disruption and damaging to the semipermeable membrane functions and allowing for components leakage which causes death to the cell [12].
2. Fluoride: The tooth enamel hydroxyapatite is originally composed of phosphate ions ( $\text{PO}_4^{3-}$ ) and calcium ions ( $\text{Ca}^{2+}$ ). Under ordinary situation, there is a stable equilibrium between the crystalline hydroxyapatite that comprises 96% of tooth enamel with the calcium and phosphate ions in saliva [13]. The acidic environment on the tooth surface result in the combination of phosphate that occurs in the oral fluids with hydrogen ions ( $\text{H}^+$ ) to form a species of hydrogen phosphate. Phosphate under these conditions is "pulled" from tooth enamel in order to restore levels of phosphate in the saliva and the hydroxyapatite dissolves in a procedure called demineralization. In case of using fluoride, the calcium and phosphate in saliva can be recrystallized into the fluorapatite rather than hydroxyapatite during the remineralization process. Fluoride ions ( $\text{F}^-$ ) exchange hydroxyl groups ( $\text{OH}^-$ ) to form fluorapatite which is less soluble than hydroxyapatite and also more resistant to subsequent demineralization under acid challenge. The rate of remineralization, in fact, increases in the presence of fluoride [14] [15].
3. Phenols and essential oils: Mouthwashes contain four phenol-conjugated essential oils (eucalyptol, thymol, menthol and methyl salicylate in up to 26% alcohol). Their action mode leads to the destruction of the bacterial cell, which happens either by the inhibition of bacterial enzymes or the extraction of Gram-negative bacterial endotoxin [16].
4. Cetylpyridinium chloride: It is a compound of quaternary ammonium (cationic) with antiseptic and antimicrobial functions. It can bind to bacterial surfaces leading to disruption of the cell membrane, resulting in leakage to the intracellular components and metabolism disruption. Inhibition and reduction of plaque build-up could be achieved by mouthwashes containing cetylpyridinium chloride [16].

## Conclusions

The study revealed that both Colgate and ZAK mouthwash solutions have antibacterial activities against the gram positive bacteria *S.lentus*, compared with Listerine which showed no or very weak inhibition activity, and that these differences are related to the differences in the chemical compositions. These results indicate that not all mouthwashes have the same capacity of antibacterial activity, and that some of them might not have such activity at all .

## Recommendation

According to the results of our study we recommend that Listerine solution should not be used for mouth gargling and, instead, Colgate or ZAK solution could be used.

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