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Evaluation of the Effect of Some Antibiotics, Crystal Violet Dye and Clove Oil on Bacterial Organisms Isolated from Human Teeth Affected With Caries Disease

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TWENTY-SIX bacterial organisms were isolated from nine samples taken from human teeth affected with caries disease. These organisms were identified as *Lactobacillus lactis* (30.8%), *Streptococcus mitis* (19.2%), *Streptococcus salivarius* (15.4%), *Staphylococcus aureus* (11.5%), *Staphylococcus epidermidis* (11.5%) and *Staphylococcus mutans* (11.5%). Sensitivity of the bacterial strains to the antibiotics cefuroxime (zinnat), ampicillin (ampicillin), amoxycillin (amoxil), norfloxin (noroxin) and erythromycin (erythromycin) were conducted. *Staphylococcus epidermidis* was found to be sensitive to all of the tested antibiotics, while *Staphylococcus aureus* shows sensitivity to amoxycillin and erythromycin only. The two strains were choosen for further studies. Crystal violet dye solution was found to inhibit the growth of the two bacterial choosen strains at concentration ranged between 0.1 and 0.6 µg/ml.While as, the complete growth inhibition was attained at 0.6 µg/ml. Sensitivity of *Staphylococcus aureus* and *Staphylococcus epidermidis* to the tested antibiotics in the presence of crystal violet solution at concentration ranged between 0.1 and 0.6 µg/ml increased with ampicillin, amoxycillin and norfloxin, while the sensitivity decreased with cefuroxime and erythromycin. The minimum inhibitory concentrations (MIC_S) of clove oil against *Staph. aureus* and *Staph. epidermidis* were found to be 7% and 6% (v/v), respectively. While, the (MIC_S) of clove oil were observed in the presence of crystal violet dye to depend on the concentration of the dye in the agar medium.

Keywords: Human teeth, Bacterial contamination, Antibiotics, Crystal violet, Clove oil.

The indigenous microbiota constitute a reservoir of potentially pathogenic bacteria that may infect host tissues (Theilade, 1990 and Marsh & Martin, 1992). In the oral cavity, indigenous bacteria are often associated with the etiology of caries and periodontal diseases (Van-Houte, 1994). Oral diseases seem to appear after an imbalance among the indigenous microbes, leading to the imergence of potenially pathogenic bacteria (Marcotte & Lavoie, 1988).

Caries is a disease that is caused by the activity of microorganisms on fermentable carbohydrates in dental plaque (Kidd *et al.*, 1993 and Boston & Graver, 1994). *Streptococcus mutans* has been associated with the initiation of dental caries (Van-Houte, 1994). Salivary immunoglobulin A deficient humans were found to be more or less susceptible to caries disease (Roberston *et al.*, 1980).

The procedures used when treating caries do not always eliminate all of the microorganisms in residual tissues (Kidd *et al.*, 1993 and Boston & Graver, 1994). Therefore, antibacterial solutions for preventing harmfull effects of bacterial activity has been recommended after cavity preparation to disinfect dentin (Meiers & Kresin, 1996). Moreover, Yoshii *et al.* (2001) assessed the antimicrobial agents against *Streptococci* isolated from odontogenic infections.

Euginol, the main chemical constituent of the volatile oil from clove (*Syzigium aromaticum*) has been used for a long time by dentists through intracanal route, as a dressing in dentistry, as an analgesic in painful and infective diseases of the oral cavity and for general oral hygiene (Elujoba *et al.*, 2005).

The aim of the present study was to elucidate the effect of some antibiotics, crystal violet dye and the natural compound, essential oil of clove, on bacterial strains isolated from teeth affected with caries disease.

Materials and Methods

Samples

Nine samples were taken from teeth of patients affected with caries disease. Samples in swabs were taken from the patients, in the Dentistry Department at a polyclinic in Cairo.

Microorganisms

Twenty six bacterial organisms, isolated from the nine clinical samples, were used in the present investigations.

Chemicals

The chemicals used in the present investigations were:

A. *In vitro* antibiotic discs of :
   1-Zinnat, CXM, 30 μg (Cefuroxime).
   2-Ampicillin, AM, 10 μg (Ampicillin)
   3-Amoxil, AMX, 25 μg (Amoxycillin)
   4-Noroxin, NOR, 10 μg (Norfloxcin).
   5-Erythromycin, E, 10 μg (Erythromycin).
   The discs were obtained from Pasteur Lab., Egypt.
B. Crystal violet was obtained from Merck, Germany and the other chemicals were reagent grade.

Media

The following media were used:

- TGY broth medium (gm/l): Tryptone, 5 gm; yeast extract, 3 gm; glucose, 1 gm and distilled water, 1000 ml.
- TGY agar medium (gm/l): TGY broth with the addition of 20 gm agar.
- Blood agar medium : peptone, 1 gm; Lab. Lemco, 1 gm; sodium chloride, 0.5 gm; agar 2 gm; 10 ml human blood and 100 ml distilled water.

Isolation of bacterial organisms from the clinical samples

Swab of each clinical sample was spreaded onto the surface of TGY-agar plate and blood-agar plate. All plates were incubated for 18-24 hr at 37 °C. After incubation, individual colonies were purified on the surface of TGY –agar medium and kept on slants of the same medium.

Identification of bacterial isolates

Identification of the isolated bacterial organisms was carried out according to Cowan & Steel (1974).

Sensitivity of the bacterial isolates to some antibiotis

The sensitivity of the twenty six bacterial isolates to the antibiotics cefuroxime (zinnat), ampicillin (ampicillin), amoxycillin (amoxil), norfloxin (noroxin) and erythromycin (erythromycin) was carried out using the disc diffusion method according to the method of Arvidson *et al.* (1981) and as recorded by Krivoshein (1989).

Suspension of 24 hr TGY broth culture (0.1 ml) of each bacterial isolate was spreaded uniformly on the surface of solidified TGY –agar plates. Antibiotic discs were applied onto the surface of the inoculated plates at a distance of 25 mm from its center. The plates were incubated for 16-18 hr at 37°C. After incubation, the results of the test were read by measuring the zone of growth inhibition around the discs, including the diameter of the disc itself.

The bacterial strain which showed sensitivity to two antibiotic only (*Staphylococcus aureus*, No. 7) and the strain which showed sensitivity to all of the tested antibiotics (*Staphylococcus epidermidis*, No. 11), were choosen for further studies.

Effect of various concentrations of crystal violet dye on the viability of the two choosen bacterial strains

The effect of various concentrations of the dye solution (crystal violet/H_2O) on the viable count of the two bacterial strains (*Staph. aureus* and *Staph. epiderimis*) were conducted. Fresh TGY culture of *Staph. aureus* and *Staph. epidermidis*, separately, (10^6-10^7, cfu/ml) were used to prepare ten fold dilutions in sterile saline solution (0.89% sodium chloride/dist. H_2O). From each appropriate dilution, 0.1 ml was spreaded uniformly onto the surface of TGY-agar plate. The seeded TGY-agar plates contain various concentrations of crystal violet

dye solution in distilled water which ranged from 0.1 to 1.0 µg/ml. Duplicate plates were used for each dye concentration.

All plates were incubated for 18-24 hr at 37°C, after which, the viable count of each test strain at the various dye concentrations were counted as c.f.u./ml.

Effect of combination treatment of various concentrations of crystal violet and antibiotics on the sensitivity of the two choosen bacterial strains

This study was carried out as follows:

Two groups of TGY-agar plates, each containing increasing concentrations of crystal violet solution ranged between 0.1 and 0.6 µg/ml agar, were used.

A group of the plates, in duplicates, were seeded, each with 0.1 ml of fresh culture of (*Staph. epidermidis*) and the other group of plates, in duplicates, were seeded each with 0.1ml of fresh culture of *Staph. aureus* as mentioned before. The antibiotic discs under test were applied on each plate, incubated for 16-18 hr at 37 °C, after which, zones of growth retardation of each bacterial strain around the discs were measured as mentioned before.

Antimicrobial activity of the essential oil of clove against the two test strains

The minimum inhibitory concentrains of the essential oil of clove against *Staph. aureus* and *Staph. epidermidis* was conducted by the agar dilution method, applied by Hammer *et al.* (1999) with some modifications.

A final concentration of 0.5% (v/v) Tween 20 (Sigma) was incorporated into the agar after autoclaving to inhance oil solubility. A series of dilutions of the clove oil ranging from 1% to 7% (v/v) was prepared in the TGY-agar containing the tween. The dried plates, in duplicates were seeded, separately, with *Staph. aureus* and *Staph. epidermidis*. TGY-agar with 0.5% (v/v) tween 20 but no oil, was used as a positive growth control.

Inoculated plates were incubated at 37 °C for 24 hr and the minimum inhibitory concentrations were determined.

Effect of combination treatment of clove oil and crystal violet dye on the viability of the two test strains

The same previous experiment was done except that the agar plates for each tested bacterial strain contains crystal violet dye at concentrations ranging from 0.1 to 0.5 µg/ml combined with concentrations of clove oil from 0.2 to 5% (v/v).

Results and Discussion

The oral microbiota of humans is highly complex and diverse. Some of the more frequently isolated microorganisms which their distributions vary according to the habitat, are the *Streptococcus* species (Smith *et al.*, 1993). There is considerable evidence that *Streptococcal* sp. and *Lactobacillus* sp. are involved in

the initiation and progression of caries disease (Loesche, 1986 and Nyvad, 1993).

In the present study, nine samples were taken from teeth of patients affected with caries disease. A total of 26 bacterial organisms were isolated from the nine samples (Table 1).

TABLE 1. Identification of bacterial organisms isolated from samples taken from human teeth affected with caries disease.

Sample	Isolate No.	Bacterial identification
A	1	*Lact. lactis*
	2	*Strep, salivarius*
	3	*Sterp. mitis*
B	4	*Lact. lactis*
	5	*Strep. salivarius*
	6	*Strep. mutans*
	7	*Staph. aureus*
C	8	*Lact. lactis*
	9	*Strep. salivarius*
	10	*Strep. mitis*
	11	*Staph. epidermidis*
D	12	*Lact. lactis*
	13	*Strep. mitis*
	14	*Strep. mutans*
	15	*Staph. epidermidis*
E	16	*Lact. lactis*
	17	*Strep. mitis*
	18	*Staph. aureus*
F	19	*Lact. lactis*
	20	*Strep. mitis*
G	21	*Lact. lactis*
H	22	*Strep. salivarius*
	23	*Strep. mutans*
	24	*Staph. aureus*
I	25	*Lact. lactis*
	26	*Staph. epidermidis*
9 samples	26 isolated strains	

The results of identification of the bacterial organisms isolated from the samples (Table 1) reveal that *Lactobacillus* sp., *Streptococcus* sp. and *Staphylococcus* sp., were the predominant bacterial strains in the nine clinical samples.

The percentage of the isolated bacterial strains in relation to the total strains (26) was presented in Table 2.

TABLE 2. Percentage of the bacterial strains in relation to the total isolated strains.

Bacterial strain	Number of strains	% of isolated strains
Lactobacillus lactis	8	30.8
Sterp. mitis	5	19.2
Strep, salivarius	4	15.4
Staph. aureus	3	11.5
Staph. epidermidis	3	11.5
Sterp. mutans	3	11.5
Total No.	26	~100%

The percentage of *Lactobacillus lactis*, *Strep. mitis* and *Strep. salivarius* were 30.8%, 19.2% and 15.4%, respectively. While each of *Staph. aureus*, *Strep. epidermidis* and *Strep. mutans* was found to be 11.5%.

Sensitivity of the bacterial strains isolated from samples affected with caries disease to some antibiotics

In many cases, susceptibility of a pathogenic organism to a specific antimicrobial drug is unpredictable (Scott, 1989). Investigators evaluate various antimicrobial agent levels in teeth extraction sites. They assessed the antimicrobial activity of the antimicrobial agents against *Streptococci* isolated from odontogenic infections (Yoshii *et al.*, 2001).

In the present study, sensitivity of the isolated twenty six bacterial strains to some antibiotics is carried out. The results (Table 3) reveal that *Lactobacillus lactis* shows sensitivity to the tested antibiotics and it is stable to ampicillin and norfloxin. *Strep. salivarius*, *Strep. mutans* and *Strep. mitis* are sensitive to amoxycillin, norfloxin and erythromycin. On the other hand, *Staph. aureus* show sensitivity to amoxycillin and erythromycin only and *Staph. epidermidis* is found to be sensitive to all of the tested antibiotics.

Regarding to the susceptibility of the antibiotics to the tested bacterial strains, amoxycillin (25μg) and erythromycin (10 μg) are susceptible to the 26 bacterial strains. On the other hand, cefuroxime (30 μg) is susceptible to *Lactobacillus lactis* and *Staph. epidermidis* while ampicillin (10 μg) is susceptible to *Staph. epidermidis* only (Table 3).

From the above results it is concluded that *Staph. aureus* is sensitive to two antibiotics only and *Staph. epidermidis* is sensitive to the five antibiotics under test. Therefore, these two strains were choosen for further studies.

Effect of various concentrations of crystal violet dye solution on the viability of Staph. aureus and Staph. epidermidis

It was reported that caries can be reduced or prevented through the use of antiseptics. Faliure to remove all infected tooth structure or to disinfect an area containing microorganisms, can lead to superinfections with bacteria (Newburn, 1992). Disinfecting the oral cavity preparation prior to its restoration eliminates the chance of bacteria in a cavity and reduces the potential for both recurrent caries and postoperative sensitivity caused by residual bacteria (Meiers & Kresin, 1996).

TABLE 3. Sensitivity of the bacterial strains, isolated from the human teeth affected with caries disease to some antibiotics.

Sample	Isolate No.	Bacterial Strain	The antibiotics				
			Cefur-oxime	Ampi-cillin	Amoxy-cillin	Norflo-xcin	Erythro-mycin
A	1	*Lactobacillus lactis*	3.7	S	4.5	S	2.8
	2	*Strep, salivarius*	S	S	3.7	1.2	1.9
	3	*Sterp. mitis*	S	S	2	2.5	2.5
B	4	*Lact. lactis*	3.6	S	4.3	S	2.6
	5	*Strep. salivarius*	S	S	3.5	1.1	1.7
	6	*Strep. mutans*	S	S	3.5	2.4	3.5
	7	*Staph. aureus*	S	S	2.3	S	1.8
C	8	*Lact. lactis*	3.7	S	4.3	S	2.6
	9	*Strep. salivarius*	S	S	3.6	1.0	1.7
	10	*Strep. mitis*	S	S	2.1	2.5	2.5
	11	*Staph. epidermidis*	2.3	1.5	2.5	1.5	2.8
D	12	*Lact. lactis*	3.5	S	4.5	S	2.7
	13	*Strep. mitis*	S	S	2.2	2.5	2.5
	14	*Strep. mutans*	S	S	3.3	2.2	3.4
	15	*Staph. epidermidis*	2.3	1.4	2.2	1.5	2.7
E	16	*Lact. lactis*	3.5	S	4.4	S	2.6
	17	*Strep. mitis*	S	S	1.9	2.5	2.5
	18	*Staph. aureus*	S	S	2.3	S	1.7
F	19	*Lact. lactis*	3.6	S	4.5	S	2.8
	20	*Strep. mitis*	S	S	2.0	2.5	2.5
G	21	*Lact. lactis*	3.5	S	4.4	S	2.8
H	22	*Strep. salivarius*	S	S	3.6	1.1	1.9
	23	*Strep. mutans*	S	S	3.4	2.3	3.5
	24	*Staph. aureus*	S	S	2.1	S	1.6
I	25	*Lact. lactis*	3.6	S	4.5	S	2.5
	26	*Staph. epidermidis*	2.4	1.7	2.4	1.5	2.6

< 10 MM : (s) stable
11-15 mm : Weakely sensitive
15-25 mm : Moderately sensitive
> 25 mm : Higly sensitive
according to (Krevoshein, 1989).

In the present study, the antimicrobial effect of crystal violet dye was studied. The viable count (c.f.u. /ml) against *Staph. aureus* and *Staph. epidermidis* on TGY agar supplemented with crystal violet at concentrations ranged between 0.1 and 0.5 μg/ml was conducted.

The results in Table 4 reveal that, the viable count (c.f.u./ml) of *Staph. aureus* and *Staph. epidermidis* decrease with increasing the concentrations of crystal violet dye solution at concentration ranged between 0.1 and 0.5 μg/ml in TGY agar.

Complete growth inhibition of the two strains was noticed on TGY agar containing 0.6 μg of the dye / ml medium.

Okamato *et al.* (1992) studied the bactericidal effect of various concentrations of crystal violet on *Strep. sorbinus* AHT. They observed the microbial growth at concentrations of 8, 0.8 and 0.08 μg/ml, while no growth when the concentration was greater than 80 μg/ml.

TABLE 4. Effect of various concentrations of crystal violet dye on the viability of *Staph. areus* and *Staph. epidermidis*.

Concentration of crystal violet (μg/ml)	*Staph. aureus*		*Staph. epidermidis*	
0.0 Control	$3.5X10^8$	8.312	$9.0X10^7$	7.954
0.1	$1.6X10^7$	7.025	$1.0X10^6$	6.000
0.2	$5.7X10^5$	5.756	$1.1X10^5$	5.041
0.3	$5.0X10^4$	4.699	$6.0X10^3$	3.788
0.4	$2.0X10^4$	4.301	$2.5X10^3$	3.398
0.5	$1.0X10^3$	3.00	$1.0X10^3$	3.000
0.6	ND	-	ND	-

C.F.U.: Colony forming init

The presented data (Table 4) and that obtained by the mentioned investigators reveal that, crystal violet solution may be used to disinfect the oral cavity prior to tooth restoration, also to reduce or prevent caries disease regarding to the effect of combination treatment of various concentrations of crystal violet dye (from 0.1 to 0.6 μg/ml TGY agar) and the tested antibiotics on the sensitivity of *Staph. aureus* and *Staph. epidermidis*.

The results (Tables 5 and 6) show that the two bacterial strains can tolerate crystal violet concentration up to 0.5 μg/ml and no detected growth observed when the concentration is 0.6 μg/ml.

The data illustrated in Table 5 reveal that *Staph. aureus* growth in the presence of various concentrations of crystal violet dye, show sensitivity towards amoxycillin (25μg) and erythromycin (10 μg). The bacterial strain is stable to cefuroxime, ampicillin and norfloxin. It is also noticed that the sensitivity of the bacterial strain increases with increasing the dye concentration. It becomes highly sensitive to amoxycillin (25μg) on growth in the presence of crystal violet dye at concentrations ranged between 0.1 and 0.5 μg/ml. While, the zones of inhibition due to erythromycin (10μg) increase on growth in the presence of 0.5 μg/ml, although the level of sensitivity does not change at concentrations ranged between 0.1 and 0.4 μg/ml. On the other hand, sensitivity of *Staph. epidermidis* to cefuroxime (30μg) and erythromycin at 10μg (Table 6) decreases when it is grown on TGY agar containing from 0.1 to 0.5 μg/ml crystal violet. The bacterial sensitivity increases towards ampicillin, amoxycillin and norfloxin in the presence of the dye solution.

The presented investigation and that reported by other investigators (Scott, 1989) ensure that for the choice of drugs for the treatment of certain infection in the oral cavity, sensitivity or resistance of the pathogen isolated from the patient to an appropriate range of antimicrobial drugs must be taken into consideration.

TABLE 5. Effect of combination treatment of some antibiotics and various concentrations of crystal violet dye on the sensitivity of *Staphylococcus aureus*.

Antibiotic	Zone of inhibition in mm at various concentrations of crystal violet (µg/ml)						
	0.0	0.1	0.2	0.3	0.4	0.5	0.6
Cefuroxime	S	S	S	S	S	S	ND
Ampicillin	S	S	S	0.6	0.7	1.0	ND
Amxoycillin	2.3	2.5	2.5	2.5	2.5	3.0	ND
Norfloxcin	S	S	S	S	S	S	ND
Erythromycin	1.8	1.8	1.8	1.8	1.8	2.0	ND

ND : not detected growth
< 10 MM : (s) stable
11-15 mm : Weakely sensitive
15-25 mm : Moderately sensitive
> 25 mm : Higly sensitive

TABLE 6. Effect of combination treatment of some antibiotics and various concentrations of crystal violet dye on the sensitivity of *Staph. epidermidis*.

Antibiotic	Zone of inhibition mm at various concentrations of crystal violet (µg/ml)						
	0.0	0.1	0.2	0.3	0.4	0.5	0.6
Cefuroxime	2.3	1.5	1.5	1.5	1.5	1.5	ND
Ampicillin	1.5	1.8	2.0	2.5	2.5	2.5	ND
Amoxycillin	2.5	2.5	2.5	3.0	3.2	3.5	ND
Norfloxcin	1.5	1.5	1.8	2.0	2.0	2.0	ND
Erythromycin	2.8	2.0	2.0	2.0	2.0	2.0	ND

ND : not detected growth
* < 10 MM : (s) stable
11-15 mm : Weakely sensitive
15-25 mm : Moderately sensitive
> 25 mm : Higly sensitive

Antimicribial activity of clove oil against the two test strains

The effect of different concentration of the essential oil of clove on the growth of *Staph. aureus* and *Staph. epidermidis* was studied. The results in Table 7 indicate that the MIC of the oil against *Staph. aureus* is 7% (v/v) and that against *Staph. epidermidis* is 6% (v/v).

Effect of combination treatment of clove oil and crystal violet dye on the two test strains

The antimicrobial activity of clove oil together with crystal violet dye against *Staph. aureus* and *Staph. epidermidis* was indicated in Table 8. It is noticed that the MIC of the oil decrease with increasing the crystal violet dye concentrations with both bacterial strains. *Staph. epidermidis* was found to be more sensitive than *Staph. aureus* to the combination treatment of the clove oil and crystal violet dye.

TABLE 7. Effect of different concentrations of clove oil on the growth of *Staph. aureus* and *Staph epidermidis*.

Clove oil conc. (% v/v)	Microbial growth	
	Staph. aureus	*Staph. epidermidis*
0(control)	*D*	*D*
1	*D*	*D*
2	*D*	*D*
3	*D*	*D*
4	*D*	*D*
5	*D*	*D*
6	*D*	*ND*
7	*ND*	*ND*

D : Detected growth
ND : Not detected growth

TABLE 8. Effect of combination treatment of clove oil and different concentrations of crystal violet dye on the growth of *Staph. aureus* and *Staph. epidermidis*.

Dye concentration (µg/ml)	Clove oil (% v/v)	Microbial growth	
		Staph. aureus	*Staph. epidermidis*
0.1	0.2	D	D
	0.5	D	D
	1	D	D
	2	D	D
	3	D	D
	4	ND	ND
	5	ND	ND
0.2	0.2	D	D
	0.5	D	D
	1	D	D
	2	D	D
	3	D	ND
	4	ND	ND
	5	ND	ND
0.3	0.2	D	D
	0.5	D	D
	1	D	D
	2	ND	ND
	3	ND	ND
	4	ND	ND
	5	ND	ND
0.4	0.2	D	D
	0.5	D	D
	1.0	D	ND
	2	ND	ND
	3	ND	ND
	4	ND	ND
	5	ND	ND
0.5	0.2	D	ND
	0.5	D	ND
	1.0	ND	ND
	2	ND	ND
	3	ND	ND
	4	ND	ND
	5	ND	ND

D : Detected growth
ND : Not detected growth

The percent concentrations of the clove oil combined with the crystal violet dye solution (μg/ml) that inhibit the growth of *Staph aureus* was found to be 4% with 0.1 and 0.2 μg/ml, 2% with 0.3 and 0.4 μg/ml and 1% with 0.5 μg/ml of the dye solution. On the other hand, *Staph epidermidis* was inhibited by clove oil at concentrations of 4%, 3%, 2%, 1% and ≤ 0.2 with 0.1, 0.2, 0.3, 0.4 and 0.5 μg/ml of the dye solution, respectively.

In agreement with the obtained data, other investigators (Hammer *et al.*, 1999 and Lopez *et al.*, 2005) found that the essential oil of clove inhibits the growth of *Staph. aureus*.

The obtained data indicated that crystal violet dye may inhance the effect of antibiotics and also the effect of the essential oil of clove against the test bacterial organisms used in the present investigation.

Conclusion

For elimination of the chance of bacteria in the oral cavity, prior to its restoration and to reduce the potential for both recurrent caries and post operative sensitivity caused by residual bacteria, crystal violet dye in combination with a suitable antibiotic or with the essential oil of clove may be used.

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References

Arvidson, S., Dornbusch, K. and Ericsson, H. (1981) Interpretation of the agar diffusion method for bacterial susceptibility testing. *J. of Antimicrobial Chemotherapy*, **7**, 5.

Boston, D.W. and Graver, H.T. (1994) Histobacteriological analysis of acid red dye stainable dentin found beneath intact amalgam restorations. *Oper Dent.* **19**, 65.

Cowan, S.T. and Steel, K.L. (1974) "*Cowan and Steel's Manual for the Identification of Medical Bacteria*". Cambridge Univ. Press, London.

Elujoba, A.A., Odeleye, O.M. and Ogunyemi, C.M. (2005) Traditional medicine development for medical and dental primary health care delivery system in Africa. *Afr. J. Trad. CAM,* **2**, 46.

Hammer, K.A., Carson, C.F. and Riley, T.V. (1999) antimicrobial activity of essential oils and other plant extracts. *J. of Applied Microbiology*, **86**, 985.

Kidd, E.A., Joyston-Bechal, S. and Beighton, D. (1993) The use of a caries detector dye during cavity preparation: A Microbiological Assessment. *Br. Dent.* **174**, 245.

Krivoshein, Yu. S. (1989) *"Handbook on Microbiology Laboratory Diagnosis of Infection Diseases"*. p. 36, Mir. Pub. Moscow.

Loesche, W.J. (1986) Role of *Sreptococcus mutans* in human dental decay. *Microbiol. Rev.* **50**, 353.

Lopez, P., Sanchez, C., Batlle, R. and Nerin, C. (2005) Solid and Vaporphase antimicrobial activities of six essential oils: Susceptibility of selected foodborne bacterial and fungal strains. **53**, 6939.

Marcotte, H. and Lavoie, M.C. (1998) Oral microbial ecology and the role of salivary immunoglobulin A. *Microbiol. Mol. Biol. Rev.* **62**, 71.

Marsh, P. and Martin, M. (1992) "*Oral Microbiology*", 3rd ed., Chapman and Hall, Ltd., London, United Kingdom.

Meiers, J.C. and Kresin, J.C.(1996) Cavity disinfectants and dentin bonding. *Oper Dent.* **21**, 153.

Newburn, E. (1992) Preventing dental caries: Breaking the chain of transmission. *J. Am. Dent. Assoc.* **123**, 37.

Nyvad, B. (1993) Microbial colonization of human tooth surfaces. *Acta Pathol., Microbiol. Jmmunol. Scand.* **101**, 7.

Okamoto, H., Lwase, T. and Morioka, T. (1992) Dye- mediated bactericidal effect of He-Ne laser irradiation on oral microorganisms. *Lasers in Surg. & Med.* **12**, 450.

Robertson, P.B., Mackler, B.F., Wright, T.E. and Levy, B.M. (1980) Periodontal status of patients with abnormalities of the immune system. II: Observation over a 2- year period. *J. Periodontal Res.* **51**, 70.

Scott, A.C. (1989) Laboratory control of antimicrobial therapy. In: "*Practical Medical Microbiology*" Coli, J.G., Duguid, J.P., Fraser, A.G. and Marmion, B.P. (Ed.). p. 161. Churchill Livingstone, Edinburgh, London, Melbourne and New York.

Smith, D.J., Anderson, J.M., King, W.F., Houte, J.V. and Taubman, M.A. (1993) Oral Streptococcal colonization of infants. *Oral Microbiol. Immunol.* **8**, 1.

Theilade, E. (1990) Factors controlling the microflora of the healthy mouth, In: "*Microbial Ecology*" M.J.Hill and P.D. Marsh (Ed.), p. 2-56. Human. CRC Press, Inc., Boca Raton, Fla.

Van-Houte, J. (1994) Role of microorganisms in caries etiology. *J. Dent. Res.* **73**, 672.

Yoshii, T., Yoshikawa, T., Furudoi, S., Yoshioka, A., Ohtsuka, Y. and Komori, T. (2001) Evaluation of oral antimicrobial agent levels in tooth extraction sites. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol., Endod.* **91**, 643.

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تقييم فاعلية بعض المضادات الحيوية ،الصبغة البللورية البنفسجية وزيت القرنفل على البكتيريا المعزولة من الأسنان البشرية المصابة بمرض التسوس

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تم فصل ٢٦ ميكروب بيكتيرى من ٩ عينات تم أخذها من أسنان الأنسان المصابة بمرض التسوس وتم تعريف هذه الميكروبات وهى *لاكتوباسيلس لاكتيز* (٣٠,٨%) ، *استربيتوكوكاس ميتز* (١٩,٢%) ، *استافيللوكوكاس سالفاريس* (١٥,٤%) ، *ستافيللوكوكاس اوريس* ١١,٥% ،*استافيللوكوكاس ابيدرميدز* (١١,٥%) و *استافيللوكوكاس ميتانس* (١١,٥%). أيضا حساسية البكتريا المختبرة للمضاد الحيوى سيفروكسيم(زينت) ، امبيسلين (امبيسلين) ، الاموكسيسللين (اموكسيل) ، نورفلوكسين (نوروكسين) وارثيروميسين (ارثيروميسين) قد تضمنت أيضا. ووجد أن *استافيللوكوكاس ابيدرميدز* له حساسية لكل المضات الحيوية المستخدمة ، بينما *استافيللوكوكاس اوريس* ابدى حساسية ضد الاموكسيسللين و ارثيروميسين فقط. وقد تم اختيار هاتين العزلتين لدراسات أخرى . ووجد أن الحساسية تزيد *للاستافيللوكوكاس اوريس* و *استافيللوكوكاس ابيدرميدز* فى وجود الصبغة البللورية البنفسجية مع كل من الامبيسلين ، الاموكسيسللين و النورفلوكسين بينما تقل الحساسية مع كلا من السيفروكسيم و الارثيروميسين . ووجد ايضا أن أقل تركيز يحدث وقف لزيت القرنفل ضد *استافيللوكوكاس اوريس* و *استافيللوكوكاس ابيدرميدزبنسبة* ٧% و ٦% (مجم / مجم) على التوالى بينما أقل تركيز للوقف لنفس الزيت مع الصبغة البللورية البنفسجية متوقفا على تركيز الصبغة المستخدمة فى بيئة الأجار .