

# Isolation and identification of *Streptococcus mutans* from periapical lesion of extracted teeth

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## ABSTRACT

A cross-sectional study done on 200 patients (rang from 20-55 years) attended to dentist unit in Azadi teaching hospital in Kirkuk city-Iraq from March 2018 to April 2019. After successful assessment of available laboratory tests and preparation of patients. Sample of periapical lesion were collected from all patients one week after the teeth extraction for bacteriological examination of these swabs for evaluation the antibiotic resistance of *S. mutans* and reporting of the most appropriate antibiotic used for eradication of this causative agent of wound infection. Sampling of the periapical lesion in the wing was done by the resident dentist using the sterile swab sticks according to the guidelines in the sections. Swabs collected on blood agar and McConkey agar were wiped out by sterile sterilization ring. Agar plates are incubated at 37 ° for 24-48 hours. The bacterial colonies were then stained on an agar plate and subjected to biochemical tests to identify and classify them. The study showed that 12.5% (25 of 200) of patients enrolled in the study had positive culture of periapical lesion swab and 87.5% was negative , the study found that 20% of patients with positive periapical lesion culture were diabetics comparing with 8.57% of patients with negative periapical lesion and 12% of patients with positive wound culture were smokers comparing with 6.86% of patients with negative wound culture. The study showed that 68% of isolated bacteria from periapical lesion culture was *S. mutans* followed by 20% *S. aureus* and the lowest rate of isolated bacteria was *E. coli* (12%). The study showed that all isolates of *S. mutans* (100%) were resistant to ciprofloxacin, tetracycline and levofloxacin followed by 94.12% of isolates were resistant to gentamicin and tobramycin as well as 88.24% of isolates were resistant to ceftazidime and amoxicillin.

Keywords: Periapical lesion; Teeth extraction; *S. mutans*.

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## Introduction

Dental caries is a pandemic disease affecting all the age groups of humans. Several studies indicated that >90% of the residents in developed and undeveloped countries are affected by dental caries <sup>(1,2)</sup>. Accumulative reports showed that among mutans streptococci (MS), *Streptococcus mutans* and *Streptococcus sobrinus* were the most isolated microorganisms from the majority of human dental caries <sup>2</sup>. *S. mutans* is also known to cause systematic diseases such as cardiovascular and infective endocarditis <sup>3</sup>. Recently, *S. mutans* have been considered as novel Gram-positive model bacteria due to its various virulence factors and direct association with the human host <sup>4</sup>. Several studies have shown an association between painful exacerbation of periapical lesions and the presence in the root canal of specific bacteria. Black-pigmented bacteria belonging to the genera *Porphyromonas*, *Prevotella*, and *Bacteroides* have been cultured from root canals in a significant proportion of cases in those studies and are frequently present in the same canals as members of the genera *Peptostreptococcus* and *Fusobacterium* <sup>(5,6)</sup>. However, the findings of different studies based on culturing of canal contents vary significantly. This may be due, at least in part, to the reduced reliability and sensitivity of culturing techniques. The persistence or further expansion of a periapical lesion, despite seemingly adequate endodontic treatment and timely restoration of the tooth, is usually attributed to the persistence of pathogenic microorganisms in the root canal system <sup>6</sup>. Recent investigations have documented that the presence of

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cultivable bacteria from canals at the time of obturation was critical in predicting failure of treatment <sup>(7,8)</sup>. Although enterococci were the most prevalent microorganisms in the last three studies, being present in 54, 70, and 38% of the cases, respectively, the percentages of different strains identified again vary significantly among the studies, and in a considerable number of cases there were no cultivable microorganisms <sup>9</sup>. Therefore, the aim of this study was to evaluate the rate of *S. mutans* in periapical lesion of extracted teeth and its antibiotic profile

## Materials and Methods

### Patients:

In this hospital based study, a total number of 200 patients (rang from 20-55 years) attended to dentist unit in Azadi teaching hospital in Kirkuk city-Iraq from March 2018 to April 2019. After successful assessment of available pre-operation laboratory tests and preparation of patients to enter the surgical unit. Sample of periapical lesion were collected from all patients one week after the teeth extraction for bacteriological examination of these swabs for evaluation the antibiotic resistance of *S. mutans* and reporting of the most appropriate antibiotic used for eradication of this causative agent of wound infection.

### Sampling process

A questionnaire was used to obtain data from the patient after obtaining informed consent from the patient and parents. Sampling of the open wound in the wing was done by the resident dentist using the sterile swab sticks according to the guidelines in the sections. Only one swab was collected for each patient after carefully cleaning the wound and surrounding skin to avoid surface contamination. The samples were then transferred to the microbiology laboratory within one hour of collection using a sterile sealed vial.

### Culture and identification

Swabs collected on blood agar and McConkey agar were wiped out by sterile sterilization ring. Agar plates are incubated at 37 ° for 24-48 hours. The bacterial colonies were then stained on an agar plate and subjected to biochemical tests to identify and classify them.

### Antibiotic sensitivity

Antibiotics have been exposed by the Kirby Power Disc diffusion method and interpreted in accordance with the recommendations of the National Clinical Laboratory Standards Committee. The drugs tested for both Gram-positive and Gram-negative bacteria were gentamicin, trimethoprim, ciprofloxacin, Ampoxicalve, cefexime, tetracycline, ceftazidime, tobramycin, cefotaxime, ceftriaxone, imipenem vancomycin, cefepime, amikacin, amoxicillin and levofloxacin. The antibiotics selected were based on the availability and prescription frequency of these drugs in the study area.

### Statistical analysis

Computerized statistically analysis was performed using Mintab ver 18.0 statistic program for determination of the *P*. value (*P* <0.05 considered significant).

### Finding

The study showed that 12.5% (25 of 200) of patients enrolled in the study had positive culture of periapical lesion swab and 87.5% was negative , Table 1.

**Table 1:** Result of swab cultures of surgical sites

Culture result	No.	%
Positive	25	12.5
Negative	175	87.5
Total	200	100

Table 2 shows that 20% of patients with positive periapical lesion culture were diabetics comparing with 8.57% of patients with negative periapical lesion

**Table 2:** Relation of diabetes with result of periapical lesion

Culture result	Total No.	Diabetes		Non-diabetes	
		No.	%	No.	%
Positive	25	5	20	20	80
Negative	175	15	8.57	160	91.43

P. value >0.05

Table 3 shows that 12% of patients with positive wound culture were smokers comparing with 6.86% of patients with negative wound culture.

**Table 3:** Relation of smoking with result of periapical lesion

Culture result	Total No.	Smoker		Non-smoker	
		No.	%	No.	%
Positive	25	3	12	88	80
Negative	175	12	6.86	163	93.14

P. value >0.05

The study showed that 68% of isolated bacteria from periapical lesion culture was *S. mutans* followed by 20% *S. aureus* and the lowest rate of isolated bacteria was *E. coli* (12%), Table 4.

**Table 4: Type of isolated bacteria**

Culture result	No.	%
<i>Staphylococcus aureus</i>	5	20
<i>S. mutans</i>	17	68
<i>E. coli</i>	3	12
Total	25	100

The study showed that all isolates of *S. mutans* (100%) were resistant to ciprofloxacin, tetracycline and levofloxacin followed by 94.12% of isolates were resistant to gentamicin and tobramycin as well as 88.24% of isolates were resistant to ceftazidime and amoxicillin and 70.59% of isolates were resistant to ceftriaxone and imipenem. The isolates represented rate of resistance (35.29%) toward amikacin and vancomycin, Table 6.

**Table 6:** Sensitivity and resistance rate of antibiotics used in the study against *S. mutans*

Antibiotic discs	Sensitive		Resistance	
	No.	%	No.	%
Gentamicin	1	5.88	16	94.12
Trimethoprim	8	47.06	9	52.94
Ciprofloxacin	0	0	17	100
Amoxiclav	9	52.94	8	47.06
Cefexime	8	47.06	9	52.94
Tetracycline	0	0	17	100
Ceftazidime	2	11.76	15	88.24
Tobramycin	1	5.88	16	94.12
Cefotaxime	8	47.06	9	52.94
Ceftriaxone	5	29.41	12	70.59
imipenem	5	29.41	12	70.59
Vancomycin	11	64.71	6	35.29
Cefepime	7	41.18	10	58.82

Amikacin	11	64.71	6	35.29
Amoxicillin	2	11.76	15	88.24
Levofloxacin	0	0	17	100
Ampicillin	7	41.18	10	58.82

P<0.05

## Discussion

Dental caries is a major public health concern affecting all age groups of human beings (6,7). The present study agreed with several studies around the world proved that *S. mutans* is the most isolated bacteria from patients with dental problem (8,9). However, despite the importance of epidemiological studies in these patients, limited research has been carried out to view such infection and its role in inflammation (10). In addition, 80% of Indian population dental caries and periapical lesion was with the same causative agent (11). As *S. mutans* and *S. aureus* are most commonly isolated species from caries subjects, the study focused further on these organisms. Among the sampling methodologies, plaque sample was preferred over saliva as detection levels of MS species were stated to be higher in plaque (12). Based on bacteriological examination, the present study revealed that the prevalence of *S. mutans* was higher than *S. aureus*. Although the differences in the geographical areas, detection methods and the age groups of the subjects employed in the previous studies, the prevalence and the distribution of *S. mutans* showed a similar tendency to those of the current study (7-9). Earlier studies (10,11) reported on the high variation in prevalence of microorganisms in root-filled teeth. Thus these might be due to inclusion of high numbers of teeth with persistent symptoms or a history of symptoms in some studies whereas other studies only included asymptomatic teeth showing radiographic evidence of periradicular lesions. An Indian investigation revealed that *S. mutans* isolates of the swab samples contained the highest levels of resistance against ciprofloxacin (87.93%), ceftazidim (73.27%), cefexim (84.48%), gentamicin (78.44%) and afloxacin (87.93%), which was similar to our results (13). In an other study (14), 40% of *S. mutans* isolates were resistant to seven antibiotics, including amoxicillin, ampicillin, ciprofloxacin, norfloxacin, and gentamicin. In cases of burn infections, 70% of *S. mutans* isolates were positive for beta-macrolase-metallase, with antibiotic resistance against ceftazidim (70%), chloramphenicol (68%) and intamycin (62.5%). A recent investigation revealed that *S. mutans* strains isolated from the apical lesion were resistant to coccacillin (91.8%), cotrimoxazole (86%), cephalazone (83.7%), carbinecilin (74.4%), (68.9%), ciprofloxacin (66.3%), tupramycin (58.2%), amikacin (48.8%) and gentamicin (37.2%). It was similar to our results (15). The prescription of unregulated and immoral antibiotics and even self-treatment by strong antibiotics cause high levels of resistance in *S. mutans* strains in our investigation. Differences in the idea of medical practitioners in the prescription of antibiotics cause differences in levels of antibiotic resistance against various antibiotics (13). In addition, the differences in antimicrobial activity of antibiotics and also the difference in the difficulty of developing resistance against different antibiotics are two other causes of differences in levels of antibiotic resistance (14).

## Conclusions:

It was concluded that was *S. mutans* highly isolated from infected periapical lesion and their isolates were highly resistant to most available antibiotics

## CONCLUSION

### Financial disclosure

There is no financial disclosure.

### Conflict of interest

None to declare.

### Ethical Clearance

All experimental protocols were approved under the Kirkuk health Directorate and all experiments were carried out in accordance with approved guidelines.

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