

# Antibiotic Resistance of *Streptococcus mitis* Isolated from Dental Caries Patients in Missan City

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**Summary:** Twenty six isolates of *Streptococcus mitis* bacteria collected from clinical of dental disease in missan city , then analyzed between (October and December in 2014).The samples taken in order to determine the bacterial profile and antibiotic susceptibility . Isolates of *Streptococcus mitis* were tested against eight different antibiotics and crude of crude aqueous extracts from flowers of *Matricaria chamomilla* L., by a disk diffusion method. 100% of isolates were resistat to the Tetracycline ,84%resistant to Ampicillin , 96% resistant Nitrofurantion ,100% resistant to Optochin ,80% resistant to Bacitracin ,96% resistant to, 92% resistant to Vincomycin , 73% resistant to Benzypenicillin where found to be the least effective antibiotics against MDR *Streptococcus mitis*. Also the antimicrobial suscepitility of isolates where tested against crude of crude aqueous extracts from flowers of *Matricaria chamomilla* L. with different concentration (62,5, 125, 250 , 500 mg / ml) detected by agar well diffusion methods , the aqueous extracts from flowers of *Matricaria chamomilla* L. have more effective in 500 mg / ml . Finally cytotoxicity evaluating toward human RBC , the results revealed these crud extracts of *Matricaria chamomilla* L. have not any cytotoxicity in all concentration . The good antimicrobial potency of crud extracts of *Matricaria chamomilla* L. indicates the treatment of MDR *Streptococcus mitis* as an alternative to the costly antibiotic.

**Keywords:** Antibiotic, MDR *Streptococcus mitis*, Minimum Inhibitory Concentration (MIC), *Matricaria Chamomilla* L. Flowers

## Introduction

The oral cavity is a complex ecosystem comprising many coated with a wide variety of species (Chopde et al.,2012). Viridans *Streptococcus* and other alph —hemolytic *Streptococcus* have been reported with increasing frequency to cause infection . *Streptococcus* characteristics are Gram positive cocci (spherical or ovoid ) often occurring in pairs and chains *Streptococcus* are facultatively anaerobic and catalase negative, on blood agar , the species exhibit various degrees of haemolysis , which can to be used as an early step in identifying clinical isolates . *Streptococcus mitis* ,one of the species included among viridians *Streptococci* , is the most resistant to beta-lactam antibiotics of this group (Doern et al., 1996). *Streptococcus mitis* groups, although surgical drainage is of primary importance , administration of antimicrobial therapy becomes necessary in serious infections and . (Lewis et al.,1995). However, many

attempts for prevention of dental caries were of on practical use up to the present .

Antibiotics are chemical substances considered as secondary metabolites producing by microorganisms during stationary phase. They have the ability to kill or inhibit other microorganisms (Atlas,1996). The antibiotics are divided into groups according to their mode of action , include the bacterial cell wall , cell membrane ,protein synthesis and nucleic acid synthesis (Elliott et al.,2007). Antibiotics such as penicillin and erythromycin have been reported to effectively prevent dental caries in animal and humans (Jonathan et al.,2000) ,but they are never used clinically because of many adverse effects such as hypersensitivity reaction , suprainfections and teeth staining.The most frequently isolated facaltatively anaerobic microorganisms were the oral streptococci, in particular those belonging to the

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former *Streptococcus mitis* group and milleri groups.(Ghiteanu,1997) .

Normal products have been used for thousands of years as most of the oral diseases are due to bacterial infection and it has been well documented that medicinal plants confer considerable antibacterial activity against various microorganisms(Saeki et al ., 1989). This study investigated evaluate the antibacterial activity of crude extract of the leaves of *Matricaria chamomilla* L., against *Streptococcus mitis*. With found minimum inhibition concentration of extracts with Biofresh mouth washes.

#### Material and Methods

The project was approved by the Department of biology in college of sciences /Missan university / Iraq

#### Isolation and Identification of *Streptococcus mitis*

26 isolates of *Streptococcus mitis* were isolated from caries patients . a sterile cotton swab was used . Samples were cultured on blood agar medium , incubated in jar under CO<sub>2</sub> at 37°C overnight, the colony appeared then subcultured and purified. Identification of the isolates based on many characters such as colonial morphology, Gram stain , motility , oxidative , fermentation test , catalase citrate and oxidative tests , *Streptococcus mitis* isolates were stored in Nutrient broth (John et al.,2003 ) .

#### Antibiotic susceptibility testing :

Agar disc diffusion test were done for all the isolated (Kirby Bauer, 1966 ). A suspension of each isolate was made at turbidity to 0.5 McFarland standard and then plated on to Muller – Hinton agar Plate. Antibiotic disc , was applied to each plate . The plates incubated at 37°C for 24 h .After incubation the inhibition zone was measured , the results of all isolates compared with standard isolates of *Streptococcus mitis* were isolated from dental caries patients. Plant Material *Matricaria chamomilla* L. [ Figure 1] was collected from the market in Amara city , and taken to the laboratory . The Plant materials (Fruit ) were washed separately under running tap water , followed by rinse using sterilized distilled water. Excess of was removed from the plant material

using filter paper before they were used for extraction.

#### Antibiotic susceptibility assay :

The well diffusion assay is suitable for aqueous extracts because they are difficult to dry on paper discs (Tadeg et al.,2005). A suspension of each isolate was made at turbidity equal to 0.5 McFarland standards and then plated on Muller- Hinton agar. Antibiotic disc was applied to each plate .The plate incubated at 37°C for 24 h in rich CO<sub>2</sub> . After incubation the inhibition zone was measured the results of all isolates compared with standard isolates of *Streptococcus mitis* were isolated from caries patients.

#### Preparation of aqueous extracts:

The extracts were prepared according to (Ahmad et al., 1998). 25gram of finely powdered material and 400ml of distilled water were refluxed in 500ml flask and suspension was put on Magnetic stirrer plate for 24 hours at room temperature, the extract then filtered by using filter paper type Whatman , No1. And dried at room temperature 25°C. then scratched by sterilized clean knife . After that it has been weighted to be kept in refrigerator until it has been used.

#### Determination of MIC by agar plate dilution method:

According to the methods of NCCLS ,(2002) , agar plate dilution test was used to determine the Minimum Inhibitory Concentration (MIC) of an antimicrobial agent.

#### Cytotoxicity assay :



According to the methods of Xian – Guo and Ursula (1994), human red blood cells were used for toxicity test. (Figure 1) *Matricaria chamomilla* L. Flowers

Table [1] :Antibiotics: eight type of antibiotics used in this study:

| No | Antibiotic       | Concentration | Company    |
|----|------------------|---------------|------------|
| 1  | Tetracycline     | TE10 (mcg)    | Bioanalyse |
| 2  | Ampicillin       | AM (mcg)      |            |
| 3  | Nitrofurantion   | F100(mcg)     |            |
| 4  | Optochin         | Op5(mcg)      |            |
| 5  | Bacitracin       | B10(mcg)      |            |
| 6  | Erythromycin     | E15(mcg)      |            |
| 7  | Vincomycin       | VA30(mcg)     |            |
| 8  | Benzylpenicillin | Be10 (mcg)    |            |

**Table (2 )** Distribution of bacterial isolates diagnosed by species *Streptococcus viridans* bacterial oral cavity.

| Total Number | Total Number of isolated | Number &type of <i>Streptococcus viridans</i> strains |                                   |
|--------------|--------------------------|---|-----------------------------------|
| 44           | 26                       | (11) 42.3 %   | <i>Streptococcus mitis</i>        |
|              |                          | (10) 38.4%  | <i>Streptococcus orails</i>       |
|              |                          | (2) 7.6 %   | <i>Streptococcus suis</i>         |
|              |                          | (1) 3.8 %   | <i>Streptococcus canis</i>        |
|              |                          | (1) 3.8 %   | <i>Streptococcus gallolyticus</i> |
|              |                          | (1) 3.8 %   | <i>Streptococcus pneumonia</i>    |

**Table (3):** Susceptibility of *Streptococcus mitis* to eight type of antibiotics

| No | Antibiotics      | Resistant (NO) | Resistant (%) |
|----|------------------|----------------|---------------|
| 1  | Tetracycline     | 26             | 100 %         |
| 2  | Ampicillin       | 22             | 84%           |
| 3  | Nitrofurantion   | 25             | 96%           |
| 4  | Optochin         | 26             | 100%          |
| 5  | Bacitracin       | 21             | 80%           |
| 6  | Erythromycin     | 25             | 96%           |
| 7  | Vincomycin       | 24             | 92 %          |
| 8  | Benzylpenicillin | 19             | 73%           |

Table (4 ) The mean of inhibition zone of aqueous of crude extracts from flower of ***Matricaria chamomilla L*** against multi-drug isolated against *Streptococcus mitis*

\*clinical strain

\*\* mean of three value each number

Table (5 ). The MIC of the aqueous extracts of crude extracts from flower of *Matricaria chamomilla* L.

| Sample                     | Dilution of aqueous extracts of crude extracts (mg/ml)<br><i>Matricaria chamomilla</i> L. |     |     |    |    |    |    |
|----------------------------|---|-----|-----|----|----|----|----|
|                            | ≥64   | ≥32 | ≥16 | ≥8 | ≥4 | ≥2 | ≥1 |
| <i>Streptococcus mitis</i> | -   | -   | +   | +  | +  | +  | +  |

Table (6).The MIC of Biofresh mouth wishes

| Sample                     | Dilution of Biofresh |     |     |    |    |    |    |
|----------------------------|----------------------|-----|-----|----|----|----|----|
|                            | ≥64                  | ≥32 | ≥16 | ≥8 | ≥4 | ≥2 | ≥1 |
| <i>Streptococcus mitis</i> | -                    | -   | -   | -  | +  | +  | +  |

| Bacterial strain isolated    | ** The mean of inhibition zone of aqueous of crude extracts (mm) |           |           |           |
|------------------------------|--|-----------|-----------|-----------|
|                              | 62.5 mg/ml   | 125 mg/ml | 250 mg/ml | 500 mg/ml |
| * <i>Streptococcus mitis</i> | 10.6   | 19.3      | 21.0      | 24.8      |

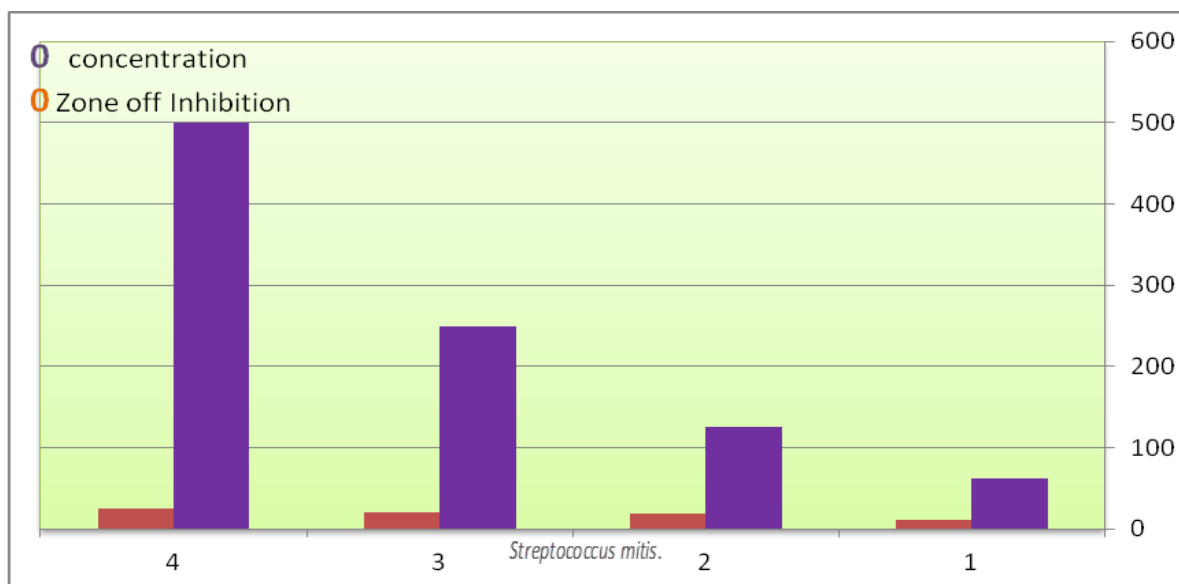


Figure (5 ): Effect of different concentration of crude extracts from flower of *Matricaria chamomilla* L. against pathogenic *Streptococcus mitis*.

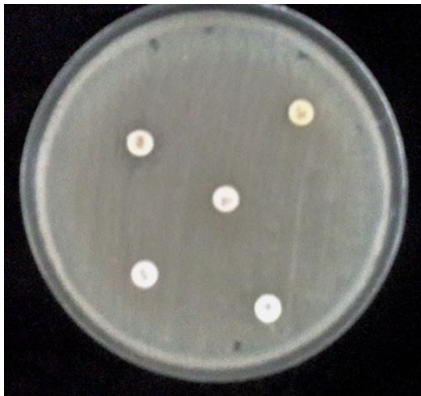


Figure 2: Mueller Hinton agar media with antibiotic sensitive disc showing *Streptococcus mitis* resistant to all antibiotics

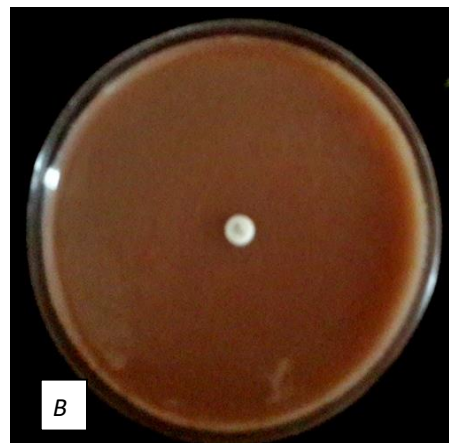
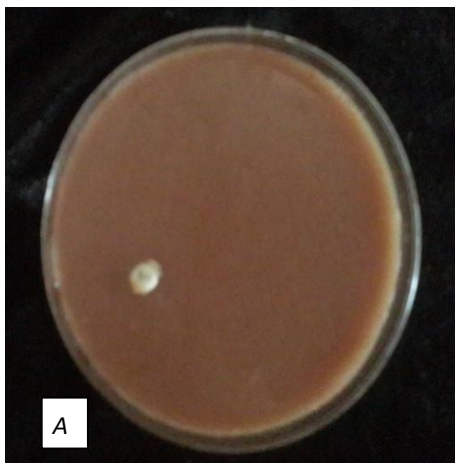
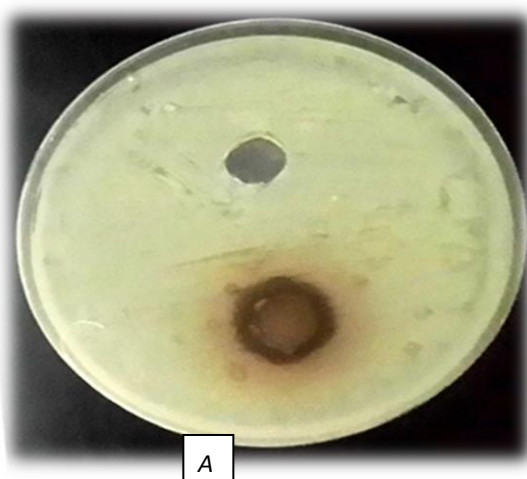


Figure 3 showing *Streptococcus mitis* resistant Optachin antibiotics (A ) on chocolate agar( B) on blood agar media.





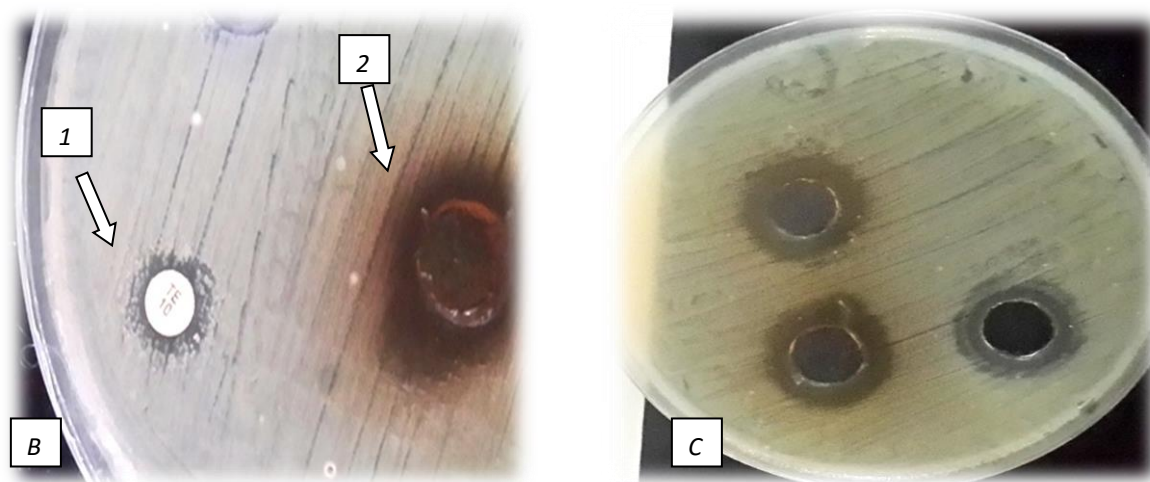


Figure 4: (A) Shown aqueous extract of *Matricaria chamomilla* L. , Tetracycline resist ant(arrow ) and sensitive (arrow 2 shown aqueous extract in 500mg /ml ) , (c) shown aqueous extract in 250 , 125 , 62.5 mg /ml against *Streptococcus mitis* MDR isolates.

## Results

Table (2 ) shown the distribution of 26 bacterial isolates were diagnoses by species of *Streptococcus viridans* ,42.3% *Streptococcus mitis* , 38.4% *Streptococcus orails* , 7.6 % *Streptococcus suis* , 3.8 % *Streptococcus canis* , 3.8 % *Streptococcus gallolyticus* , 3.8 % *Streptococcus pneumonia*.

Table (3 )showed high resistance to Optochin (100) and Tetracycline (100%) , Ampicillin (84%) ,Nitrofurantion (96%) , Bacitracin (80%) ,Erythromycin (96%) , Vincomycin (92%) , Benzylpenicillin (73%) . Multi – Drug resistant strain of *Streptococcus mitis* were recorded from dental caries patient.

All collected *Streptococcus mitis* isolated from dental caries patient were exposed to different concentration of aqueous extract of *Matricaria chamomilla* L. as described in table (4 ) .

The results represented in table (4 ) . Showed that inhibition zone of *Streptococcus mitis* isolated bacteria increased as the concentration of aqueous extract of *Matricaria chamomilla* L. increased , the susceptibility pattern to extracts on *Streptococcus mitis* isolated bacteria maximum inhibitory zone at 500 mg / ml which was mean 24.8 mm but in low concentration which was mean 10.6mm against Multi – Drug isolated bacteria *Streptococcus mitis*.

In same time the results represented in table (5 ) ,(Figure 5 ) , showed the MIC of the aqueous extracts of crude extracts from flowers of *Matricaria chamomilla* L recorded as ( $\geq 16$  mg / ml ) , whale the

results represented in table (6 ) showed the MIC of Biofresh mouth wishes recorded as ( $\geq 4$  mg / ml).

## Discussion

Our study showed high prevalence of bacterial infection especially MDR- *Streptococcus mitis* among dental caries patient. This study was focused on the susceptibility testing of clinically isolates belonging to mitis group since these microorganisms are frequently isolated from oral and maxillofacial infaction (Renneberg et al .,1997).

Viridans *Streptococcus* are normal inhabitants of the human oral cavity . The portal of entry for the organisms is usually an oral lesion . Systematic infections can still occur in patient without overt oral lesions . The Viridans *Streptococcus* highly resistant to penicillin , *Streptococcus mitis* in particular , as a cause of sepsis in patients . The relevant mechanism of penicillin resistace in Viridans *Streptococcus* might be the patients with lowered affinity for beta – lactam antibiotics (Doern et al.,1996). The results prevalence of *Streptococcus mitis* isolated and new resistant strains , the resistant was detected against Tetracycline (100%) , Ampicillin (84%) ,Nitrofurantion (96%) , Optochin (100%) ,Bacitracin (80%) , Erythromycin (96%) ,Vincomycin (92%) , Benzylpenicillin (73%) [ Table 3 Figure 3 , 4 , 5] this results are agreement with other studies carried out in Romania (Bancesu et al.,2004) 85 isolated of the 151 sample studied belonged to *Streptococcus mitis* group .

There are four main mechanisms by which bacteria become resistant to antibiotics agents, Destruction and inactivation of the antibiotic by blockage of

transport of the agent into the cell providing the cell with a replacement for the metabolic step inhibited by the drug, and protection of the target site by a bacterial protein therefore it is imperative to seek other sources of therapeutic agents (Lambert, 2005). In this study, agar well diffusion method was used to determine the antibacterial activity of the crude extracts of flowers of aqueous extract of *Matricaria chamomilla* L. in [table 4, Figure 4,5] shows the activity of different concentration (62.5, 125, 250 and 500 mg/ml) which increased the inhibition zone against MDR *Streptococcus mitis* bacteria, the growth inhibition of bacteria increased as the concentration of extract increased, the susceptibility pattern to the extract on *Streptococcus mitis* isolated expressed maximum inhibitory zone at concentration 500 mg/ml which was 24.8mm, while the low concentration of aqueous extract of *Matricaria chamomilla* L. recorded 10.6 mm as in Table 4 Figure 4, 5], that's result agreement with (2008 شوكت , واخرون).

Finally a test was also carried out to examine the cytotoxicity assay by using (Xian-guo and Ursula 1994), methods towards human red blood cells in which the crude extracts of aqueous extract of *Matricaria chamomilla* L., where found that they are not having any cytotoxicity at (1-500mg/ml). Results of this study suggest that the crude extracts of aqueous extract of *Matricaria chamomilla* L. may be useful either alone or when combined with antimicrobial agents to treat (MDR) *Streptococcus mitis* bacteria.

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