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Zohreh Zamanian
Department of Microbiology, Islamic Azad University, Kazeroon Branch, Iran

Nahid Arian Pour
Department of Microbiology, AJA University of Medical Sciences, Tehran, Iran, Arianpourn@yahoo.com

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ORIGINAL ARTICLE

Effect of Zoocin A on Growth of the Biofilm Producing Cariogenic Oral Bacteria

Zohreh Zamanian¹, Nahid Arian Pour²

¹Department of Microbiology, Islamic Azad University, Kazeroon Branch, Iran
²Department of Microbiology, AJA University of Medical Sciences, Tehran, Iran
Correspondence e-mail to: Arianpourn@yahoo.com

ABSTRACT

Zoocin A has a potential antibacterial properties and its use as an anti-cariogenic agent needs to be explored. 

Objective: Dental caries is an infectious disease, caused mainly by mutans streptococci (MS). The aim of the present study is to evaluate the antimicrobial effect of zoocin A on the biofilm producing cariogenic oral bacteria compared with antibiotics.

Methods: The samples were collected from dental caries and plaques of 130 cases referring to eight government dental clinics of Hamedan- Iran for treatment. The isolated bacteria were identified on the basis of morphological, biochemical and molecular methods. The antimicrobial effects of the zoocin A and antibiotics were compared.

Results: Zoocin A showed varying degrees of inhibition on the most common oral biofilm producing bacteria we isolated which were identified as S. mutans, S. mitis, S. sanguinis, S. gordonii, Lactobacillus gasseri, and Granulicatella adiacens by PCR using16S rRNA gene sequence. Minimum inhibitory concentration (MIC) of 2.2 mg/l and 3.2 mg/l was observed against S. mutans and S. gordonii respectively.

Conclusion: In Hamedan, S.mitis and S. mutans are dominant species in decayed teeth of cases referring to government dental clinics. Isolated bacteria showed varying sensitivity to different antibiotics. Zoocin A inhibited the growth of some Streptococcal species like S. mutans and S. gordonii. Antimicrobial property of Zoocin A against cariogenic agents collected from high risk caries patients is equal to antibiotics tested in this study.

Key words: bacteriocin, biofilm, dental decay, mutans streptococci, Zoocin A

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INTRODUCTION

Dental decay is a common preventable, oral infectious disease associated with increased consumption of sugar and carbohydrates.¹ Dental caries has been known since recorded history, but was not an important health problem until sucrose became a major component of the human diet.¹ Tooth decay is the result of irreversible solubilization of tooth mineral by acid produced by certain bacteria that reside in dental plaques.¹

In 1890s, Miller was the first to relate bacteria with dental decay.² The mouth is colonized by various bacterial species, but only some species participate in dental decay. Streptococci form 20% of the oral bacteria that participate in biofilm formation.³ Streptococcus mutans is the main etiologic agent of dental decay.³ It is a gram positive, non-motile, facultative anaerobic coccus which can metabolize carbohydrates.³ Lactobacilli are associated with progression of the decay.³ They are gram positive, facultative anaerobic or microaerophilic, rod shaped, non-spore forming bacteria.³

Tooth decay is a controllable infectious disease. Rigorous debridement of teeth surfaces is the standard treatment.¹ Short-term use of antimicrobial agents, especially antibiotics is beneficial.¹ Antibiotics are constantly in use for treating dental caries and have been used for inhibition of biofilm formation.⁵ However, certain bacteria have developed mechanisms for resisting antibiotics.⁷ The emergence of resistant bacteria is occurring rapidly worldwide, endangering the efficacy of antibiotics.⁸ The number of multi-drug resistant pathogens is increasing, which is a serious problem. Thus, developing a new generation of antibacterial agents to treat infectious diseases is becoming important.⁹ On the other hand, development of new generation of antibiotics by the pharmaceutical industry has been stalled due to various obstacles including economic obstacle.⁸
Many new antibacterial substances have the potential to replace the antibiotics. Bacteriocins are proteinaceous toxins found in almost all bacteria. They often inhibit the growth of related organisms. A bacteriocin-like inhibitory substance called zoocin A is an extracellular enzyme secreted by Streptococcus equi subsp. zooepidermicus 4881 which hydrolyzes peptidoglycan cross bridges of susceptible Streptococci, most notably Streptococcus mutans. Thus, it acts as a potential antibacterial agent for reducing the population of the competitors, suggesting its use as an anti-cariogenic agent.

The aim of the present study is to evaluate the antimicrobial effect of Zoocin A on the biofilm producing cariogenic oral bacteria isolated from study cases compared with antibiotics.

**METHODS**

**Study Cases**
130 cases referring to eight government dental clinics of Hamedan- Iran were randomly selected. Oral cavity and teeth of every case was checked by the dentist. A questionnaire was filled which contained questions regarding their demographic features, oral cavity hygiene and teeth conditions. To achieve an overall view of oral and teeth health conditions of the participants, questions scored zero to 10. 10 indicates excellent oral and teeth health conditions and zero shows poor oral and teeth conditions, requiring emergency treatment. The oral hygiene questions were evaluated on the basis of IDC-M version. Written informed consent was obtained from every participant. Data were analyzed using SPSS software. Students’ T-test and Chi square test were some of the statistical tests used for analysis of the data.

**Sample collection**
Cases were asked to rinse their mouth with sterile distilled water prior to sample collection. Sterile swabs were used to collect samples from dental caries and plaques. Used swabs were inserted in sterile test tubes containing 5 ml sterile phosphate buffer saline and were transported to the laboratory in ice box within 3 hours.

**Isolation of the bacteria**
In the laboratory, collected samples were cultured on Brain and Heart infusion agar (Sigma Aldrich), Columbia agar (Sigma, Aldrich), blood agar (Oxoid) containing 5% de-fibrinated sheep blood, Trypticase AzolecTwinTmouse (TAT) (Hi Media), Hewitt agar (Sigma Aldrich), and Luria Bertani broth (LB) (Hi Media) incubated at 37°C for 24 - 48 hours under aerobic and anaerobic conditions. To maintain anaerobic conditions we used both glass jar as well as anaerobic incubator (Anaerobic Incubator BJPX-G Series, Biobase).

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**Table 1. Primers used for PCR**

| Microorganism | Sequence | Length (bp) |
|---------------|----------|-------------|
| S. mitis      | TGAATCGAGGTGGCTAC | 259         |
|               | TCCCCCTCTAAAGAAGAC   |             |
| S. salivarius | GGTGTCACATCTAGTCCTGG | 544         |
|               | CGTTGATGTCTTAGAAAGGCC |          |
| S. sanguinis  | GGATAGTGGTCAGGCACTCAGT | 313         |
|               | GAACAGTTGCTGGATCTGAGGC |        |
| S. oralis     | TCGGCTAGCAACTCCAGCC | 374         |
|               | GCAACCTTTGGATTGCAAC |             |
| S. gordonii   | CTATCGGATGCTAATCAAGTG | 440         |
|               | GAGACGCTTAATTCGTCAAGAA |         |
| S. mutans     | GGCACCACAAACTGGGAAAGCTCAGTT | 433       |
|               | GGAAGCGCCGCTAATGCAACAGGAT |     |
| S. sobrinus   | GATGATTTGGCTAGGATCAATCCTC | 328       |
|               | ACTGAGCCAGTGAGATCTGGGAACTG |    |
| Universal2    | GATTAGATCCGTGTCAGCC | 733         |
|               | TACCTTGTACGACTT |             |
| Universal 2   | TCCTACGGAGGCCAGCAT | 466         |
|               | GAGAATCCAGGGATATCCTTACGT |       |
| Universal 3   | CGCTAGTATCGTGATCGAATG | 69          |
|               | TGTGACGAGGGGTGGTA |             |
Identification of the isolated bacteria
The isolated bacteria were identified on the basis of their phenotypic and biochemical characteristics including microscopic examination of their stained smears, biochemical tests like Indole, Methyl red, Voges-Proskauer, Citrate utilization (IMViC), oxidase, catalase, nitrate reduction, and motility tests. Biochemical characteristics of isolated Streptococci were verified using API 20 Strep kits- France. Viability of the isolated bacteria was determined by serial dilution of the broth prepared from their first cultivation on selective media.

Molecular identification of the isolated bacteria
DNA extraction kit (Sinaclon - Iran) was used to extract the DNA of the isolated samples. Primers used were provided by Bioneer, South Korea. Their sequences used are given in Table 1. PCR was carried out using universal primers employing GeneAmp PCR system 9700 (Thermo Fisher Scientific). The test was carried out with initial denaturing stage adjusted at 95°C for 4 minutes and 45 seconds respectively. Annealing took 45 seconds at 60°C and extension took 90 seconds at 72°C. Tris/Borate/EDTA (TBE) 1x was used as buffer. The sequencer used in the present study was marked ABI3730XL.

Biofilm formation
Ability of the isolated bacteria to form biofilm was estimated both by dyed test tube and microtiter plate methods. Experiments for biofilm formation were repeated thrice. The density of the stained biofilm of the bacteria sticking to the wall of the glass tubes, dyed by crystal violet, indicated the biofilm producing ability of the bacteria. In microtiter procedure, control and samples were placed in alternate wells of a microtiter plate.

Antibiotic sensitivity of the isolated strains
Isolated bacteria were cultured on Muller-Hinton agar. Antibiogram test was performed by Kirby-Bauer disc diffusion method using Vancomycin, Tetracycline and Erythromycin Difco discs. Whonet 5.6 software was used to measure the zone of inhibition of tested antibiotics.

Effect of Zoocin A on the isolated strains
Effect of Zoocin A was studied by well diffusion and disc diffusion methods on isolated strains using Muller-Hinton agar. In the well diffusion method, 20µl Zoocin A solution (Sigma Aldrich) was poured in 6x3 mm well cut on solid Muller-Hinton agar on which each one of the isolated bacteria were grown and covered by soft agar gel. Effect of Zoocin A on the isolated strains were measured after 24 hours incubation at 30°C. Distilled water was used as control. In disk diffusion method, 6mm disks impregnated with 2-3µg Zoocin A were placed on agar plates on which isolated bacteria were grown and incubated for 24 hours at 30°C. Zone of inhibition on each isolate was measured.

For comparing the effect of Zoocin with antibiotics, Vancomycin, Tetracycline and Erythromycin disks were also used. Minimum Inhibitory Concentration (MIC) of Zoocin A was determined by pouring 200 µl of the two fold serially diluted Zoocin A in 3x6mm wells cut on cultured Muller-Hinton agar. The zone of inhibition was measured.

RESULTS
In the present study, 130 cases, aged 7 to 78 years with mean age of 34.83±16.61 years, participated in the study. Maximum number of participants were placed in the age range of 16-30- years (36.92%) followed by 31-45 years group (30%). 75% of the participants had good oral health condition (Table 1).

Maximum number of samples were collected from small mandibular molar teeth i.e. teeth number 19 & 28 (10.8% cases each). 29.2% participants had no history of filling their teeth. Students’ T test was used with 95% confidence interval to show the significance of the differences. Between-sample-distribution is normal in this study with minimum expecting count of 0.02. \( \chi^2 \) value of 0.012 between the samples is significant. Students’ T-test co efficient 20.97 with 99 degrees of freedom and 95 percent interval reveals in-between-group uniformity.

Participants’ teeth defects were categorized on the basis of ICD-M10, a subclass of WHO classification version 10 (ICD10). Pain was the main reason for 85.4% cases to visit the dentist which is grouped under K02.1. Based on the 10 score system, in the 34th of the cases oral and dental health was in good condition. Maximum distribution i.e. 21.5% belonged to score 8. Demographic features of the cases is presented in Table 1.

Bacterial isolation and identification
Gram stained slide smears were checked for microscopic characteristics. Routine general and specific biochemical tests, mobility, etc. revealed the presence of different species of Streptococcus like mitis, mutans, Sanguinis, gordonii and S. salivarius. Lactobacilli gasseri, and iners, Rothia mucilaginosa, Gemella haemolysans and Granulicatella adiacens could also be identified. S. mutans was isolated in the preforming decays and lactobacilli were identified in deep caries. Confirmatory identification using API 20-strep test kit revealed Streptococcus mitis, S.mutans, S. gordonii, S. sanguinis and S. salivarius were the most commonly isolated Streptococci. Screening of the isolates for biofilm formation revealed that six isolates i.e. Streptococcus mutans, S. mitis, S.sanguinis, S. gordonii, Lactobacillus gasseri, and Granulicatella adiacens were capable of forming biofilm and were numbered CF1 to CF6. These were
all gram positive bacteria with negative oxidase and nitrate reduction tests and were non-motile. Figure 1a shows the dyed test tubes containing these isolates and Figure 1b shows attachment of the biofilm to the bottom of the wells of microtiter plate.

In microtiter procedure, control and samples were placed in alternate wells of a microtiter plate (Figure 1b). Control wells were colored pale yellow and wells containing cases’ samples colored dark blue. Color appearance on the wall of the tube observed by naked eye was qualitative way of biofilm formation.

**Molecular identification of the isolated species**

The isolated species capable of forming biofilm in vitro were identified by PCR using 16s rRNA gene. Purified pieces of 16s rRNA obtained by PCR using ABI Prism kit (Orchid BioSciences, Inc. Princeton, U.S.A.) was prepared. Pattern sequence was ABI 3730 XL DNA (ABI PRISM® 3730 XL Genetic Analyzer Sequencing,
AB Applied Biosystems Hitachi). Primers used were provided by Bioneer, South Korea (Table 4). PCR was carried out using GeneAmp PCR system 9700. Comparison was made with sequences present in the Human Oral Microbiom Database (HOMD) employing its software. Sequences with less than 90% similarity were deleted from the study. After DNA preparation and phylogenic analysis of the isolated species was carried out and were compared with HOMD, employing the HOMD online software). Abundance of bacteria belonging to phylum Firmicutes and Actinobacteria which were found almost in all samples, followed by comparatively lesser distribution of Proteobacteria, Fusobacteria and Bacteroidetes (present only in one sample) are shown in Table 3. & Dendogram 1 & 2. Dominant bacteria belonged to the Phylum Firmicutes with redundancy of S.mitis, and S.mutans followed by Lactobacilli indicating that reduction of pH attracts

Table 3. Bacteria isolated from dental caries by 16S rRNA sequencing technique

| No. | Sample No. | Similar isolates recorded in data bank | Similarity % | Grouping of samples | Phylum |
|-----|------------|--------------------------------------|--------------|---------------------|--------|
| 1   | AJ234052.1 | Actinomyces naeslundii               | 99           | A1                  | Actinobacteria |
| 2   | X81063.1   | Actinomyces sp.                      | 99           | A2                  | Actinobacteria |
| 3   | AY349363.1 | Actinomyces sp.oral clone IO076      | 99           | A3                  | Actinobacteria |
| 4   | AJ234056.1 | Actinomyces viscosus                 | 98           | A4                  | Actinobacteria |
| 5   | AJ717364.1 | Rothia dentocariosa                 | 100          |                     | Actinobacteria |
| 6   | DQ409140.1 | Rothia mucilaginosa                  | 99           |                     | Actinobacteria |
| 7   | AB271749.1 | Bacillus smithii                     | 99           |                     | Firmicutes    |
| 8   | L14326.1   | Gemella haemolysans                  | 99           |                     | Firmicutes    |
| 9   | AY005051.1 | Gemella sp.oral strainC24KA          | 99           |                     | Firmicutes    |
| 10  | AY879304.1 | Granulicatella adiacens              | 100          |                     | Firmicutes    |
| 11  | EF460495.1 | Lactobacillus gasseri                | 100          |                     | Firmicutes    |
| 12  | AY283269.1 | Lactobacillus iners                  | 99           |                     | Firmicutes    |
| 13  | DQ256277.1 | Lactobacillus reuteri                | 96           |                     | Firmicutes    |
| 14  | AB185767.1 | Lactobacillus vaginalis              | 99           |                     | Firmicutes    |
| 15  | AY281088.1 | Streptococcus gordonii               | 99           |                     | Firmicutes    |
| 16  | AY485603.1 | Streptococcus sinensis               | 99           |                     | Firmicutes    |
| 17  | DQ232531.1 | Streptococcus intermedius            | 100          |                     | Firmicutes    |
| 18  | AY518677.1 | Streptococcus mitis                  | 100          |                     | Firmicutes    |
| 19  | DQ677788.1 | S.mutans strain ChDCYM15             | 100          |                     | Firmicutes    |
| 20  | CP000410.1 | Streptococcus pneumonia              | 97           |                     | Firmicutes    |
| 21  | AY188352.1 | Streptococcus salivarius             | 99           |                     | Firmicutes    |
| 22  | CP000387.1 | Streptococcus sanguinis              | 99           |                     | Firmicutes    |
| 23  | DQ67784.1  | S. mutans                           | 93           |                     | Firmicutes    |
| 24  | AB294730.1 | S. mutans                           | 96           |                     | Firmicutes    |
| 25  | DQ677734.1 | S. mutans                           | 90           |                     | Firmicutes    |
| 26  | AF287782.1 | Veillonella sp.oral cloneAA050       | 99           |                     | Firmicutes    |
| 27  | DQ440557.1 | Fusobacterium nucleatum              | 99           |                     | Fusobacteria  |
| 28  | AY008309.1 | Leptotrichia sp.oral cloneBU064      | 99           |                     | Fusobacteria  |
| 29  | AB291890.1 | Sphingomonas sp.Pd-S-(l)-m-D-3(6)     | 100          |                     | α-Proteobacteria |
| 30  | AJ90755.2  | Haemophilus pittmaniae               | 99           |                     | γ-Proteobacteria |
| 31  | AM411997.1 | Pseudomonas sp                       | 100          |                     | γ-Proteobacteria |
| 32  | EU009183.1 | Shigella dysenteriae                | 100          |                     | γ-Proteobacteria |
| 33  | AY807157.1 | Uncultured Haemophilus sp.clone IOS28B87 | 99   | 1 1 1 1 1 | γ-Proteobacteria |

| Total | 286 52 83 79 72 |
### Table 4. Ten common oral bacteria, their sequences and HOMD code Number

| No | Taxonomy ID | Genus. Species          | NCBI Taxonomy ID | Oral Taxon ID (HOT) | 16S rRNA Alignment                                      |
|----|-------------|-------------------------|-----------------|---------------------|--------------------------------------------------------|
| 1  | 28037       | Streptococcus mitis     | NCTC 12261      | 677                 | GAGTTTGATCCTGGCTCAGGACGAACGCT-GGCGG                    |
|    |             |                         |                 |                     | CGTGCCCT-AATACAT                                      |
| 2  | 1596        | Lactobacillus gasseri   | ATCC 33323      | 615                 | GAGTTTGATCCTGGCTCAGGACGAACGCTGGCGG                    |
|    |             |                         |                 |                     | CGTGCCCTAATACAT                                      |
| 3  | 147802      | Lactobacillus iners     | LMG 18914       | 838                 | GAGTTTGATCCTGGCTCAGGACGAACGCTGGCGG                    |
|    |             |                         |                 |                     | CGTGCCCTAATACAT                                      |
| 4  | 1309        | Streptococcus mutans    | NCTC 10449      | 686                 | GACGAACGGCTGGCCGCGGTGCTAATACATGCAAG                   |
|    |             |                         |                 |                     | TGGGACGCAAGGAAACACACTGTGCTGACACCTG                   |
| 5  | 125704      | Streptococcus mutans    | ATCC 25175      | 686                 | GACGAACGGCTGGCCGCGGTGCTAATACATGCAAG                   |
|    |             |                         |                 |                     | TGGGACGCAAGGAAACACACTGTGCTGACACCTG                   |
| 6  | 1305        | Streptococcus sanguinis | ATCC 10556      | 758                 | GAGTTTGATCCTGGCTCAGGACGAACGCTGGCGG                    |
|    |             |                         |                 |                     | CGTGCCCTAATACAT                                      |
| 7  | 1302        | Streptococcus gordonii  | ATCC 10558      | 622                 | GAGTTTGATCCTGGCTCAGGACGAACGCTGGCGG                    |
|    |             |                         |                 |                     | CGTGCC-TAATACAT                                      |
| 8  | 43675       | Rothia mucilaginosa     | ATCC 25296      | 681                 | GAGTTTGATCTGCTCAGGACGACGCTGGCGG                      |
|    |             |                         |                 |                     | CGTGCTAAACACATGCAAGTCGACGAC                        |
| 9  | 1304        | Streptococcus salivarius| NCTC 8618      | 755                 | GAGTTTGATCCTGGCTCAGGACGACGCTGGCGG                    |
|    |             |                         |                 |                     | CGTGCC-TAATACAT                                      |
| 10 | 46124       | Granulicatella adiacens | ATCC 49175     | 534                 | GAGTTTGATCCTGGCTCAGGACGACGCTGGCGG                    |
|    |             |                         |                 |                     | CGTGCCCT-AATACAT                                      |

*Figure 1. Phylogenetic Tree of Actinobacteria*
acidophilic bacteria. Table 4 shows 10 common oral bacteria along with the sequences used and their code number in HOMD database.

Antibiotic sensitivity of the isolates
The inhibition zone measured using Who net 5.6 software showed mean diameter of inhibition zone obtained in case of erythromycin (15μg) was 22.80 ± 6.00 mm, for vancomycin (30μg) it was 9.0 ± 0.13 while for tetracycline (30ug) the diameter was 22.3 ± 15mm. Based on the diameter of inhibition zone of tested antibiotics, erythromycin and tetracycline were both effective against isolated bacteria but vancomycin did not inhibit their growth.

Effect of Zoocin A on the isolated species
Zoocin A inhibited the growth of biofilm producing bacteria with maximum effect on some Streptococci particularly S. mutans and S. gordonii. MIC of 2.2 and 3.2 μg were effective against S. mutans and S. gordonii.
respectively. Table 5 shows the effect of Zoocin A on the isolated bacteria. Zoocin A at lower concentration than antibiotics inhibits the growth of Streptococcus mutans and S. gordonii.

**DISCUSSION**

After Miller stated that oral biofilm producing bacteria are responsible for dental decay, various researches worked on the identification of oral bacteria isolated from tooth decay, based on their culture and phenotypic characteristics. The advances in bacterial isolation techniques, led researches to the identification of bacteria by molecular procedures. Samples for identification of oral bacteria were collected from dental cavities and biofilms; According to previous researches, biofilm formed on the teeth surfaces attracts more colony forming bacteria. Similar to our previous study, this adopted 16SrRNA as a reliable method for identification of the isolated bacteria and to differentiate between the MS species.

In the present study, identification of the bacteria isolated from oral cavity of the participants was carried out by all available procedures. Comparing the efficacy of the different techniques for bacterial identification, our findings indicate a precise and accurate identification by molecular procedure. Among the diagnostic techniques employed in this study, it is evident that at present, 16SrRNA is the most precise way of bacterial identification. Similar to our findings, Munson et al, Chhour et al, Corby et al., Chandrabhan et al and Aas et al concluded from their studies that S. mutans and Lactobacillus spp. are dominant in caries formation. According to them a diversity of bacterial species, primarily gram-positive species, are involved in caries formation and bacterial profile changes with the progression of disease. They believe that caries is the result of a shift in the balance of the resident microflora driven by changes in local environmental conditions like acidic conditions produced by species that live in decayed tooth which supports the “ecological plaque hypothesis”. Loesche also believes S.mutans was involved with the initiation of decay, whereas the lactobacilli were associated with the progression of the lesion. We noticed that some of the isolated bacteria were common with bacteria reported by other researchers in different parts of the world while some species were different indicating that not only bacterial population differs in different stages of tooth decay but also it is influenced by different epidemiological factors including race or geographical distribution of the population.

Amongst those who had their own permanent teeth, decayed teeth per person in the elderly was 1.2 which was comparatively but non-significantly less than the average number of decayed teeth per person in the elderly in Thai population reported by Supaporn et al. In contrast to previous study that reported all species they isolated were susceptible to the tested antibiotics, we isolated resistant species as well. In the present study we came across vancomycin resistant Streptococci which is rarely reported.

The present controversy leads to the conclusion that antibiotic susceptibility is not a constant and fixed bacterial specificity and inspection of antibiotic susceptibility is required for prophylaxis and treatment. In Hmedan, S.mitis, and S. mutans are dominant species in decayed teeth of the cases referring to governmental clinics and isolated bacteria are sensitive to tetracycline and erythromycin but resistant to vancomycin.

This study also proved that zoocin A like antibiotics has antibacterial activity with maximum inhibitory effect on S. mutans and S. gordonii. Based on the findings of the present study, tooth decay starts with colonization of tooth with Mutans streptococci which are affected by zoocin A. Zoocin A by inhibiting the growth of Mutans streptococci which are the initiating agents in tooth decay can stop tooth decay even at lower concentrations compared to antibiotics. Further clinical researches may lead to extrapolation of our findings; protection and prevention of dental decay by zoocin A rather than antibiotics will be feasible in near future. The findings of this study are in accordance with Lester and Simmond’s findings. Akesson et al have the same opinion and believe much of the potential for use of such agents as enzybiotics stems from their ability to target related bacteria.

**CONCLUSION**

At present, MS bacteria are known as etiological agents of dental caries of which S. mutans is the leading cause of tooth decay and the most cariogenic streptococcus. By eliminating the established MS populations from the oral cavity, the chance of caries development reduces. Many new antibacterial substances have been found to replace the old antibiotics; however, finding and identifying new antimicrobial substances is a difficult task. Zoocin A is a bacteriocin which is able to inhibit the growth of S. mutans and S. gordonii, the main tooth decaying agents. Thus, it may be a potential

| Bacteria   | Bacterial count before Zoocin A application | Bacterial count 24 hrs after Zoocin A application |
|------------|--------------------------------------------|--------------------------------------------------|
| S. mitis   | 1.5x10⁶                                    | 4.1x10⁷                                          |
| S. sanguis | 1.3x10⁶                                    | 1.1x10⁶                                          |
| S. mutans  | 2.7x10⁷                                    | 1.0x10⁶                                          |
| L. gasseri | 2.8x10⁴                                    | 1.8x10⁴                                          |

**Table 5. Effect of Zoocin A on the number of bacteria grown on culture media**
drug candidate for replacing antibiotics in order to substitute antibiotics and possibly eliminate the chance of multiple drug resistance in the future.

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CONFLICT OF INTEREST

Authors declare no conflict of interests.

REFERENCES

1. Loesche WJ. Microbiology of Dental Decay and Periodontal Disease: General Concepts. In: Medical Microbiology. 4th ed. 1996.
2. He XS, Shi WY. Oral microbiology: past, present and future. Int J Oral Sci. 2009; 1:47-58.
3. Pereira-Cenci T, Cenci MS, Fedorowicz Z, Azavedo M. Antibacterial agents in composite restorations for the prevention of dental caries. Cochrane Database Syst Rev. 2013,CD007819.
4. Fani MM, Kohanteb J, Dayaghi M. Inhibitory activity of garlic (Allium sativum) extract on multidrug-resistant Streptococcus mutans. J Indian Soc Pedod Prev Dent. 2007;25:164-8.
5. Makarova K, Slesarev A, Wolf Y, Sorokin A, Mirkin B, Koonin E, et al. Comparative genomics of the lactic acid bacteria. Proc Natl Acad Sci U S A. 2006;103:15611-6.
6. Dinesh MD, Shaheena A, Abdul Bari KK, Neethu A. 2006;103:15611-6.
7. Chen Y. Solution structure of the target recognition domain of zoocin A: An antibacterial enzyme and the metal binding site of zoocin A. [Dissertation]. 2009. University of Alabama. Tuscaloosa. Available from: http://acumen.lib.ua.edu/content/u0015/0000001/0000200/ u0015_0000001_0000200.pdf
8. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. P T. 2015 Apr;40(4):277-83.
9. Yang SC, Lin CH, Sung CT, Fang JY. Antibacterial activities of bacteriaicins: application in foods and pharmaceuticals. Front Microbiol. 2014; 5:241.
10. Kuramitsu HK, Xuesong He, Renate Lux, Maxwell H. Anderson, Wenyuan Shi. Interspecies interactions within oral microbial communities. Microbiol Mol Biol Rev. 2007; 71(4): 653–70.
11. ICD-10. International Statistical Classification of Diseases and Related Health Problems. 10th Revision. 2010. [Internet]. Available from: http://www.who.int/classifications/icd/en/
12. Jain K, Sheetal P, Neelam M, Hirak RD, Das S. Isolation and characterization of biofilm-forming bacteria and associated extracellular polymeric substances from oral cavity. Ann Microbiol. 2013;63:1553–62.
13. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clin Infect Dis. 2009;49:1749-55.
14. Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen J, Dewhirst FE, et al. Bacteria of dental caries in primary and permanent teeth in children and young adults. J Clin Microbiol. 2008;46:1407-17.
15. Salman HA, Senthikumar R. Identification and antibiogram profile of Streptococcus mutans and Streptococcus sobrinus from dental caries subjects. J Appl Pharmaceut Sci. 2015;5:054-7.
16. Claire J, Ling CL, Ciesielezuk HL, Lockwood J, Hopkins S, et al. Detection and identification of bacteria in clinical samples by 16SrRNA gene sequencing: comparison of two different approaches in clinical practice. J Med Microbiol. 2012; 61: 483–8.
17. Chandrabhan D, Rajmani H, Bhatt R, Verma P. Isolation of dental caries bacteria from dental plaque and effect of tooth pastes on acidogenic bacteria. Open J Med. Microbiol. 2012;2:65-9.
18. Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. J Clin Microbiol. 2007;45:2761–4.
19. Srinivasan R, Karaoz U, Volegova M, MacKichan J, Kato-Maeda M, Miller S, Nadarajan R, Brodie EL, Lynch SV. Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. PLoS One. 2013;10:e0117617.
20. Munson MA, Banerjee A, Watson TF, Wade WG. Molecular analysis of the microflora associated with dental caries. J Clin Microbiol. 2004;42:3023-9.
21. Chhour KL, Nadkarni MA, Byun R, Martin FE, Jacques NA, Hunter N. Molecular analysis of microbial diversity in advanced caries. J Clin Microbiol. 2005;43:843-9.
22. Corby PM, Lyons-Weiler J, Bretz WA, Hart TC, Aas JA, Boumenna T, et al. Microbial risk indicators in early childhood caries. J. Clin. Microbiol. 2005;43:5753-9.
23. Argimon S, Caufield PW. Distribution of putative virulence genes in Streptococcus mutans strains does not correlate with caries experience. J Clin Microbiol. 2011. Mar;49(3):984-92.
24. Sanguan S, Wongsriya K, Ratanadheeradhorn S, Channetkit A. The oral health status and hygiene of the dependent elderly in Muang, Phitsanulok, Thailand. J Dent Indones. 2016;23:64-8.
25. Park C, Nichols M, Schrag SJ. Two cases of invasive vancomycin-resistant group B streptococcus infection. N Engl J Med. 2014;370:885-6.

26. Bagga B, Shenep JL. Management of infections caused by vancomycin-resistant gram-positive bacteria. Pediatr Infect Dis J. 2010;29:662-4.

27. Lester K, Simmonds RS. Zoocin A and lauricidin in combination reduce Streptococcus mutans growth in a multispecies biofilm. Caries Res. 2012;46:185-93.

28. Akesson M, Dufour M, Sloan GL, Simmonds RS. Targeting of streptococci by zoocin A. FEMS Microbiol Lett. 2007;270:155-61.

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