Journal of Advanced Zoology
ISSN: 0253-7214
Volume 45 Issue 2 Year 2024 Page 986-993

Minimum Inhibitory Concentration Of Different Effective Antibiotics Against Oral Staphylococcus Aureus Isolated From Diabetic Patients

Niloufarkeshtehgar[1], Faraz Mokhtari[2], Haleh Zokaee[3]*, Tanin Nourollahian[4]

[1,2]Dentist, Golestan University of Medical Sciences, Gorgan, Iran.
*[3]*Oral Medicine Dept, Dental Research Center, Golestan University of Medical Sciences, Gorgan, Iran.*
[4]Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran.

***Corresponding Author:** Haleh Zokaee,*
**Oral Medicine Dept, Dental Research Center, Golestan University of Medical Sciences, Gorgan, Iran.*
Email: Haleh.zokaee@gmail.com.

Abstract

Bacterial sialadenitis is common in diabetics, and Staphylococcus aureus (SA) is the causative agent. Prescription of empiric antibiotics to eliminate bacteria is the best treatment in these cases. The purpose of this study was to investigate the effect of Minimum Inhtionibitory Concentration (MIC) of second, third, and fourth generation of cephalosporins as well as oxacillin against SA.

Methods: In this study, 247 samples were collected from the parotid duct opening from type II diabetes patients. The samples were transferred to the laboratory in BHIB medium and were subjected to culture and biochemical diagnostic tests to isolate and detect SAMIC of Cefuroxime, Ceftriaxone, cefepime, cloxacillin, oxacillin against SA determined by broth microdilution method. The results were analyzed by SPSS18.

Results: Among 247 type 2 diabetic patients, Staphylococcus aureus was isolated from 61 patients (39 women, 22 men). 51, 46, 52 and 60 people were sensitive to 2nd, 3rd, 4th generation cephalosporin antibiotics and oxacillin, respectively. Of these, the lowest to the highest average MIC values were related to oxacillin, cefuroxime, cefepime and ceftriaxone. The average MIC in three antibiotics, oxacillin, methicillin, and cloxacillin, respectively was 0.71±0.69, 6.24±4.04, 40.08± 33.23. The type of antibiotic used had a significant relationship with resistance and sensitivity. Sensitivity to oxacillin, methicillin and cloxacillin was observed in in 98.4%, 85.2% and 75.4% of patients.

Conclusions: Oxacillin showed significantly the lowest MIC compared to methicillin and cloxacillin. Therefore, it can be a recommended antibiotic for infections caused by staph aureus to be administered to diabetics. Based on our results, oxacillin can be a suitable treatment option in diabetic patients with bacterial sialadenitis. If oxacillin is not available, second generation cephalosporin (cefuroxime) can be used against SA.

Introduction

Diabetes mellitus (DM) is a common chronic disease that is defined by defective carbohydrate metabolism due to relative or absolute lack of insulin and hyperglycemia (1).Diabetes is a disorder characterized by hyperglycemia, which can be associated with various types of disorders such as retinopathy, ocular diseases, nephrosis, renal diseases and neuralgia (pain in the distribution of nerves)(2). Diabetes is classified into two types: type I and type II.D Mis associated with fatal tissue damage and weakness of various body functions, which increases microbial population in oral cavity and causes oral cavity diseases such as tooth decay, periodontitis, gingivitis, dry mouth and sialadenitis(3, 4). Acute purulent sialadenitis can affect both children and adults, and the parotid gland is mainly affected by this disease(5). Sialadenosis is frequently related with diseases such as diabetes mellitus, alcohol abuse and malnutrition (6). Sialadenosis is an asymptomatic bilateral enlargement of the parotid gland, which is also observed in diabetics (4, 7). In fact, sialadenitis is a general term that includes acute, chronic, or recurrent infection and/or inflammatory conditions involving the salivary glands (8). Acute sialadenitis usually occurs as a result of secondary viral and bacterial infections, whereas chronic and recurrent sialadenitis occurs in the setting of salivary duct obstruction, mainly due to stone obstruction and inflammatory ductal stenosis (9). Medical management of sialadenitis involves supportive care and antibiotics, while gland-sparing surgical intervention by sialendoscopy may enable stone extraction and ductal opening in appropriately selected patients (10). The microbial flora of human oral cavity is diversified and consists of different types of microorganisms.

The oral cavity is infected by *SA* bacteria as well as other anaerobic Gram-negative species(11), and most oral infections in diabetic patients are associated with a high number of microorganisms (12). Decreased salivary flow allows bacteria, which is the main factor in this condition, to enter into the gland (12). *SA* is the most frequent bacterium cultured in these infections (13, 14). *SA* is a gram-positive, facultative anaerobic, non-motile bacterium lacking spores, which is catalase, nitrite and sometimes coagulase positive and shows large, round and golden-yellow colonies on blood agar medium often accompanied by hemolysis (15).

The best treatment for acute bacterial sialadenitis is the use of antibiotics that can defend against *SA*(16). Resistance to antibiotics has turned into a global challenge, and SA has such a this ability, too.(17). Beta-lactam antibiotics are the first line of treatment for *SA* infections (18). Considering the prevalence of diabetes, antibiotics such as beta-lactamase-resistant penicillins and cephalosporinshas been suggested to treat sialadenitis in diabetics; however comparison of antibiotic effect against SA from cephalosporin family and beta-lactamase-resistant penicillins (with the least complications and maximum effect has not been attempted in a study so far (19). Therefore, the aim of this study was to investigate the minimum inhibitory concentration (MIC) of three generations of cephalosporins, Oxacillin, Methicillin and Cloxacillin against *SA* isolated from oral cavity of patients with type II diabetes.

Materials and Methods

Study Population

This study was conducted on 247 patients with controlled and uncontrolled DM referred to Deziani Medical Education Center of Golestan University of Medical Sciences (GOUMS) in 2019. Demographic information of patients was collected by a checklist, including age, gender, medical history, patient complaint, medication, last recorded hemoglobin A1c(HbA1c) and fasting blood sugar (FBS) values, and the use of mouthwash. Ethics approval and consent to participate was confirmed by GOUMS Ethical Committee by the codeIR.GS.REC.1398.347 an OUMd IR.GOUMS.REC1399.096.

Inclusion criteria of this study were as follows:

1) Presentation of informed consent by patients over 40 years of age
2) Type II diabetes confirmed by a physician and laboratory tests (FBS≥126 mg/dL)
3) No use of antibiotics over the past six months and not using mouthwash three weeks before the start of the study
4) No gingival surgery or teeth scaling.

Exclusion criteria of this study was as follows:

1)Failure of SA isolation from patient sample

Sampling Method

The patients were asked to sit on the unit. Samples were taken from the opening of parotid duct on the right and left side of patients using Paper Point No. 30 **(Figure 1).**Afterward, the samples were placed in Brain Heart Infusion (BHI) Broth culture medium and transferred to the laboratory.

Figure 1. Sampling by Paper Point No. 30 from the opening of parotid duct.

Bacteriological tests

After transferring the samples to the laboratory, samples were kept in a 37-degree incubator for 12-18 hours, and then samples were cultured in 4 regionsfrom the BHIB medium with a loop in the blood agar medium containing 7.5% salt, to isolate and better growth of staphs. From the colonies grown in blood agar, 4 areas were cultured in mannitol salt agar medium to isolate mannitol-positive colonies (colonies that change the color of the culture medium from pink to yellow). We first stained these colonies and heated them to isolate Gram-positive cocci, then we performed the catalase test on these colonies to isolate micrococci and confirmed with Oxidative/fermentation glucose test (OF). We separated from micrococci. To diagnose Staphylococcus aureus, we performed coagulase **(figure2)** and DNase tests, and when these two tests were positive, we identified Staphylococcus aureus The antibiotics used included second, third, and fourth generation Cephalosporins, as described below: second generation: Cefuroxime / third generation: Ceftriaxone / fourth generation: Cefepime.

It should be noted that a sterile swab was used to inoculate the test organism on the Mueller Hinton Broth culture medium(20). In this study, for the mentioned antibiotics, the antibiotic sensitivity test was performed using the Minimum Inhibitory Concentration (MIC) method in the form of serial dilutions (Serial Dilution Method) according to CLSI standards **(figure 3)**. To perform the MIC test of the evaluated antibiotics, we prepared the required concentrations of 612 to 0.12 μg/ml, and for bacteria, the concentration of 1.5×10^5, and in less than 20 minutes, we mixed the bacterial suspension and different concentrations of antibiotics in the wells of the 96-well plate(21).

Then we read the light absorbance of all the wells at 570 nm wavelength and then put them at 37 degrees for 18 to 24 hours and read their light absorbance again. In the negative control plate, the light absorbance should be less than 0.100 ,and in the positive control plate, the absorbance should be more than 0.250. To determine the MIC, we found the first well whose optical absorbance was more than 0.250 and considered the previous well as MIC**.** Schematic figures of has been shown in figures 4&5.

In this research, oxacillin was used as standard.

Figure 2. Blood agar culture for SA

Figure 3. Coagulase test for differentiating staphylococcus from other micrococci

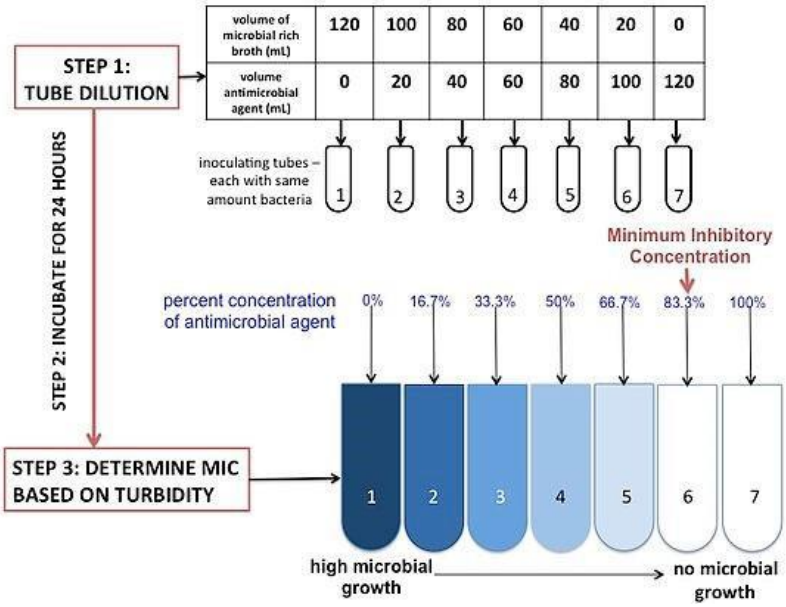

Figure4. Schematic figure of serial dilution technique

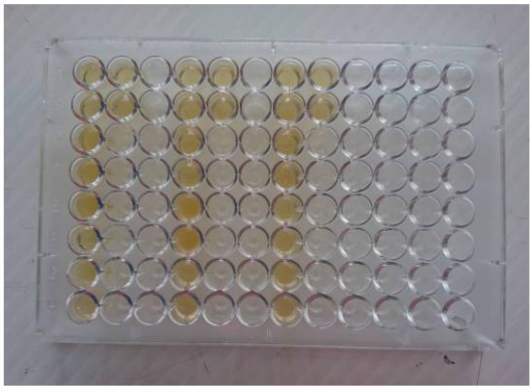

Figure5. Serial dilution technique

Statistical Analysis

The data obtained from the study was entered into SPSS software (version 18).Chi-square test was subsequently used to compare the sensitivity between the groups, and $P<0.05$ was considered the significance level.

Results and Discussion

SA was isolated from 61 (24.69%, 22 men and 39 women) out of 247 patients who were in the age range of 40-70 years. The average age of type 2 diabetes patients with Staphylococcus aureus participating in the study was 50.98 ± 7.71 years. In this study, the highest level of sensitivity to Oxacillin was observed (60

patients, 98.4%), and there was only 1.6% resistance (1 case) to this antibiotic among bacteria isolated from patients with diabetes. In addition, there was 15 cases (24.6%) of resistance to Cloxacillin, 75.4% sensitivity to Cloxacillin (46 cases), 14.8% resistance to Methicillin (9 cases) and 85.2% sensitivity to Methicillin (52 cases). The results of this study also showed that the type of antibiotic (Cloxacillin, Oxacillin, Methicillin) is related to resistance and sensitivity in patients (P=0.001).

Regarding cephalosporin family antibiotics, there was 16.4% resistance to Cefuroxime (10 cases) and 83.6% sensitivity to this antibiotic (51 cases), 24.6% resistance to Ceftriaxone (15 cases) and 75.4% sensitivity to this antibiotic (46 cases), 14.8% sensitivity to Cefepime (9 cases) and 85.2% sensitivity to this antibiotic (52 cases) among the isolated bacteria. However, there was no significant relationship between these three types of antibiotics with resistance and sensitivity in patients (P>0.05). Besides, in pairwise comparison of Cefuroxime, Ceftriaxone, and Cefepime antibiotics with Oxacillin, the latter had a greater effect on bacteria (P<0.05).

In this study, no significant correlation was found between sensitivity with age, sex, duration of diabetes, type of treatment (drug, insulin, drug/insulin) and also with control of the disease (HbA1c). In this study, Oxacillin had a better performance compared to other antibiotics and had the lowest mean MIC value (P<0.05). Considering 50% and 90% sensitivity of patients to antibiotics (Oxacillin, Cloxacillin, Methicillin, Cefuroxime, Ceftriaxone, Cefepime), mean MIC valuesare shown in **Table 1**.

Table 1. Sensitivity percent, MIC50 and MIC90 of *SA*isolated from Patients diabetes (n = 61)

Antimicrobial		**Standard deviation ± meanor sensitivity breakpoint**	**Cut-off or Range test**	**N= 61**
Oxacillin	MIC $_{50}$	0.7 ± 0.57	0.25-2	**30**
	MIC $_{90}$	0.68 ± 0.57		**54**
Cloxacillin	MIC $_{50}$	23.3 ± 8.66	8-32	**23**
	MIC $_{90}$	23.41 ± 8.65		**41**
Methicillin	MIC $_{50}$	2.07 ± 1.16	1-4	**26**
	MIC $_{90}$	1.93 ± 1.05		**47**
Cefuroxime	MIC $_{50}$	2.96 ± 1.07	1-4	**26**
	MIC $_{90}$	2.97 ± 1/1		**46**
Ceftriaxone	MIC $_{50}$	6.26 ± 2.02	4-8	**23**
	MIC $_{90}$	6.09 ± 2.09		**41**
Cefepime	MIC $_{50}$	3.98 ± 2.8	0.5-8	**26**
	MIC $_{90}$	3.81 ± 2.4		**47**

Diabetes is associated with infections such as gingivitis, candidiasis, tooth decay and sialadenitis. The widespread incidence of these infections in diabetics leads to poor oral and dental hygiene (22). Prevention and control of periodontal disease should be considered an integral part of diabetes control (23). Human oral cavity where many bacterial species form biofilm structures is the most common and active habitat for these bacteria. Several species of *SA ,Streptococcus*, *Corynebacterium*, *Enterococcus* and other bacteria are found in the human oral cavity (22).

Sialadenitis is common in diseases such as diabetes, and due to antibiotic resistance of diabetics, the choice of primary (empiric) antibiotic is of high importance to limit the infection because diabetic patients have a greater tendency to disseminated infection due to compromised immune system (10).

Treatment of infections caused by *SA* has been complicated because of resistance to Methicillin(24). Penicillin and its derivatives are always considered in the treatment of *SA* infections, and finally, beta-lactamase-resistant cephalosporins and penicillins are suggested in lack of coverage due to the presence of Methicillin-resistant SA(25). Therefore, the aim of our study was to determine MIC of a number of antibiotics (Oxacillin, Cloxacillin, Methicillin, Cefuroxime, Ceftriaxone, Cefepime) against *SA* isolated from diabetics. Out of 5005 oral swab samples, A. J. Smith et al. in Scotland (2003) isolated *SA*bacteria from 1017 samples (26). In Pakistan(2020), Sumaira and colleagues isolated *SA* in 37 cases (74%) out of 50 diabetic patients from among 100 oral swab samples (50 diabetics and 50 healthy subjects)(27), In Iraq, Al-Abdul et al.(2017)managed to isolate 13 cases (29.5%) of *SA*bacteria from 21 diabetic patients by sampling gingival crevicular fluid (GCF)(28), which was consistent with our study. Abbaset al.(2011) isolated 15.5% *SA* from 200 oral samples from type II diabetic patients in Iraq (29). In 2019, Al-Farhan et al. in Iraq were able to isolate 55.5% *SA*bacteria in 20 diabetic samples among 73 periodontitis samples (30). In our study, among 247 samples collected from diabetics, 61 cases (24.69%) of *SA* were isolated. We can point to things such as

difference in sampling (our sampling was from parotid gland), individual oral hygiene of diabetics, patients' lifestyle and immune system status to account for the difference in percentage of these studies with ours. According to the results of this study and given the MIC value for Oxacillin, Cloxacillin, and Methicillin, most of the isolated strains showed sensitivity to these three antibiotics; however, Oxacillin (with a sensitivity of 0.71±0.69 and a cut-off of 0.25-4) showed sensitivity in a lower medical dose than the other two antibiotics. Moreover, in relation to the state of resistance and sensitivity to the antibiotic, the highest sensitivity (21.4%), and the lowest resistance (1.6%) was observed for Oxacillin(only one patient resistant to Oxacillin) compared to other two antibiotics.

According to the findings of this research, 14.8% of SA species in type II diabetes patients were resistant to Methicillin (MRSA). In 1995, Teixeira et al. stated that from 152 samples taken from patients in hospitals of Brazil, 85 MRSA strains were identified, all of which showed resistance to Oxacillin, penicillin, and gentamicin (31). Bueris and colleagues isolated 25.9% MRSA strains in 48 saliva samples from Brazil in 2005 (32). In 2012, Batabyal et al. identified 109 cases (48.9%) of *SA* in 223 samples of patients from India with oral problems, 5.5% of which were MRSA strains (33). In the study of Godebo and colleagues in India (2013), among 322 wound samples, 76.7% of the isolated *SA* samples were resistant to Methicillin and Oxacillin(34). In the study of A. J. Smith et al., 95% of the strains were sensitive to Methicillin and 5% were resistant to it (26). In our study, among 247 samples, resistance to Methicillin was seen in 9 samples (14.8%) from diabetic patients. Furthermore, in our research, among Cefuroxime, Ceftriaxone and Cefepime, the lowest mean MIC was related to Cefuroxime (second-generation cephalosporin) and the highest to Ceftriaxone; therefore, Cefuroxime had a better performance against *SA* strains among these three antibiotics. However, in a pairwise comparison of Cefuroxime, Ceftriaxone, and Cefepime antibiotics with Oxacillin, the results showed that Oxacillin had a better performance against *SA*. In his study, Tartaglione mentioned the effect of Cefuroxime against *SA* strains (35), and Klein NC also stated weaker effect of Ceftriaxone on Gram-positive bacteria (36). These cases can confirm our findings regarding Cefuroxime and Ceftriaxone. In the present study, no significant relationship was found between the age and sex of diabetics with resistance or sensitivity to antibiotics (Oxacillin, Cloxacillin, Methicillin, Cefuroxime, Ceftriaxone, Cefepime). In their study, Batabyal and colleagues also did not observe a significant correlation between age and gender with Methicillin resistance or sensitivity (33). In 2016, Lee et al. in their study on the effect of age and gender on antibiotic resistance in Korean patients with febrile urinary tract infections showed that age had no effect on resistance to antibiotics, but gender was effective on sensitivity to cefotaxime and cefoxitin (37), which was similar to our study. The resistance of bacteria, especially *SA*, to antibiotics is increasing due to frequent and unreasonable use of antibiotics; therefore, it is important to choose an antibiotic with a suitable spectrum of effect in diabetics with oral and dental problems.

Conclusion

Diabetics are prone to bacterial infections to a higher degree due to a weak immune system, and sialadenitis is also common in these patients. *SA*is a causative agent of sialadenitis, and antibiotic resistance is increasing to this bacterium. According to the results of our study, Oxacillin has a better performance against *SA*than the second, third, and fourth generation cephalosporins, and it can be a suitable treatment option for the treatment of sialadenitis. Cefuroxime can be used as a proper treatment option in case of unavailability of Oxacillin and resistance to first-generation cephalosporin.

Acknowledgments: The authors are grateful for the scientific and financial support of Dental Research Center, Golestan University of Medical Sciences, Gorgan, Iran.
Conflict of interest: None
Financial support: None
Ethics statement: None

References

1. Kumar V, Cotran RS, Robbins Sl. Basic Pathology. 7th ed. New Delhi, India: Elsevier Publication; 2004. pp. 33–34.
2. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2010;33(suppl 1):S62-S69. doi:10.2337/dc10-S062.

3. Harris MI, Eastman RC. Early detection of undiagnosed diabetes mellitus: a US perspective. Diabetes Metab Res Rev. 2000 Jul-Aug;16(4):230-6. doi: 10.1002/1520-7560(2000)9999:9999<::aid-dmrr122>3.0.co;2-w. PMID: 10934451.
4. Russotto SB. Asymptomatic parotid gland enlargement in diabetes mellitus. Oral Surg Oral Med Oral Pathol. 1981 Dec;52(6):594-8. doi: 10.1016/0030-4220(81)90075-x. PMID: 6947184.
5. Sheppard DC, Chambers HF. Suppurative parotitis. West J Med. 1998 Aug;169(2):116-7. PMID: 9735697; PMCID: PMC1305186.
6. Henry-Stanley MJ, Beneke J, Bardales RH, Stanley MW. Fine-needle aspiration of normal tissue from enlarged salivary glands: sialosis or missed target? Diagn Cytopathol. 1995 Nov;13(4):300-3. doi: 10.1002/dc.2840130405. PMID: 8599912.
7. Tüzün E, Hatemi AC, Memişoğlu K. Possible role of gangliosides in salivary gland complications of diabetes. Med Hypotheses. 2000 Jun;54(6):910-2. doi: 10.1054/mehy.1999.0978. PMID: 10867739.
8. KRIPPAEHNE WW, HUNT TK, DUNPHY JE. Acute suppurative parotitis: a study of 161 cases. Ann Surg. 1962 Aug;156(2):251-7. doi: 10.1097/00000658-196208000-00010. PMID: 14459677; PMCID: PMC1466328.
9. Delcea C, Rad D, Gyorgy M, Runcan R, Breaz A, Gavrilă-Ardelean M, Bululoi AS. A Network Analysis Approach to Romanian Resilience-Coping Mechanisms in the Covid-19 Era. Pharmacophore. 2023 Aug 28;14(4):57-63.
10. Trujillo O, Rahmati RW. Acute and chronic salivary infection.En: Gland- Preserving Salivary Surgery: A Problem-BasedApproach. Springer International Publishing; 2018. pp. 109-18.
11. Boggess KA, Edelstein BL. Oral health in women during preconception and pregnancy: implications for birth outcomes and infant oral health. Matern Child Health J. 2006 Sep;10(5 Suppl):S169-74. doi: 10.1007/s10995-006-0095-x. PMID: 16816998; PMCID: PMC1592159.
12. Negrato CA, Tarzia O. Buccal alterations in diabetes mellitus. Diabetol Metab Syndr. 2010 Jan 15;2:3. doi: 10.1186/1758-5996-2-3. PMID: 20180965; PMCID: PMC2843640.
13. Durand, M.L.; Deschler, D.G. Infections of the Ears, Nose, Throat, and Sinuses; Springer: Berlin/Heidelberg, Germany, 2018; pp.1–393.
14. Wilson KF, Meier JD, Ward PD. Salivary gland disorders. Am Fam Physician. 2014 Jun 1;89(11):882-8. PMID: 25077394.
15. Gulzar M, Zehra A. SA: A brief review. Int J Vet Sci Res. 2018;4(1):020-2.
16. Greenberg M, Glick M. Ship JA Burket's Oral Medicine11th Edition. Hamilton, Ontario, BC Decker Inc. 2008.
17. Diekema DJ, BootsMiller BJ, Vaughn TE, Woolson RF, Yankey JW, Ernst EJ, Flach SD, Ward MM, Franciscus CL, Pfaller MA, Doebbeling BN. Antimicrobial resistance trends and outbreak frequency in United States hospitals. Clin Infect Dis. 2004 Jan 1;38(1):78-85. doi: 10.1086/380457. Epub 2003 Dec 8. PMID: 14679451.
18. Centers for Disease Control and Prevention (CDC). Staphylococcus aureus resistant to vancomycin--United States, 2002. MMWR. Morbidity and mortality weekly report. 2002 Jul 5;51(26):565-7.
19. Troeltzsch M, Pache C, Probst FA, Troeltzsch M, Ehrenfeld M, Otto S. Antibiotic concentrations in saliva: a systematic review of the literature, with clinical implications for the treatment of sialadenitis. J Oral Maxillofac Surg. 2014;72(1):67-75.
20. Tille P. 2015. Bailey & Scott's diagnostic microbiology, 13th ed. Mosby Elsevier, St. Louis, MO.
21. Clinical Laboratory Standards Institute. Reference Method for Broth Dilution Antifungal Susceptibility Testing ofFilamentous Fungi; Approved Standard, 2nd ed.; CLSI document M38-A2; Clinical and Laboratory StandardsInstitute: Wayne, PA, USA, 2008.
22. Rogers AH. Molecular Oral Microbiology. Caister.Academic Press 2008;65-108.
23. Matthews DC. The relationship between diabetes and periodontal disease. J Can Dent Assoc. 2002 Mar;68(3):161-4. PMID: 11911811.
24. Velasco D, del Mar Tomas M, Cartelle M, Beceiro A, Perez A, Molina F, Bou G (2005) Evaluation of different methods for detecting methicillin (oxacillin) resistance in Staphylococcus aureus. J Antimicrob Chemother 55:379–382
25. Kali A, Stephen S, Umadevi S, Kumar S, Joseph NM, Srirangaraj S. Changing Trends in Resistance Pattern of Methicillin Resistant Staphylococcus aureus. J Clin Diagn Res. 2013 Sep;7(9):1979-82. doi: 10.7860/JCDR/2013/6142.3377. Epub 2013 Sep 10. PMID: 24179914; PMCID: PMC3809653.
26. Smith AJ, Robertson D, Tang MK, Jackson MS, MacKenzie D, Bagg J. Staphylococcus aureus in the oral cavity: a three-year retrospective analysis of clinical laboratory data. Br Dent J. 2003 Dec 20;195(12):701-3; discussion 694. doi: 10.1038/sj.bdj.4810832. PMID: 14718964.

27. Awan S. Isolation and PCR Based Identification of SAfrom Oral Cavity of Diabetic Patients in Quetta City. Pak-Euro J. Med. Life Sci. 2020;3(4):156-67.doi: 10.31580/pjmls.v2i4.1712.
28. Al-Abdul AA, Hussein IK. Isolation and Identification of Bacteria from Diabetic and Non-Diabetic Patients With Periodontitis. Donish J of Microbiol And Biotech Res. 2017;4(2):4-9.
29. Abass VT, Omer SA. Oral findings and microflora in type II diabetes mellitus in Sulaimani city. Journal of Sulaimani Medical College. 2011;1(1):13-28.
30. Al-Farhan S.R, AL-Abdullah A.A. and Al-Moussawi A.A. Isolation and Diagnosis of Anaerobic bacteria of Periodontitis byMolecular Methods in Diabetic and Non-Diabetic Patients in Basra Province/Iraq. Sci. J. Med. Res. 2019; 3 (10): 53-63.
31. Teixeira LA, Resende CA, Ormonde LR, Rosenbaum R, Figueiredo AM, de Lencastre H, Tomasz A. Geographic spread of epidemic multiresistant Staphylococcus aureus clone in Brazil. J Clin Microbiol. 1995 Sep;33(9):2400-4. doi: 10.1128/jcm.33.9.2400-2404.1995. PMID: 7494036; PMCID: PMC228423.
32. Bueris V, Pimenta FC, Ito IY, Marin JM. Oral incidence of SAand antimicrobials agents resistance. Braz J Oral Sci. 2005 Jan;4(12):676-9.
33. Batabyal B, Biswas S, Mandal B, Desai PD, D Sarkan N. Oral suffering and antimicrobial susceptibility of SAin a dental hospital in Kolkata, India. Int J Pharm Bio Sci. 2012;3(4):620-9.
34. Godebo, G., Kibru, G. & Tassew, H. Multidrug-resistant bacterial isolates in infected wounds at Jimma University Specialized Hospital, Ethiopia. Ann Clin Microbiol Antimicrob 12, 17 (2013). https://doi.org/10.1186/1476-0711-12-17
35. Tartaglione TA, Polk RE. Review of the new second-generation cephalosporins: cefonicid, ceforanide, and cefuroxime. Drug Intell Clin Pharm. 1985 Mar;19(3):188-98. doi: 10.1177/106002808501900304. PMID: 3884304.
36. Klein NC, Cunha BA. Third-generation cephalosporins. Med Clin North Am. 1995 Jul;79(4):705-19. doi: 10.1016/s0025-7125(16)30034-7. PMID: 7791418.
37. Lee DS, Choe HS, Kim HY, Yoo JM, Bae WJ, Cho YH, Kim SW, Han CH, Bae SR, Jang H, Park SB, Yoon BI, Lee SJ. Role of age and sex in determining antibiotic resistance in febrile urinary tract infections. Int J Infect Dis. 2016 Oct;51:89-96. doi: 10.1016/j.ijid.2016.08.015. Epub 2016 Aug 26. PMID: 27575938.