

Microbiological Studies on Bacteria Associated with Several Oral Disease And Dental Caries

Muna Jalal Ali BSc.,MSc. ⁽¹⁾

Key words

Dental arch perimeter, dental arch dimensions, malocclusion, and Kurdish sample

Abstract

A total of (80) swabs were collected from mouth of patients suffering from dental and oral disease of both sexes and their ages ranged from 1-60 years which are (referred) to consult the dental clinics and specialized centers of dental in AL-Hawija city from 2009.12.1 to 2010.4.30, In order to isolate and identify the causative agents that associated with oral disease and its antibiotics sensitivity against some antibiotics in addition to study the some factors that affected and aided the occurrence and prevalence of these agents in mouth. Gram positive bacteria were a high number especially Streptococci – viridans which get of (4) species, from it the more important it's the Streptococcus mutans (20) isolates and in rate 25%, then S. sanguis (14) isolates 17.5%, S. mitis (13 isolates) 16.3% and S. salivarius (12 isolates) 15%. For the Staphylococci were isolated Staphylococcus epidermidis (8 isolates) rate 10%, for the Lactobacillus, two species were isolated (6 isolates) in rate 7.5% for L. acidophilus, the other species L. casei (5 isolates) 6.2%, the most other Gram positive bacteria were rare in isolation and at different (2 isolates) rates 2.5% Actinomyces viscosus. The ability of some bacteria to tolerate different salt concentrations & PH level. It was found that Staph. epidermidis was capable of toleration of the highest salt concentrations (10%) while L. acidophilus was sensitive for all of the utilized concentrations, but it showed its high capability to grow on acidic medium (PH=4) more than other bacteria. The antibiotic sensitivity test for isolates against (9) antibiotics showed that the Cefotaxim was the best antibiotics and have higher effect on bacterial isolates, then Ciprofloxacin and Amikacin. While the antibiotics Trimethoprim, Ampicillin and Amoxicillin were less effect on these isolates.

Introduction

Tooth decay and Periodontal Disease around teeth are considered the most common chronic diseases in the world ⁽¹⁾. Tooth decay and Periodontal Disease are not like other diseases for important reasons, including: the cases are chronic

and progress slowly over many years, and many non-specific bacteria are already existed in the oral cavity (microflora) cause these infections to all people ⁽²⁾. The dental plaque bacteria form decay caries lesion or primary initial caries, If they are not treated, they form cavity due to the out puts ferment carbohydrates, and soluble layer tooth enamel due to acids leading to

(1) Assistant lecturer, Technical Institute Hawija

organic and inorganic demineralization and then saliva-laundering and the removal of sugars suspended to teeth, equivalent to the acid, calcium and phosphate enters again to the teeth in the process of remineralization 1, which is better existed with fluoride ⁽³⁾.specialists and researchers in the field of dentistry were interested in the subject of tooth decay for being a very difficult problem for the specialists, in addition to causing severe pain more than other infectious diseases, besides it remains the critical factor responsible for the loss of most teeth in the all ages other than other causes . It was found that more than two-thirds of children age (5-17 years) have with tooth decay and about (94%) of adults suffer from tooth decay not treated or that their teeth filled and that is an evidence of decay at earlier time. ⁽⁴⁾. The infection of the oral cavity may occur due to microbial activity in normal oral flora in the mouth, these infections include tooth decay dental caries and abscesses , periodontitis and gingivitis ⁽⁵⁾. Streptococcus mutans is a type of bacteria that causes tooth decay in humans, in conjunction with the bacterium Lactobacilli, these species are the main causes for dental disease ⁽⁶⁾, and recent studies assured the involvement of Actinomycetes in the spread of decay root caries ⁽⁷⁾. Despite the presence of Candida spp, commonly in the mouth, it is an important factors causing candidiasis ⁽⁸⁾. Because there are few studies that dealt with this disease in the district of Hawija in terms of biological and implications for oral health in particular and the body in general and the importance of microbes in causing tooth decay, this study drew its goals as follows: isolation and identification of important bacteria related to tooth decay and diseases of the mouth, determining the sensitivity of some bacteria of certain types of antimicrobial agents, studying the effect of some salt concentrations and pH in the growth of some bacteria.

Materials and Method

Collection of samples: A total of (80) swabs was collected from dental plaque from mouths of patients which are

suffering from dental and oral disease of both sexes and their ages range from 1-60 years which are (referred) to consult the dental clinics and specialized centers of dental in AL-Hawija city from 2009.12.1 to 2010.4.30 and by using sterile swabs into the middle nutritious carrier and then transferred to laboratory.Samples plant / samples from the mouth of patients were planted on growth media (Blood agar, Mannitol salt agar, MaCconkey agar, Crystal - violate blood agar, Actinomyces selective medium) and the dishes were incubated in the incubator 37C° for 24 hours.

Diagnosis of Bacterial Isolates

a-Phenotypic characteristics: These include the study of the characteristics of colonies and morphological characteristics, including shape, size, height, strength, and ability to produce pigments,smell,and others.

b-Biochemical characteristics: the tests included catalase, oxidase,urease, nitrate reduction test, motility test, degradation of gelatin, methyl red, voges- proskauer, indole test, analyzing the blood, the consumption of citrates.

Brearing Tests

a-Growth in different concentrations of salt (NaCl). The following concentrations are used in this test (5,6, 7,8, 9, 10%). Inoculated tubes containing the center of the heart and brain infusion liquid containing the salt concentration is required, and the tubes incubated for 24 hours at 37C°. The presence of turbidity in the medium is a sign of the growth of micro-organism under test. Then (0.1) ml of each tube was taken and spread on the surface of blood agar using glass spreader, and the dishes incubated for a period (24-48 hours) to get a noticeable growth of the colonies and colonies were calculated and the average number of colonies was taken (there have been three replicates for each concentration)⁽⁹⁾.

b-Growth in different (pH): the following pH's are used in this test (pH= 3, 4,5,6,7,8), pH had been controlled By

using the solution (40%) of sodium hydroxide (NaOH), or a solution of hydrochloric acid (HCl), Inoculated tubes containing the center of the heart and brain infusion liquid containing the pH concentration is required, and the tubes incubated for 24 hours at 37°C. The presence of turbidity in the medium is a sign of the growth of micro-organism under test. Then (0.1) ml of each tube was taken and spread on the surface of blood agar using glass spreader, and the dishes incubated for a period (24-48 hours) to get a noticeable growth of the colonies and colonies were calculated and the average number of colonies was taken (there have been three replicates for each concentration)⁽⁹⁾.

Antibiotic Susceptibility Test

The modified Kirby – Bauer method that was recommended by the⁽¹⁰⁾ was used as follows:-

a-Muller- Hinton agar was prepared from a dehydrated base according to the manufacturers recommendations.
b-The medium was cooled to 45-50°C and poured into the plates, allowed to set on a level surface to a depth of approximately 4mm.

c-Inoculums from primary culture plates were prepared by touching 3-5 colonies with a loop and transferred to a tube of saline.

d-The saline – bacteria mixture was compared with the turbidity standard and density of the test suspension was adjusted to be equal to 0.5 tube of Maccfarland .

e-By dipping a sterile swab into the inoculum, Muller- Hinton plates were inoculated. Inoculated plates were left to dry for a few minutes at room temperature.
f-The antibiotic discs were placed on the inoculated plates .

g-Plates were placed in incubator at appropriate incubation temperature within 30 minutes of preparation .

h-After overnight incubation, the diameter of each zone was measured and recorded in mm.

Results

Insulation: In the study, (80) isolates of bacteria isolated has been obtained from the mouths of patients, as shown in the table (1). It has been shown that Streptococcus spp. represented the highest percentage of the rates of isolation, which include four types: Streptococcus mutans, S. sanguis, S. mitis, S. salivarius 25%, 17.5%, 16.3%, 15%, respectively, followed by Staphylococcus epidermidis (10%), while the bacteria Lactobacilli has accounted for 7.5% and 6.2% for each of the L. acidophilus, L. casei, The lowest percentage was for the bacteria Actinomyces viscosus which was 2.5%. Table (2) shows that the Staphylococcus epidermidis bear the highest concentrations of salt (10%). Table (3) shows that Staphylococcus epidermidis do not have the ability to grow at acidic pH (pH = 5), and the results show as well, the ability of Lactobacillus acidophilus to grow in the middle acid more than S. mutans. Table (4) shows the percentage of bacterial isolates resistance to nine antibiotics, S. mutans, shows the highest resistance to antibiotics:

trimethoprim, chloramphenicol, tetracycline erythromycin, amoxicillin, and ampicillin while sensitivity to antibiotics cefotaxim, ciprofloxacin, amikacin by 10%, 25%, 30%. As for the Staphylococcus epidermidis, it showed high resistance to trimethoprim, chloramphenicol, tetracycline erythromycin, amoxicillin, ampicillin. While the bacterial isolates showed lowest resistance to Amikacin and ciprofloxacin by 25% and by cefotaxim 12.5%. With regard to Lactobacillus acidophilus, it showed the highest resistance to trimethoprim, chloramphenicol, tetracycline erythromycin, amoxicillin, ampicillin. While the bacterial isolates showed a resistance to cefotaxim and ciprofloxacin 16.6%, while the of Amikacin rate 33.3%.

Discussion

Insulation: This agrees with what⁽¹¹⁾ who stated, that Streptococcus mutans constituted the highest isolate proportion (33%) from a Streptococcus bacteria,

followed by *S. sanguis* ratio (15%), while *S. salivarius* recorded the lowest percentage of 13%. These results corresponds with the results^(12, 13), as these studies indicated that the *Streptococcus mutans* is prevailing in the vicinity of the mouth of the bacterial species *Streptococcus* with the following proportions 42%, 33%, 31% respectively. It has been observed during the study, that the emergence of positive bacteria and special causes of decay and cariogenic bacteria in the mouth of man played an important role in assessing the cavity and caries.

Carrying Salt Concentrations

This finding corresponds with the results obtained by⁽¹¹⁾ which stated that the *Staphylococcus epidermidis* bear the salt concentration (9%), and *Lactobacillus acidophilus* showed sensitivity to all concentrations of salt used in the study. This result agrees with⁽¹¹⁾ findings about the inability of these bacteria to withstand the salt concentration (4%), the differences in carrying concentrations of salt may be attributed to the existence of a difference in permeability of the cell membrane.

Hydrogen Base Bearing

This corresponds with the result obtained by⁽¹⁴⁾ who found out that the viability of the *Lactobacillus* are more acidic in the middle of the viability of the bacterium *Streptococcus mutans*.

The Sensitivity of Bacteria to Antibiotics

The current study showed that the cefotaxim is the best of antibiotic in its influence on the bacterial isolates taken from the mouth, followed by the antibiotic ciprofloxacin and Amikacin. The antibiotic trimethoprim, chloramphenicol, tetracycline, erythromycin, amoxicillin, ampicillin are less effective on the bacterial isolates. One of the results we have noticed that the bacterial isolates showed variation in their resistance to antibiotics of the group of

aminoglycosides, the ratio of resistance to chloramphenicol 75% The percentage of resistance to Amikacin is less than 25%. The resistance to aminoglycosides antibiotics increased notably in recent times, this resistance which is due to the formation of the enzyme by resistant bacteria modifies the antibiotic because of loss of outer membrane proteins, which reduces the permeability of the antibiotic inside the bacterial cell⁽¹⁵⁾.

The results of this study also showed the high resistance shown by the bacterial isolates to trimethoprim, which amounted to 90% and explain the mechanism of the resistance to this antibiotic, either because of genes carried on plasmid, as this plasmid provides bacterial cell metabolically enzyme new non-sensitive to the antibiotic, which replaces the biochemical enzyme with the presence of this drug, which leads to the production of folic acid⁽¹⁶⁾.

The study also shows an increasing resistance to tetracycline 75%, it is believed that this resistance resulted from the presence of plasmids that encode resistance to the antibiotic which moves significantly, and that the presence of high resistance in isolates of local class of β -lactames may be due to the production of β -lactames enzymes either by some plasmids or by bacterial chromosome⁽¹⁷⁾. It is noted during the study that the lowest resistance showed by the bacterial isolates was to cefotaxim (10%) and ciprofloxacin (16.6%). It appeared that most of the bacterial isolates were sensitive to these adversaries and may be due to response to the majority of the isolates of these two adversaries to being of limited use antibiotics at the present time in hospitals, leading to increased resistance to antibiotics can be passed as determinants responsible for drug resistance to antibiotics by Alaguetranip plasmids.

Conclusion

From the results of the present study we can conclude the following :-

1-Among bacterial pathogens, *S. mutans* caused the highest number of cases of oral disease and dental caries in these ages.

2-Staph. epidermidis was capable of toleration of the highest salt concentrations(10%).

3-L. acidophilus showed its high capability to grow on acidic medium (PH=4) more than other bacteria .

4-The Cefotaxim was the best antibiotics and have higher effect on bacterial isolates ,then Ciprofloxacin and Amikacin .

1-The molecular studies included polymerease chain reaction technique for the detection of plasmid – and chromosome – borne virulence genes in S. mutans , to investigate the distribution of these genes in isolates from oral disease and dental cariesin patients .

2-Infectivity and virulence factors in S. mutans in relation to oral disease and dental cariesin.

Recommendations

Further future studies are recommended to cover the following subjects.

Table (1):- shows the numbers and percentages of bacteria isolated from the mouth of patients

Bacterial species	Number	Percentage
<i>Streptococcus mutans</i>	20	25
<i>Streptococcus sanguis</i>	14	17.5
<i>Streptococcus mitis</i>	13	16.5
<i>Streptococcus salivarius</i>	12	15
<i>Staphylococcus epidermidis</i>	8	10
<i>Lactobacillus acidophilus</i>	6	7.5
<i>Lactobacillus casei</i>	5	6.2
<i>Actinomyces viscosus</i>	2	2.5
Total	80	100

Carrying salt concentrations

Table (2):- The impact of salt concentrations of NaCl on the growth of S. mutans , Staphylococcus epidermidis,and Lactobacillus acidophilus.

Salt concentrations / Types of bacteria	5%	6%	7%	8%	9%	10%
<i>Lactobacillus acidophilus</i>	-	-	-	-	-	-
<i>Streptococcus mutans</i>	70	40	80	60	15	-
<i>Staphylococcus epidermidis</i>	100	62.5	75	87.5	50	37.5

- Does not have a growth

Table (3):- the impact of pH on the growth S. mutans, Staphylococcus epidermidis,and Lactobacillus acidophilus

PH / Types of bacteria	3	4	5	6	7	8
<i>Streptococcus mutans</i>	-	-	10	60	35	50
<i>Staphylococcus epidermidis</i>	-	-	-	-	50	25
<i>Lactobacillus acidophilus</i>	16.6	100	83.3	33.3	50	66.6

No growth

Table (4):- Percentage of resistant bacterial isolates to antibiotics

Kinds of bacteria Antibiotics	<i>Staph. epidermidis</i>	<i>S. mutans</i>	<i>L. acidophilus</i>
Ampicillin	55	87.5	83.3
Amoxicillin	60	75	66.6
Amikacin	30	25	33.3
Ciprofloxacin	25	25	16.6
Cefotaxim	10	12.5	16.6
chloramphenicol	50	75	50
Erythromycin	60	87.5	83.3
Tetracycline	55	75	66.6
Trimethoprim	90	87.5	83.3

References

- 1-Sturdevant,C.M.;Roberson,T.M.;Heymann,H.O.;Sturdevant J.R.(1995).the art and science of operative dentistry .Mosby;USA;3rd ed ;by ;Lundeen TF;Roberson TM;PP:60-128.
- 2-Youmans ,G.P.;Patterson ,P.Y.;Sommer,H.M.(1986).The biological and clinical basis of infectious diseases .W.B.sanders company ;chap.23;by Duncan JL;PP:266-286.
- 3-Bratthal,D.(1996).Dental caries . Eur . J . Oral .Sci .104 (4) :486-491. - Beck ,J.D.;Slade G.and Offenbacher , S.(2000).Oral disease , cardiovascular diseases and systemic inflammation . Periodontol.23:110-20.
- 4-Epsteine ,J.B.; McBride ,B.C. and Stevenson –moor ,P.(1991). The efficacy of chlorhexidine gel in reduction of *S.mutans* and *Lactobacillus* spp. In patients treated with radiation therapy .Oral .surg.71(2):172-178.
- 5-Holt ,R.;Robert ,G.and Scully ,C.(2000).Dental damage ,sequelae ,and prevention .B.M.J.6 (Issue 7251):1717-20.
- 6-Twetman,S.;and Grindefjord ,M.(1999).Mutans Streptococci suppression by chlorhexidine gel in toddlers .AM.J.Dent-12(2):89-91.
- 7-Quirynen ,M.;Gizani ,S.;Mongardini ,C.;Declercq,D.;Vinckier,F.and Van Steenberghe ,D.(1999).the effect of periodontal therapy on the number of cariogenic bacteria in different intra –oral niches .J.Clin.Periodontol.26:322-327.
- 8-Reo,G.G.(1998).Risk factors for the spread of antibiotic resistant bacteria .Journal of periodontology,55(3)323-330.
- 9-Sato,M.;Tsuchiya ,H.;Akagiri,M.;Takagi,N.and Inuma ,M.(1997).Groth inhibition of oral bacteria related to denture stomatitis by anti –candidal chalcones .Aus .Dent. J.72(5):343-6.
- 10-Claus ,D. and Berkeley,R.C.W.(1984).Genus Bacillus .In:Bergeys Manual of systematic bacteriology ,vol.11,pp.1105-1139.
- 11-World Health Organization (2003) .Basic Laboratory procedures in clinical bacteriology.
- 12-الحسيني ، عدي متعب هادي .2002. دراسة مايكروبيولوجية -gingivitis والتهاب اللثة dental caries لتسوس الاسنان والتهاب ماحول السن . رسالة ماجستير . كلية العلوم . جامعة الكوفة
- 13-Babaahmady ,K.G.; Challacombe ,S.J.;March ,P.D. and Newman,H.N.(1998). Ecological study of *S.mutans* , *S. sorbinus* and *Lactobacillus* spp.At sub sites from approximal denetal plaque from children . caries-Res.32(1):51-8.
- 14- Brambilla ,E.;Twetman ,S.;Felloni ,A.;Gagetti, M.G.;Canegallo ,L.;Garica ,G.F. and Strohmenger,L.(1999).Salivary mutans Streptococci and Lactobacilli in 9 – and 13- year old Italian – school – children and the relation to oral health .Clin.Oral .Investig .3(1): 7-10.
- 15-الموسوي ، علياء موسى علي .2006. دراسة حول البكتريا -والخمائر المرافقة لبعض امراض الفم في مدينة الناصرية . رسالة ماجستير . كلية التربية . جامعة ذي قار
- 16-Mingeot-Lectereq, M.P.J.Glupezynski,Y. and Tulken ,P.M.(1999). Aminoglycosides activity and resistance.Antimicrobial .Agents chemother .43(4):727-737.
- 17-Araque.M.;Nieves ,B.;Ruiz ,O.;Dager ,M.(2000).Characterization of plasmids which mediated resistance to multiple antibiotic in gram negative bacteria on nosocomial origin .Enferm .Infect microbial .Clin.15(6):299-305.
- 18-Hostacka ,A.& Karelova,E. and Peuther,J.(1997).Outer membrane protein profiles of *Pseudomonas aeruginosa* after the post antibiotic effect of imipenem microbios ,90:45-50.