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Antimicrobial Susceptibility Pattern of Some Pathogenic Bacteria Isolated from Dental Caries

Reyam Abdul Khuder Mohammed Jassam[1]; Ahmed jaber aboob[2]; Ahmed S. Abed [3]; Emad Salaam Abood [3*]

[1]*B.V.S, M.Sc. Oral Microbiology, Al-Mustaqbal University College, Department of Radiology Techniques, Hilla, IRAQ*

[2]*B.D.S., M.Sc. Oral Microbiology, Babylon health directorate, Hilla, IRAQ*

[3]*Medical Physics, Hilla University College, Babylon, Iraq*

Abstract

The Cross-Sectional study was carried out for a period of six months, from June (2020) to December (2020). 283 patients were visited a dental clinic in Hilla city suffering from dental caries. All these samples were inoculated for isolated pathogenic bacteria by identification of these bacteria by gram stain, biochemical test and compact Vitek 2 system. Out of (283) clinical samples, only 250 (88.3%) positive culture, whereas 33 (11.6%) samples showed no bacterial growth, which may be treated with antibiotics or the presence of other types of causative agents that might need special technique for their detection, such as viruses and fungus. Thus, Gram-positive bacteria were about 47% of the total isolates, whereas Gram-negative bacteria comprised about 53% of the total isolates (52.8 percent). The predominant gram-positive bacterial species found in dental caries was *Streptococcus mutans*, found in 45 individuals (18 percent of the samples), followed by Streptococcus epidermidis, found in 26 people (10.4 percent), Streptococcus pneumonia, found in 23 people (9.2 percent), Staphylococcus aurous, found in 19 people (7.5 percent), and Streptococcus oralis, found in 5 people (2.3 percent) (2 percent). In addition, *Lactobacillus acidophilus* was the most common negative bacterial species isolated from dental caries. It was found in 40 (16%), followed by *Fusobacterium nucleatum* 41 (91.11%), *E. coli* 35 (14%), 5 (2%) were found for each *Campylobacter jenjuni* and *Klebsiella pneumonia, and* 3 (1.2%) were found for each *Pseudomonas aeruginosa* and *These* bacteria found in all isolates were identified by the compact Vitek system. The Antibiotic Susceptibility Test for Gram Positive and Negative Grams Bacterial isolates were investigated. The results were compared according to the compact Vitek 2 system as susceptible, intermediate and resistant. It has been found that most Gram-positive and Gram-negative isolates are highly resistant to beta lactam groups. It was found that *Streptococcus mutans* was resistant to Penicillin at a rate of (82.2%). In addition, these isolates were highly sensitive to Amoxicillin and Ciprofloxacin at a rate of (86.6%) and (71.1%) respectively. *Streptococcus epidermidis* was highly resistant to Tetracycline at 88.4% and highly sensitive to Amoxicillin at the same rate. The results of this study showed that *Streptococcus pneumonia* was highly sensitive to Gentamycin, Ciprofloxacin, Cefotaxime, Amoxicillin and Vancomycin at a rate of 78.2%. *Staphylococcus aurous* were tested for antibiotics. It was found that these bacteria were highly sensitive to Meropenem (94.7%). *Streptococcus oralis* was highly resistant to Imperium (100%), and highly sensitive to Gentamycin, Ciprofloxacin, Cefotaxime, and Amoxicillin. In addition, gram negative bacteria were studied for antibiotic testing. It was found that *E. coli* was highly sensitive to Gentamycin, Imperium, Amoxicillin and Vancomycin (91.4%), while *Fusobacterium nucleatum* was highly sensitive to Ciprofloxacin (95.12%). *Campylobacter jenjuni*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Proteus mirabilis* were highly sensitive to most antibiotics used in this study. Finally, *Lactobacillus acidophilus* was highly resistant to Penicillin in rate (82.5%) and sensitive to Ciprofloxacin in rate (88.5%).

Keywords: Dental caries. Pathogenic bacteria, Antimicrobial Susceptibility, PCR, Compact Vitek System.

1. Introduction

A dental cavity (dental cavity) is damage to the tooth that may occur when caries produces acids that attack the surface of the tooth or enamel in the mouth. Causing this may lead to a cavity in a tooth (1). Cavities generate acid from germs that dissolves the hard teeth (enamel, dentin and cement) (2). When bacteria digest food waste or sugar on tooth enamel, they form an acid which results in tooth decay (3). A diet that consists of plenty of simple carbohydrates is a risk factor for the development of harmful bacteria in the intestines (4). In the presence of saliva, caries develops if mineral breakdown is higher than buildup from sources like saliva. While it is important to keep track of every change, this may be complicated if

*Corresponding author e-mail: amadiraq45@gmail.com

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certain diseases make it difficult to produce enough saliva: diabetes mellitus, Sjögren syndrome, and some medicines (5). Drugs that reduce the production of saliva include antihistamines and antidepressants (6). Dental caries is also linked with low socioeconomic status, inadequate oral hygiene, and receding gums, which expose the root surfaces of teeth (7). Germs such as *Streptococcus mutans* and Streptococcus sobrinus are the most frequent bacteria found in tooth cavities (8). However, cariogenic bacteria (those that may cause illness) are found in dental plaques but are typically too low to cause issues until there is a balance change (9). This is caused by local environmental changes, such as repeated intakes of sugar or poor clearance of biofilm (toothbrushing). The condition may cause discomfort, tooth loss and infection if left untreated (10). The mouth includes a broad range of oral bacteria, yet there are only a few species of dental caries which are thought to occur: *Streptococcus mutans* and Lactobacillus (11). *Streptococcus mutans* are gram-positive bacteria that form biofilms on the tooth surface. (12) These organisms, after fermentation of dietary carbohydrates, may generate high lactic acid levels that are resistant to low pH deleterious effects and necessary for cariogenic bacteria (13). Since cement from root surfaces is easier to demineralize than enamel, a broader range of bacteria, including *Lactobacillus acidophilus, Actinomyces spp., Nocardia sp., and Streptococcus mutans*, may lead to root carriages (14) Bacteria develop in a sticky, creamy substance called plaque around your teeth and gums, a biofilm (15). Some locations accumulate plaque more often than others, such as low salivary flow rates (molar fissures) (16). Grooves on the occlusal surfaces of molar and premolar teeth offer plaque bacteria tiny retention sites as well as inter-proximal sites. The plaque may also be collected above or below the gingiva, where it is known as a supra or substitute plaque (17). These bacterial strains, most especially *S. mutans*, may be acquired from the kiss of the caregiver or pre-masticated by feeding (18). In the mid-twentieth century, antibiotic therapy started with sulfa-containing medications and medicines derived from natural microbial compounds, such as penicillin, which was discovered in 1941. Antibiotics were also utilized in clinical and pharmaceutical research to address the difficulties of bacterial infections (19). Systemic antibiotics have demonstrated potential effectiveness at an early stage in the prevention or treatment of dental caries. Some systemic antibiotics have been emphasized, including penicillin, tetracyclines, metronidazole, macrolides, and clindamycin. They describe the use, mechanisms, side effects, and resistance (20).

Aim to study:

The aimed to identify and characterize some bacteria in patients with dental caries and antibiotic resistance patterns to determine the risk to public health.

Materials and methods:

A. Patients and collection of samples:

The Cross Sectional study was carried out for a period of six months, from June (2020) to December (2020). 283 patients were visited a dental clinic in Hilla city suffering from dental caries. The samples were collected from each case by disposable cotton swabs, and followed standard procedure for microscopic examination and isolation of bacteria. Specimens were collected carefully to avoid any contamination. One aliquot of collected specimen was immediately inoculated in blood agar media at the bedside for aerobic culture. The rest of the specimen was transferred to the Department of Microbiology for further investigations. It was inoculated into Blood agar, MacConKey agar, Mannitol agar and Nutrient agar medium, then incubated at ($37^{o}C$) for (24) hours aerobically. Aerobic and anaerobic bacterial isolates were diagnosed by gram stain, colony morphology, biochemical test, Compact VITEK-2 System and identification of some bacteria by *16SrRNA* technique.

Ethical Approval:

B. In order to comply with ethical standards, a permission was obtained from each participant before he or she was allowed to take part in the research.

C. Identification of bacterial isolates by gram stain, biochemical tests:

The identification tests, including cultural, morphological and biochemical characteristics were done for each isolate according to (21, 22).

D. Identification of bacterial isolates with Compact VITEK-2 System:

The Compact VITEK-2 System tested and identified all bacterial isolates (BioMerieux). This is a phenotypic identification type that relies on biochemical responses to detect isolates. The Vitek-2 card has 64 wells for various biochemical fluorescence tests. About 20% of the 64 metabolic tests assessed carbohydrate absorption; this included testing for phosphatase, urea, nitrate, and actidione. The Vitek-2 machine autonomously controls the card, including filling and screening, and then transfers the cards to the connected incubator. For each output report, an algorithmic system decodes it. The findings were recognized in the ID-GP (Gram-positive bacterium identification) and ID-GN (Gram-negative bacteria identification) databases. The relevant supporting software proposes these IDs. If the first results showed "low discrimination" or "no ID," only then were the tests repeated. Afterwards, the repeated results were utilized for data analysis. All strains were inoculated on cultivated medium and then incubated at 37°C throughout the night. A single isolated colony was utilized to identify the phenotypical VITEK-2 System technique, as directed by the company (BioMerieux). The suspension was produced on the suggestions of the fabricator of the Company BioMérieux by swabbing an adequate number of colonies from pure overnight culture and suspending the sterile micro-organisms in a (12 x 75) mm transparent plastic (polystyrene) test tube with 3.0 ml of sterile saline. The turbidity was adapted to match a McFarland No. (0.5) using a Densi Chek meter. The same suspension was utilized in VITEK-2 compact system antibiogram testing.

Identification of some bacterial isolates by *16SrRNA gene*:

The primer sequence and PCR conditions that used in study are listed in Table (1).

E. DNA extraction form bacterial culture:

A Genomic DNA purification kit coupled with (Geneaid, USA). Using a UV-trans illuminator, it is seen.

F. Primers Sequences:

A Genomic DNA purification kit purchased from (Geneaid, USA) by Using a UV-trans illuminator, it is seen.

Table (1): *16SrRNA gene*s primers sequences with their amplicon size Base pair (bp) and their condition.

16Sr RNA Genes	Primer sequence (5′-3′)	Size (bp)	PCR condition	Reference
L. acidophilus	5'-AGAGTTTGATCCTGGCTCAG-3' 5'-AAGGAGGTGATCCAGCCGCA-3'	287	Stage 1: 2 min., 95ºC, Stage 2: 30 sec., 95ºC, Stage 3: 30 sec., reduction 0.5ºC per cycle, 63.3ºC Stage 4: 30.0 sec.,72ºC Stage 5: Replication stages 2-4 14 extra periods Stage 6: 30 sec., 95ºC Stage 7: 30 sec., 56.3ºC Stage 8: 30.0 sec., 72ºC, Stage 9: Replication stages 6-8 19 extra periods Stage 10:5 min.,72ºC Step 11: hold, 4ºC	23
F. nucleatum	5'-AGA GTT TGA TCC TGG CTC AG-3' 5'-GTC ATC GTG CAC ACA GAA TTG CTG-3'	360	Stage 1: 2 min., 95ºC, Stage 2: 30 sec., 95ºC, Stage 3: 30 sec., reduction 0.5ºC per cycle, 63.3ºC Stage 4: 30.0 sec.,72ºC Stage 5: Replication stages 2-4 14 extra periods Stage 6: 30 sec., 95ºC Stage 7: 30 sec., 56.3ºC Stage 8: 30.0 sec., 72ºC, Stage 9: Replication stages 6-8 19 extra periods Stage 10:5 min.,72ºC Step 11: hold, 4ºC	24
C. jenjuni	5'-AGAGTTTGATCCTGGCTCAG-3' 5'-GATCATCCTCTCAGACCAG-3'	300	Stage 1: 2 min., 95ºC, Stage 2: 30 sec., 95ºC, Stage 3: 30 sec., reduction 0.5ºC per cycle, 63.3ºC Stage 4: 30.0 sec.,72ºC Stage 5: Replication stages 2-4 14 extra periods	25

			Stage 6: 30 sec., 95ºC Stage 7: 30 sec., 56.3ºC Stage 8: 30.0 sec., 72ºC, Stage 9: Replication stages 6-8 19 extra periods Stage 10:5 min.,72ºC Step 11: hold, 4ºC	

G. Detection of Amplified Products by Agarose Gel Electrophoresis:

Successful PCR amplification was verified by the observation of the agarose gel/electrophoresis using UV light. Gel from agarose has been produced. The comb was then attached to one end of the tile to provide the wells needed to load the DNA sample. The agarose was carefully poured into the tray and allowed to harden for 30 minutes at room temperature. The comb was then carefully removed from the tray. The plate was fixed in an EPC filled with TBE buffer covering the gel surface. 5 l of DNA samples were placed into each agarose gel well, and the five l DNA ladder was added to one well. The electric current may flow for 50 minutes at 70 volts. 280 nm was utilized to monitor DNA bands using a UV trans-illuminator, and the gel was shot using a digital camera.

Antibiogram testing by VITEK-2 Compact:

Antibiogram testing was carried out using the automated compact system VITEK-2, utilizing particular cards based on the determination of MIC techniques. The following antibiotics: penicillin, Imperium, tetracycline, gentamycin, meropenem, chloramphenicol, Amoxicillin, ciprofloxacin, cefotaxime, clindamycin, and Vancomycin were on the following cards. Special cards were infected in the way specified in the VITEK-2 compact system, which assesses the growth pattern of each organism in the presence of the antibiotic. Several factors are utilized to give a suitable input for MIC estimates based on observed growth characteristics. In order to establish an interpretation of the category, the MIC result must be connected to an organism identification.

Results:

All these samples were inoculated for isolated pathogenic bacteria by identification of these bacteria by gram stain, biochemical test and compact Vitek 2 system. Out of (283) clinical samples, only 250 (88.3%) positive culture, whereas 33 (11.6%) samples showed no bacterial growth, which may be treated with antibiotics or the presence of other types of causative agents that might need special technique for their detection, such as viruses and fungus. Based on these findings, it has been revealed that Gram-positive bacteria comprise 118/250 (47.2%) of the total isolates and have been regarded as the majority of gram-negative gram-negative bacteria (132/250 (52.8%) and Table (2) illustrates the distribution in patients of bacterial isolates.

Table (2): Distribution of gram positive and gram negative bacterial isolates from patients with dental caries

Total No. of Samples	Positive culture	Gram positive	Gram negative	No growth
283 samples	250(88.3%)	118/250 (47.2%)	132/250 (52.8%)	33(11.6%)

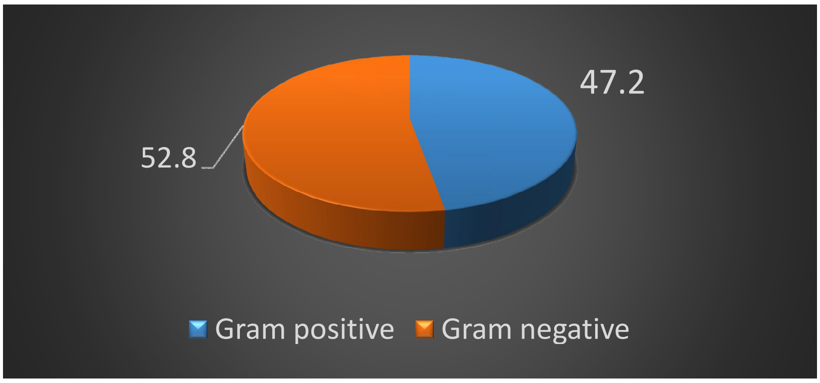

Figure (1): The percentage of gram positive and gram negative among patients with dental caries

Streptococcus mutans was the most common gram positive bacterial species isolated from dental caries. It was found in 45 (18%), followed by *Streptococcus epidermidis* found in 26 (10.4%), *Streptococcus pneumonia* 23 (9.2%), *Staphylococcus aurous* 19 (7.5%) and *Streptococcus oralis* 5 (2%), as shown in Figure (2). In addition, *Lactobacillus acidophilus* was the most common negative bacterial species isolated from dental caries. It was found in 40 (16%), followed by *Fusobacterium nucleatum* 41 (91.11%), *E. coli* 35 (14%), 5 (2%) were found for each *Campylobacter jenjuni* and *Klebsiella pneumonia, and* 3 (1.2%) were found for each *Pseudomonas aeruginosa* and *Proteus mirabilis*

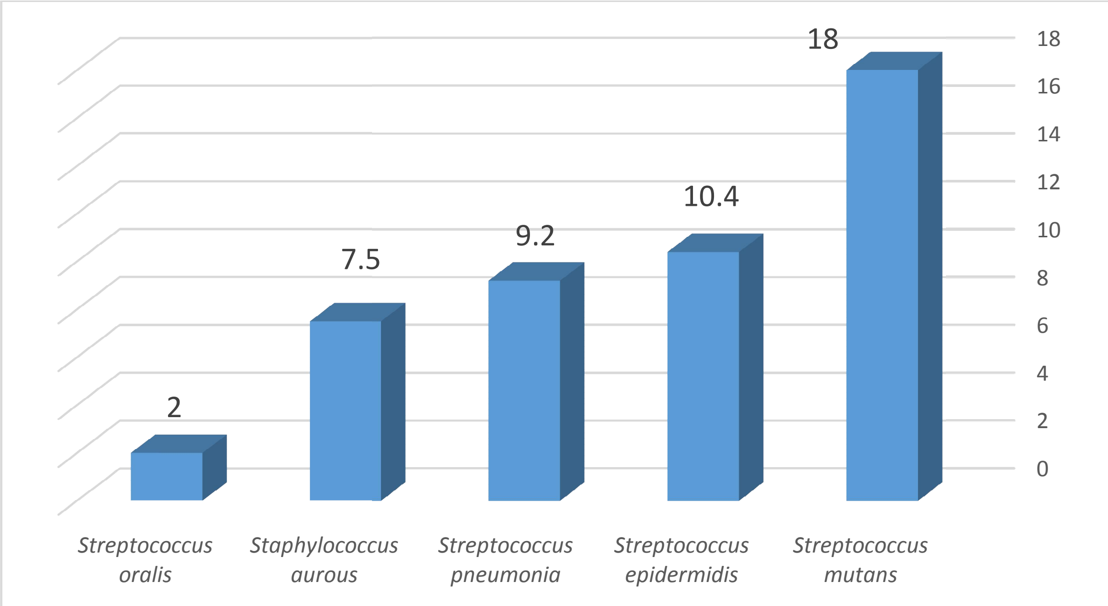

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Figure (2): Distribution of gram positive bacterial isolates from patients with dental caries

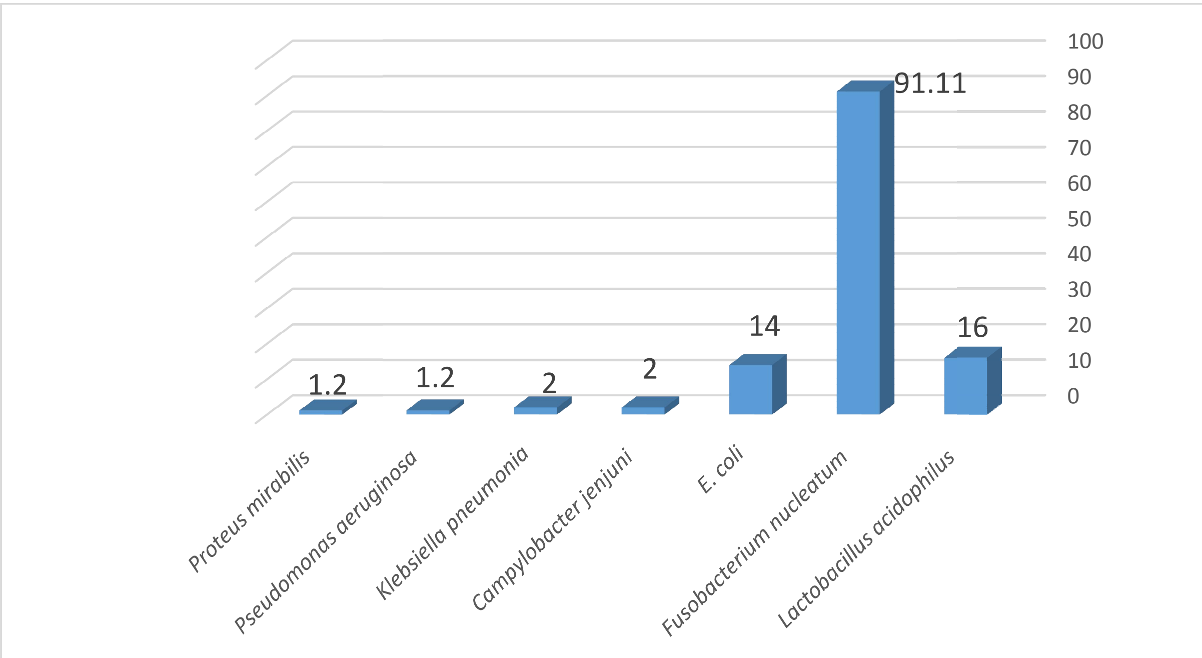

Figure (3): Distribution of gram negative bacterial isolates from patients with dental caries

Table (2): Distribution of gram positive and negative bacterial isolates from patients with dental caries

Bacteria	Total isolate	%
Gram positive		
Streptococcus mutans	45	18%
Streptococcus epidermidis	26	10.4%
Streptococcus pneumonia	23	9.2%
Staphylococcus aurous	19	7.5%
Streptococcus oralis	5	2%
Total	**118**	**47.2%**
Gram negative		
Lactobacillus acidophilus	40	16%
Fusobacterium nucleatum	41	91.11%
E. coli	35	14%
Campylobacter jenjuni	5	2%
Klebsiella pneumonia	5	2%
Pseudomonas aeruginosa	3	1.2%
Proteus mirabilis	3	1.2%
Total	**132**	**52.8%**
Total no. of bacterial isolates	**250**	**100%**

Identification of *Lactobacillus acidophilus* by PCR technique:

In this study, *Lactobacillus acidophilus* were detected by *16SrRNA* genes by PCR technique from dental caries according to Table (1), *Lactobacillus acidophilus* was found in all isolated were identified by compact Vitek system as shown in Figure (4).

Identification of *Fusobacterium nucleatum* by PCR technique:

In this study, *Fusobacterium nucleatum* were detected by *16SrRNA* genes by PCR technique from dental caries according to Table (1), *Fusobacterium nucleatum* was found in all isolated were identified by compact Vitek system as shown in Figure (5).

Identification of *Campylobacter jenjuni* by PCR technique:

In this study, *Campylobacter jenjuni* were detected by *16SrRNA* genes by PCR technique from dental caries according to Table (1), *Campylobacter jenjuni* was found in all isolated were identified by compact Vitek system as shown in Figure (6).

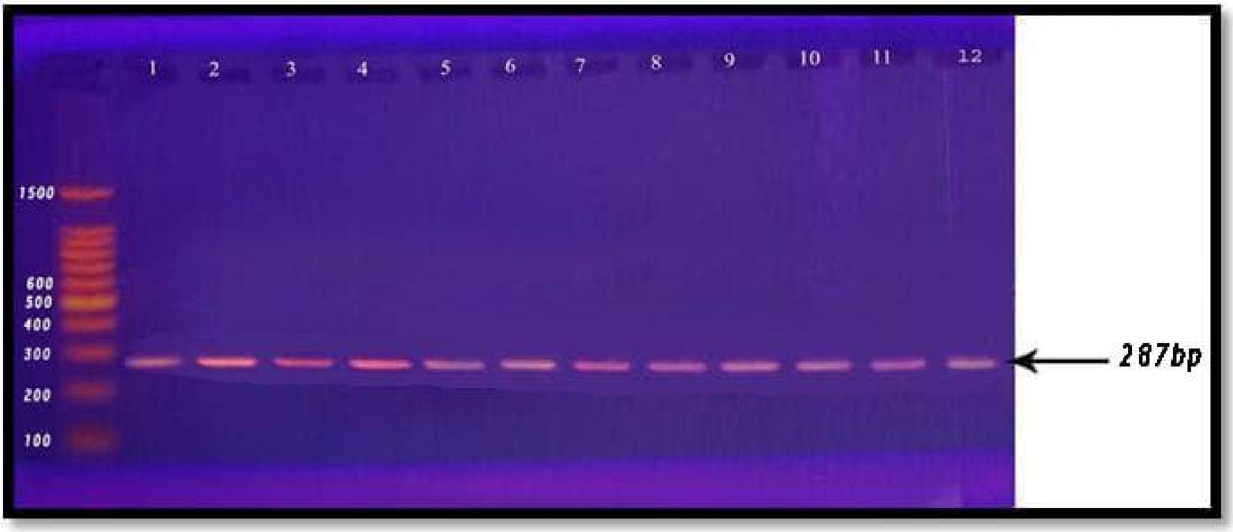

Figure (4): Agarose gel electrophoresis 50 minutes by 70 volts for 16S rRNA PCR products observed under U.V light at 301 nm after ethidium bromide staining. M: 1500 bp ladder; lane (1-12) has been positive for gene Lactobacillus acidophilus, product size (287 bp).

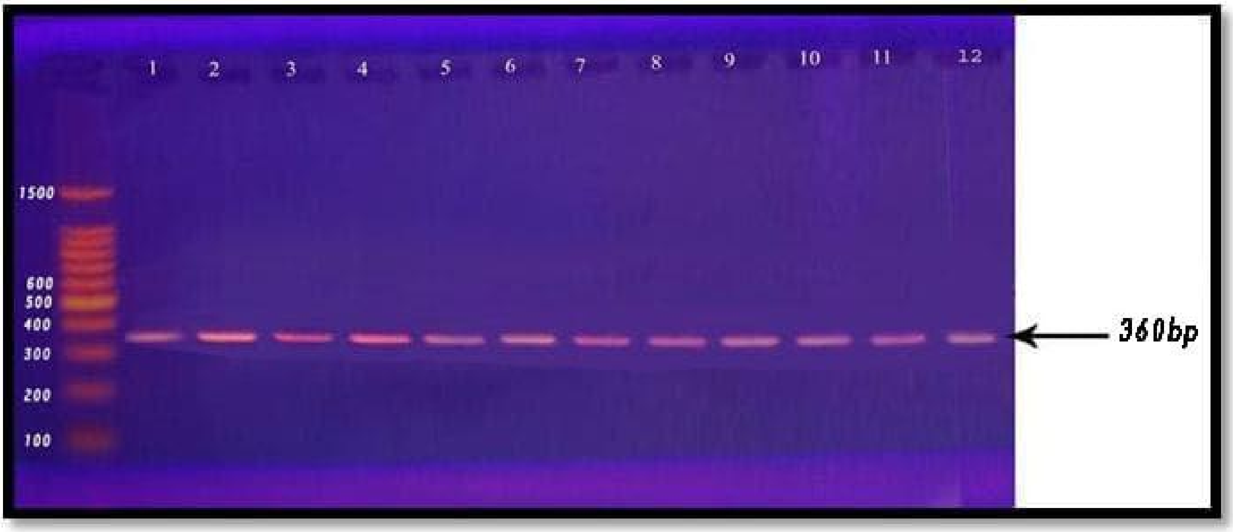

Figure (5): Agarose gel electrophoresis 50 minutes by 70 volts for 16S rRNA PCR products observed under U.V light at 301 nm after ethidium bromide staining. M: 1500 bp ladder; lane (1-12) has been positive for the nucleatum gene Fusobacterium, product size (360 bp).

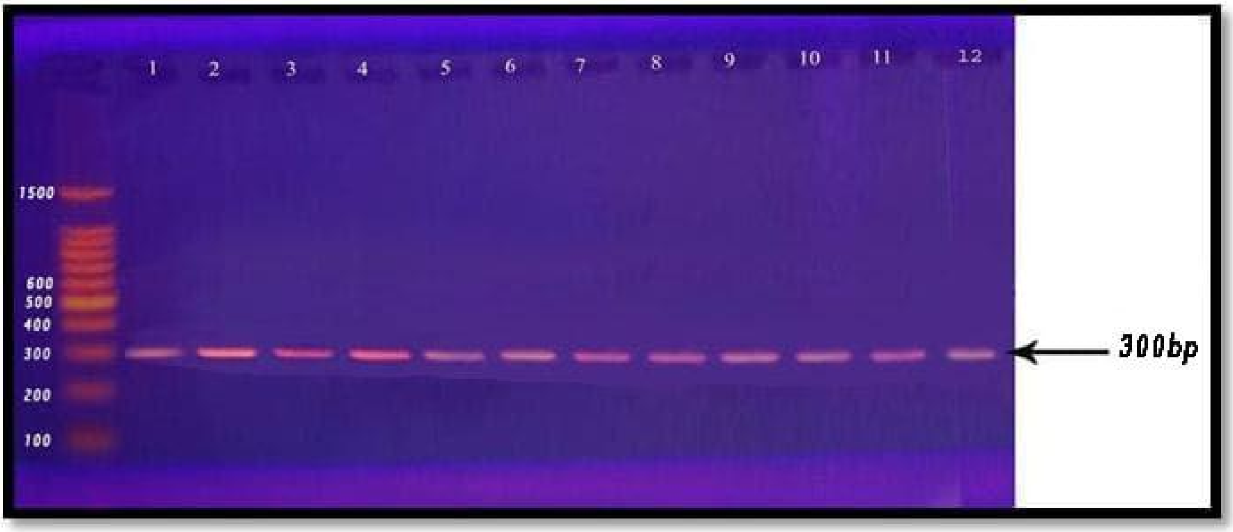

Figure (6): Agarose gel electrophoresis 50 minutes by 70 volts for 16S rRNA PCR products observed under U.V light at 301 nm after ethidium bromide staining. M: 1500 bp of ladder; lane (1-12) was positive for the jenjuni Campylobacter gene (300 bp).

Antibiotic Susceptibility Test for gram positive and negative Bacterial isolates:

In this study, the Antibiotic Susceptibility Test for gram positive and negative bacterial isolates was investigated. The results were compared according to the compact Vitek 2 system as susceptible, intermediate, and resistant. It has been found that most Gram-positive and Gram-negative isolates are highly resistant to beta lactam groups. It was found that *Streptococcus mutans* was resistant to Penicillin at a rate of (82.2%). In addition, these isolates were highly sensitive to Amoxicillin and Ciprofloxacin at a rate of (86.6%) and (71.1%) respectively, as shown in Table (3). *Streptococcus epidermidis* was highly resistant to Tetracycline at a rate of (88.4%) and highly sensitive to Amoxicillin at the same rate as shown in Table (4). The results of this study showed that *Streptococcus pneumonia* was highly sensitive to Gentamycin, Ciprofloxacin, Cefotaxime, Amoxicillin and Vancomycin at a rate of (78.2%) as shown in Table (5). *Staphylococcus aurous* were tested for antibiotics. It was found that these bacteria were highly sensitive to Meropenem at a rate of (94.7%) as shown in Table (6). *Streptococcus oralis* was highly resistant to Imperium (100%), and highly sensitive to Gentamycin, Ciprofloxacin, Cefotaxime, and Amoxicillin as shown in Table (7). In addition, gram negative bacteria were studied for antibiotic testing. It was found that *E. coli* was highly sensitive to Gentamycin, Imperium, Amoxicillin and Vancomycin (91.4%) as shown in Table (8), while *Fusobacterium nucleatum* was highly sensitive to Ciprofloxacin (95.12%) as shown in Table (9). *Campylobacter jenjuni*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Proteus mirabilis* were highly sensitive to most antibiotics used in this study, as shown in Table (10, 11, 12 and 13). Finally, *Lactobacillus acidophilus* was highly resistant to Penicillin in rate (82.5%) and sensitive to Ciprofloxacin in rate (88.5%) as shown in Table (14).

Table (3): Antibiotic Susceptibility Test for *Streptococcus mutans* isolates

Streptococcus mutans				
No.	**antibiotic**	**Resistance (45 isolates)**	**Intermediate (45 isolates)**	**Sensitive (45 isolates)**
1.	**Penicillin**	**37(82.2%)**	**6(13%)**	**2(4.4%)**
2.	**Tetracycline**	**21(46.6%)**	**4(8.8%)**	**20(44.4%)**
3.	**Imperium**	**33(73.3%)**	**4(8.8%)**	**8(17.7%)**

4.	Gentamycin	13(28.8%)	3(6.6%)	29(64.4%)
5.	Chloramphenicol	18(40%)	5(11.1%)	22(48.8%)
6.	Meropenem	20(44.4%)	7(15.5%)	18(40%)
7.	Ciprofloxacin	5(11.1%)	8(17.7%)	32(71.1%)
8.	Cefotaxime	17(37.7%)	3(6.6%)	31(68.8%)
9.	Amoxicillin	5(11.1%)	1(2.2%)	39(86.6%)
10.	Vancomycin	17(37.7%)	5(11.1%)	25(55.5%)
11.	Clindamycin	17(37.7%)	5(11.1%)	23(51.1%)

Table (4): Antibiotic Susceptibility Test for *Streptococcus epidermidis* isolates

Streptococcus epidermidis				
No.	**antibiotic**	**Resistance (26 isolates)**	**Intermediate (26 isolates)**	**Sensitive (26 isolates)**
1.	Penicillin	19(73%)	4(15.3%)	3(11.53%)
2.	Tetracycline	23(88.4%)	2(7.6%)	1(3.8%)
3.	Imperium	21(80.7%)	1(3.8%)	4(15.3%)
4.	Gentamycin	0(0.0%)	3(11.53%)	23(88.4%)
5.	Chloramphenicol	1(3.8%)	4(15.3%)	21(80.4%)
6.	Meropenem	3(11.53%)	2(7.6%)	21(80.4%)
7.	Ciprofloxacin	4(15.3%)	3(11.53%)	19(73%)
8.	Cefotaxime	5(19.23%)	4(15.3%)	17(65.3%)
9.	Amoxicillin	1(3.8%)	2(7.6%)	23(88.4%)
10.	Vancomycin	4(15.3%)	2(7.6%)	20(76.9%)
11.	Clindamycin	8(30.7%)	3(11.53%)	15(57.6%)

Table (5): Antibiotic Susceptibility Test for *Streptococcus pneumonia* isolates

Streptococcus pneumonia				
No.	**antibiotic**	**Resistance (23 isolates)**	**Intermediate (23 isolates)**	**Sensitive (23 isolates)**
1.	Penicillin	19(82.6%)	1(4.3%)	3(13.04%)
2.	Tetracycline	17(73.9%)	4(17.39%)	2(8.69%)
3.	Imperium	13(56.5%)	2(8.69%)	8(34.7%)
4.	Gentamycin	5(21.7%)	1(4.3%)	18(78.2%)
5.	Chloramphenicol	6(26.08%)	4(17.39%)	13(56.5%)
6.	Meropenem	8(34.7%)	3(13.04%)	12(52.17%)
7.	Ciprofloxacin	3(13.04%)	2(8.69%)	18(78.2%)
8.	Cefotaxime	4(17.39%)	1(4.3%)	18(78.2%)
9.	Amoxicillin	7(30.43%)	0(0.0%)	18(78.2%)
10.	Vancomycin	3(13.04%)	2(8.69%)	18(78.2%)
11.	Clindamycin	7(30.39%)	4(17.39%)	12(52.17%)

Table (6): Antibiotic Susceptibility Test for *Staphylococcus aurous* isolates

Staphylococcus aurous				
No.	**antibiotic**	**Resistance (19 isolates)**	**Intermediate (19 isolates)**	**Sensitive (19 isolates)**
1.	Penicillin	10(52.6%)	3(15.7%)	6(31.5%)
2.	Tetracycline	3(15.7%)	2(10.5%)	14(73.6%)
3.	Imperium	5(26.3%)	5(26.3%)	9(47.3%)
4.	Gentamycin	4(21.05%)	3(15.7%)	12(63.1%)
5.	Chloramphenicol	3(15.7%)	3(15.7%)	13(68.4%)
6.	Meropenem	1(5.2%)	0(0.0%)	18(94.7%)
7.	Ciprofloxacin	1(5.2%)	2(10.5%)	16(84.2%)

8.	Cefotaxime	8(42.1%)	4(21.05%)	7(36.8%)
9.	Amoxicillin	4(21.05%)	2(15.7%)	13(68.1%)
10.	Vancomycin	3(15.7%)	2(10.52%)	13(68.1%)
11.	Clindamycin	2(10.52%)	0(0.0%)	15(78.9%)

Table (7): Antibiotic Susceptibility Test for *Streptococcus oralis* isolates

Streptococcus oralis				
No.	**antibiotic**	**Resistance (5 isolates)**	**Intermediate (5 isolates)**	**Sensitive (5 isolates)**
1.	Penicillin	3(60%)	0(0.0%)	2(40%)
2.	Tetracycline	4(80%)	0(0.0%)	1(20%)
3.	Imperium	5(100%)	0(0.0%)	0(0.0%)
4.	Gentamycin	0(0.0%)	0(0.0%)	5(100%)
5.	Chloramphenicol	2(40%)	0(0.0%)	3(60%)
6.	Meropenem	1(20%)	1(20%)	3(60%)
7.	Ciprofloxacin	0(0.0%)	0(0.0%)	5(100%)
8.	Cefotaxime	0(0.0%)	0(0.0%)	5(100%)
9.	Amoxicillin	0(0.0%)	0(0.0%)	5(100%)
10.	Vancomycin	2(40%)	1(20%)	2(40%)
11.	Clindamycin	1(20%)	1(20%)	3(60%)

Table (8): Antibiotic Susceptibility Test for *E. coli* isolates

E. coli				
No.	**antibiotic**	**Resistance (35 isolates)**	**Intermediate (35 isolates)**	**Sensitive (35 isolates)**
1.	Penicillin	4(11.42%)	2(5.7%)	29(82.8%)
2.	Tetracycline	5(14.2%)	2(5.7%)	28(80%)
3.	Imperium	3(12%)	3(12%)	32(91.4%)
4.	Gentamycin	2(5.7%)	1(2.8%)	32(91.4%)
5.	Chloramphenicol	13(37.14%)	3(12%)	19(54.2%)
6.	Meropenem	15(42.8%)	1(2.8%)	19(54.2%)
7.	Ciprofloxacin	3(12%)	4(11.42%)	28(80%)
8.	Cefotaxime	6(17.1%)	2(5.7%)	27(77.1%)
9.	Amoxicillin	2(5.7%)	1(2.8%)	32(91.4%)
10.	Vancomycin	15(42.8%)	1(2.8%)	32(91.4%)
11.	Clindamycin	13(37.14%)	2(5.7%)	20(57.1%)

Table (9): Antibiotic Susceptibility Test for *Fusobacterium nucleatum* isolates

Fusobacterium nucleatum				
No.	**antibiotic**	**Resistance (41 isolates)**	**Intermediate (41 isolates)**	**Sensitive (41 isolates)**
1.	Penicillin	31(75.6%)	2(4.8%)	8(19.5%)
2.	Tetracycline	23(56.09%)	1(2.4%)	17(41.4%)
3.	Imperium	25(60.9%)	3(7.31%)	7(17.07%)
4.	Gentamycin	3(7.31%)	2(4.8%)	36(87.8%)
5.	Chloramphenicol	4(9.75%)	1(2.4%)	36(87.8%)
6.	Meropenem	3(7.31%)	3(7.31%)	35(85.3%)
7.	Ciprofloxacin	2(4.8%)	0(0.0%)	39(95.12%)
8.	Cefotaxime	3(7.31%)	1(2.4%)	37(90.2%)
9.	Amoxicillin	1(2.4%)	0(0.0%)	40(97.5%)
10.	Vancomycin	32(78.04%)	3(7.31%)	6(14.6%)
11.	Clindamycin	13(31.7%)	3(7.31%)	25(60.9%)

Table (10): Antibiotic Susceptibility Test for *Campylobacter jenjuni* isolates

Campylobacter jenjuni				
No.	**Antibiotic**	**Resistance (5 isolates)**	**Intermediate (5 isolates)**	**Sensitive (5 isolates)**
1.	Penicillin	2(40%)	1(20%)	2(40%)
2.	Tetracycline	2(60%)	0(0.0%)	2(40%)
3.	Imperium	2(40%)	1(20%)	2(40%)
4.	Gentamycin	0(0.0%)	0(0.0%)	5(100%)
5.	Chloramphenicol	1(20%)	1(20%)	3(60%)
6.	Meropenem	0(0.0%)	0(0.0%)	5(100%)
7.	Ciprofloxacin	0(0.0%)	0(0.0%)	5(100%)
8.	Cefotaxime	0(0.0%)	0(0.0%)	5(100%)
9.	Amoxicillin	2(40%)	1(20%)	2(40%)
10.	Vancomycin	2(40%)	0(0.0%)	3(60%)
11.	Clindamycin	2(40%)	1(20%)	2(40%)

Table (11): Antibiotic Susceptibility Test for *Pseudomonas aeruginosa* isolates

Pseudomonas aeruginosa				
No.	**antibiotic**	**Resistance (3 isolates)**	**Intermediate (3 isolates)**	**Sensitive (3 isolates)**
1.	Penicillin	1(33.3%)	0(0.0%)	2(66.6%)
2.	Tetracycline	0(0.0%)	0(0.0%)	3(100%)
3.	Imperium	0(0.0%)	0(0.0%)	3(100%)
4.	Gentamycin	0(0.0%)	1(33.3%)	2(66.6%)
5.	Chloramphenicol	3(100%)	0(0.0%)	0(0.0%)
6.	Meropenem	2(66.6%)	1(33.3%)	2(66.6%)
7.	Ciprofloxacin	0(0.0%)	0(0.0%)	3(100%)
8.	Cefotaxime	0(0.0%)	0(0.0%)	3(100%)
9.	Amoxicillin	0(0.0%)	0(0.0%)	3(100%)
10.	Vancomycin	3(100%)	3(100%)	0(0.0%)
11.	Clindamycin	3(100%)	3(100%)	0(0.0%)

Table (12): Antibiotic Susceptibility Test for *Klebsiella pneumonia* isolates

Klebsiella pneumonia				
No.	**antibiotic**	**Resistance (5 isolates)**	**Intermediate (5 isolates)**	**Sensitive (5 isolates)**
1.	Penicillin	0(0.0%)	0(0.0%)	5(100%)
2.	Tetracycline	5(100%)	0(0.0%)	0(0.0%)
3.	Imperium	3(60%)	0(0.0%)	2(40%)
4.	Gentamycin	2(40%)	1(20%)	2(40%)
5.	Chloramphenicol	3(60%)	0(0.0%)	2(40%)
6.	Meropenem	1(20%)	0(0.0%)	4(80%)
7.	Ciprofloxacin	0(0.0%)	0(0.0%)	5(100%)
8.	Cefotaxime	0(0.0%)	0(0.0%)	5(100%)
9.	Amoxicillin	0(0.0%)	0(0.0%)	5(100%)
10.	Vancomycin	4(80%)	0(0.0%)	1(20%)
11.	Clindamycin	3(60%)	1(20%)	1(20%)

Table (13): Antibiotic Susceptibility Test for *Proteus mirabilis* isolates

Proteus mirabilis				
No.	**antibiotic**	**Resistance (3 isolates)**	**Intermediate (3 isolates)**	**Sensitive (3 isolates)**
1.	Penicillin	2(66.6%)	0(0.0%)	1(33.3%)
2.	Tetracycline	3(100%)	0(0.0%)	0(0.0%)
3.	Imperium	1(33.3%)	2(66.6%)	0(0.0%)
4.	Gentamycin	0(0.0%)	0(0.0%)	3(100%)
5.	Chloramphenicol	0(0.0%)	0(0.0%)	3(100%)
6.	Meropenem	0(0.0%)	0(0.0%)	3(100%)
7.	Ciprofloxacin	0(0.0%)	0(0.0%)	3(100%)
8.	Cefotaxime	1(33.3%)	0(0.0%)	2(66.6%)
9.	Amoxicillin	0(0.0%)	1(33.3%)	2(66.6%)
10.	Vancomycin	3(100%)	0(0.0%)	0(0.0%)
11.	Clindamycin	3(100%)	0(0.0%)	0(0.0%)

Table (14): Antibiotic Susceptibility Test for *Lactobacillus acidophilus* isolates

Lactobacillus acidophilus				
No.	**antibiotic**	**Resistance (40 isolates)**	**Intermediate (40 isolates)**	**Sensitive (40 isolates)**
1.	Penicillin	33(82.5%)	2(5%)	5(12.5%)
2.	Tetracycline	23(57.5%)	3(7.5%)	15(37.5%)
3.	Imperium	18(45%)	2(5%)	20(50%)
4.	Gentamycin	4(10%)	3(7.5%)	33(82.5%)
5.	Chloramphenicol	30(75%)	3(7.5%)	7(17.5)
6.	Meropenem	12(48%)	4(10%)	24(60%)
7.	Ciprofloxacin	4(10%)	3(7.5%)	33(88.5%)
8.	Cefotaxime	5(12.5%)	1(2.5%)	34(85%)
9.	Amoxicillin	13(32.5%)	2(5%)	25(26.5%)
10.	Vancomycin	15(37.5%)	3(7.5%)	22(55%)
11.	Clindamycin	30(75%)	3(7.5%)	7(17.5%)

Discussion:

In this study, many types of pathogenic bacteria were isolated from dental caries. This was in agreement with many studies (26, 27, 28, 29). Mutant streptococci and lactobacilli were more frequently isolated from dental caries (26). The prevalence of cariogenic bacteria is due to dental caries. Cariogenic microorganisms are pathogenic factors that contribute to oral micro-environment acidification, linked to caries start and progression. Streptococcus mutans is a recognized cariogenic bacterium (30). Pathogenic factors have an important role in the formation of caries in oral bacteria. The alliance of various pathogens helps to trigger and develop diseases (31). The composition of oral microbiota may vary readily via food and the environment (32). Dental cavities are pathogenised by acidogenic and aciduric bacteria in the tooth biofilm (33). Esberg *et al*., (34) found that major oral bacteria that have been discovered with their rRNA 16S sequences include the lactobacillus, Streptococcus oralis, Rothia mucilagino and Kingella oralis, and Fusobacterium on the tongue surface. The formation of the oral microbial community includes both competition and synergy between the hundreds of species in the mouth cavity. In the human mouth cavity, bacterial populations are continuously changing (35). The need to identify and characterize these bacteria by suitable fast detection procedures may help create future clinical treatment strategies to improve oral health (36). Streptococci in the human oral cavity are the major bacterial species. In the oral cavity, several species of this gram-positive coccus have been discovered. These include strep, pneumonia, pneumonia, oral strep, and oral strep. All may be regarded as major oral or systemic pathogens (37). Oral bacteria have developed methods for sensing and evading or modifying the host. Both the surface of the tooth and the gingive epithel inhabit the same ecological niche (38).

A very effective innate host defense mechanism, however, continuously checks bacterial colonization and inhibits local tissue bacterial invasion. There is a dynamic balance between the dental plaque bacteria and the inherent host protection system (39). These results were in agreement with results of Nomura *et al*., (40), Vergalli *et al*., (41) who found that a small percentage of gram-negative bacteria come from the scarcity of their presence in the environment, according to what many studies have confirmed, including a study by Eberlein *et al*., (42). This is due to the fact that most gram-negative bacteria come from infections of the respiratory system or gastro-intestinal tract and appear in the mouth, and that is consistent with the findings of the study by Behzadi *et al*., (43). This may be due to poor hygiene, the intromission of contaminated tools into the mouth, and playing with soil that leads to the pathogen entering from the external environment into the mouth. Bacteria enter by water, food, air, and hands (44). An antibiotic susceptibility test was done for the bacteria isolates *Streptococcus mutans*, *Streptococcus epidermidis*, *Streptococcus pneumonia*, *Staphylococcus aurous, Streptococcus oralis*, *Lactobacillus acidophilus, Fusobacterium nucleatum, E. coli, Campylobacter jenjuni, Klebsiella pneumonia, Pse* Some antibiotics were used to show the effect on different types of bacteria isolated from dental caries patients. It has been found that there is clear variation in resistance, and most isolates show resistance to one or more of these antibiotics. The results were compared according to (45) as susceptible, intermediate and resistant. It has been found that all Gram-negative and Gram-positive isolates are highly resistant to the beta lactam group. The results are almost identical to those obtained by (46) who have pointed out that these bacteria produce chromosomally encoded beta lactamases that mediate beta lactam and cephalosporin resistance and by a decrease in cell permeation of these antibiotics through modification of the outer membrane proteins (pores) (47).

Multiple antibiotic resistance, including Penicillin and Tetracycline Imperium, has usually risen in many Gram-positive bacteria. ESBLs are a set of enzymes which hydrolyse cephamical cephamics such as Cephtazidime, Cefotaxime, Ceftriaxone, and Monobactam, like Aztreonam, but do not hydrolyse cephamicins such as Cefoxitin (48). Most ESBLs are also able to hydrolyze cephalosporins of the fourth generation, such as Cefepime (49). In Gram-positive bacteria, every known mechanism of resistance to -lactam antibiotics may be identified. These mechanisms include the -lactamase production of the -lactam ring controlled by plasmid or chromosomal regulation by different bacteria, or the absence of protein receptors on the cell walls and changes in the durability of -lactam antibiotics, preventing the use of antibiotics by blocking the pores of the external membrane (50). In the current study, the susceptibility of Ciprofloxacin bacterial isolates was tested. Most of the bacterial isolates of *St. mutans*, *K. pneumoniae*, *S. aureus,* and *E. coli* were susceptible to ciprofloxacin. These results were consistent with results achieved by Domalaon et al., (51) who found that the majority of Gram-positive and Gram-negative isolates were susceptible to Ciprofloxacin was only a medicine of choice for S. aureus owing to the blockage of DNA gyrase, which is in agreement with Sader et al. (52). Ciprofloxacin was a bactericidal medicine that impacted gram-negative and gram-positive bacteria as well as fluroquinolone resistance through chromosomal mutations or alternations that impaired fluroquinolone permeation into the bacterial cell wall. Aminoglycosides represented by Gentamicin were also utilized (53).

Conclusion:

Information on the sort of microorganisms that occupy the oral pit is important in anticipating and forestalling dental infections as well as the related fundamental entanglements brought about by them. An anti-microbial defenselessness test recognized numerous sorts of microorganisms detached from dental caries patients. Gram positive microscopic organisms were protected from beta-lactam anti-toxins.

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