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Assessment of Antibiotics and Fluoride Toothpastes against Pathogenic Bacteria associated with Dental Caries

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Abstract

Dental caries is the most contagious disease throughout the world. In the present study, a total of 100 dental caries patients were selected and samples from these patients were collected through sterile pincers for microbiological examination. A total of 9 different pathogenic bacterial isolates were identified through conventional culturing technique and among them maximum number of occurrence was by *Salmonella spp* (26.13%), followed by *E. coli* (17.39%), *Proteus spp* (17.39%), *Shigella spp* (8.69%), *Pseudomonas spp* (8.69%), *Vibrio spp* (8.69%), *Enterobacter spp* (4.34%), *Yersinia spp* (4.34%) and *S. mutans* (4.34%). Furthermore, antibiotic susceptibility tests were performed by 9 different commercially available antibiotics i.e. vancomycin, erythromycin, clindamycin, amoxicillin, tetracycline, rifampicin, ampicillin, ticarcillin and metronidazole/silver sulphadizine. Kirby Bauer disc diffusion method was used against test organisms and it was observed that all these antibiotics expressed significant activity against greater part of test organisms but few of them showed resistance to metronidazole/silver sulphadizine. In addition to this, different toothpastes encoded as TP-1, TP-2, TP-3, TP-4 and TP-5 were used at a concentration of 20 mg/mL in order to evaluate their activity against test organisms and it was observed that all these toothpastes showed significant activity against isolated bacteria. It was concluded that all these toothpastes contained fluoride which supports to avoid tooth decay and promote minty fresh breath. Furthermore, it was recommended that avoid usage of too much sweets, chocolates, candies especially in children/adults, and there must be a proper use of toothpastes twice a day at every stage of life.

Keywords: Dental Caries, Pathogenic Bacteria, Antibiotics, Fluoride Toothpastes

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1. INTRODUCTION

Dental caries and dental plaque are contagious infections caused by microorganisms, which is one of the leading chronic infections of the world [1]. They are due to bacterial decomposition of the enamel and dentin of teeth, and if left untreated, it can cause extensive pain and distress [2]. Dental caries is the disintegration

of teeth due to the actions of microorganisms and as a result the colors of cavities may be different e.g. from yellow to black and categorized through alternating stages of de-mineralization and re-mineralization, that causes the cavities in the teeth and finally tooth loss [3, 4, 5]. On the other hand, a sticky colorless covering of bacteria and sugar is termed as plaque so, it is consistently grow up on teeth, which is the primary element of decay and gum infection [6]. In both cases, high level of lactic acid is produce by bacteria after subsequent utilization of nutritional sugars; however these bacteria survive at low pH and have vital carcinogenic properties [7]. Symptoms of dental caries usually include pain and difficulty during eating, foul smelling from mouth and swelling of the face. A vast range of oral bacteria are present in mouth, among them *Streptococcus mutants* and *Lactobacillus* species are believed to be responsible for dental caries [3].

The leading risk aspect in the growth of dental caries is occupation of teeth by carcinogenic bacteria with *S. mutans* being the main species causing early dental caries development. *S. mutans* ferments sucrose to make extracellular glucan, a polysaccharide mysterious in water, enabling the bacteria to get attached to the surface of the teeth and similarly protect the bacteria from external factors e.g. mechanical disturbance, salivary clearance and antimicrobial materials [2]. Additional risk factors responsible for dental caries include (i) having no regular tooth brushing (ii) eating tobacco and (iii) deprived oral hygiene. Bacterial plaque deposited on the teeth surfaces consist of “inborn oral flora” which is the leading causative agent responsible for the formation of dental caries [2, 3].

One of the utmost common long-term contagious diseases in the developing countries is dental caries and teeth erosions [8]. The destruction of enamel or dentin is produce by dental caries due to bacteriological actions. In developed countries the frequency of dental caries is decreasing, however growing in developing countries with deprived socioeconomic status. Factors that affect dietary and oral hygiene status even in the western countries include income, education, and social status. Individuals with low socioeconomic status have more dental cavities as compared to high socioeconomic status [9]. Above 80% of the general population are infected with dental caries forming it one of the leading infections in human beings. In youngsters and adults when the teeth pain is too much due to dental caries, physician often recommended complete removal of infected teeth. The reason behind it is the excessive usage of candies, chocolates and lack of fluoride in teeth [9]. A group of researchers proposed that lack of oral hygiene and untreated dental caries may lead to stomach ulcer, gastric cancer, cardiac diseases and several other severe disorders [10]. According to a survey report, about 6-21% of adults throughout the world loss their teeth due to severe gingivitis [9]. Furthermore the physical, psychological, social impacts of dental caries are known to reduce the quality life style [5]. Dental caries being a multifactorial disease is associated with a number of factors, for example age, gender, cultural relationship, deficiency of oral hygiene and socioeconomic status. In Pakistan, dental hygiene has been ignored for years and still it is not the main problem [11].

Antibiotics are commonly prescribed for the treatment of dental caries but resistance cause by oral pathogens to commercially available antibiotics is a global issue. Resistance of bacteria to these antibiotics consists of unlimited problems in both the public and hospital setting; moreover, increase in expenditures for quality health is a universal issue. Resistance to antibiotics doesn’t always occur but generally related with significant morbidity, long hospital stay, heavy expenses and mortality. Heavy expenses related with resistant microbes might be due to, requirement of using costly drugs, being hospitalized for longer period of time, higher mortality, delay in proper treatment or necessary to do operation. Excessive usage of antibiotics has created another main problem of increase in incidence of multi-drug resistant pathogens [12].

Professional hygiene care includes the regular dental check-ups and the use of toothpaste having high concentrations of fluoride. It is the most important practice in order to prevent the dental caries. Toothpastes should have the ability to eliminate plaque, stains and debris and to prevent the scraping of tooth coating or harm gums [2]. There should be proper use of toothpaste on daily basis in order to take proper care of teeth e.g. drinking of beverages, a high application of deodorant can assist to destruction of enamel and dentine [13]. The teeth surfaces are usually stained due to large use of coffee or smoking. Whitening tooth pastes usually used to remove the stains and to make the teeth whiten [14].

The aim of the present research study is to investigate comparative assessment of antibiotics and fluoride toothpastes against pathogenic bacteria associated with dental caries in patients visiting different dental clinics in district Peshawar. Data of this research study will help in the selection of appropriate treatment regime for dental caries and dental plaque. Moreover, it will also provide a guideline to select proper nutritional diet and toothpastes in order to minimize the risks associated with dental caries.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 100 dental carries samples were collected from patients visiting different dental clinics in district Peshawar through sterile pincers and also patient's clinical history profile was noted down. Furthermore, patients were distributed into 3 different sets according to age wise i.e. Group-I contained patients having age between 11-20 years, Group-II contained patients having age between 21-30 years and Group-III included patients having age between 31-above 50 years. Dietary pattern was also planned for these groups i.e. Group-I eat extra sugar stuffs, Group-II eat all food stuffs in optimum quantity while Group-III eat intoxicating drinks. After collection of dental caries samples and history reports, samples were transferred to the Microbiology Research Laboratory (MRL), Abasyn University Peshawar and preserved at 4^{o}C within refrigerator and further microbiological analysis were carried out within one month.

2.2 Growth Media for Bacterial Isolation

Basal salt (minimal salt media) intermediate with yeast extract and nutrient agar media were used in the present study according to standard protocols for isolation of bacterial isolates from dental caries [15]. About 25 mL of basal salt broth was prepared for each sample in flask. After inoculation, basal salt broth was incubated at 35^{o}C for 2 days. After incubation, about 1 mL of recently grown culture was successively diluted up to 10^{-5} with sterile distilled water. About 100 µL of diluted samples were used to cover the surface of basal salt agar plates and after that plates were kept in incubator at 37^{o}C under aerobic conditions for about three days. After incubation, bacterial isolates were identified by pure culturing technique i.e. determining culture characteristics, Gram staining and biochemical tests [16].

2.3 Evaluation of Antibiotic Sensitivity Profile of Isolated Bacteria

Antibiotic sensitivity profiling was done for the evaluation of susceptibility pattern of bacterial isolates towards different antibiotics. List of antibiotics used in this study included Vancomycin (VA), Erythromycin (E), Clindamycin (DA), Amoxicillin (AML), Tetracycline (TE), Rifampicin (RD), Ampicillin (AM), Ticarcillin (TIC), and Metronidazole/silver sulphadizine (MTZ) according to standard guidelines [17]. The antibiotic discs were stored at 4°C for the prevention of potency loss before use.

Muller Hinton agar media was prepared and autoclaved and after autoclaving cooled to 50°C. About 50 mL agar was then transferred to the sterile petri plates. The plates were left half open for 15-20 min in order to solidify the agar and to remove the moisture on a leveled surface for uniform depth and the media was incubated overnight for sterility check. From the fresh culture, the colony was picked and immersed in 1 mL normal saline in a sterile glass tube and mixed properly. With the help of sterile cotton swab fresh bacterial isolates were picked and then then spread over the surface Muller Hinton agar plates in order to make a lawn. The antibiotic discs were then placed on the inoculated Muller Hinton's agar plates with the help of sterile fine forceps. All the antibiotics disc were placed following the standard protocol. Plates were incubated at 37°C for 24 hrs and after incubation; zone of inhibition around each disc was measured in millimeter and then compared with the corresponding values given in standard guidelines [17].

2.4 Determination of Antibacterial Activity of Toothpastes against Isolated Bacteria

About 5 different commercially available toothpastes encoded as TP-1, TP-2, TP-3, TP-4 and TP-5 were used in the current study while the outcomes of toothpastes were established on content they contained. Stock solutions (500 mg/mL) of these toothpastes were prepared and then a dilution of 20 mg/mL for each toothpaste was prepared from it by using distilled water. Agar well diffusion method was used to assess the antibacterial activity of toothpastes against pathogenic bacterial isolates. Nutrient agar plates were prepared and with the help of glass spreader approximately 25 µL inoculum of each test organism was

uniformly distributed over the surface of media plates. Wells about 8 mm diameter were bored with the help of cork borer and then about 50 µL of each toothpaste were transferred into the wells. The plates were then incubated at 37°C for 24 hrs and after incubation, zone of inhibition were measured in millimetre.

3. RESULTS AND DISCUSSIONS

In the present research study, it was observed that 42 patients were from female group and 58 patients were from male group among total 100 patients and the female to male ratio in the current study was 1:1.3 which showed that the overall prevalence of dental caries was more common in males as compared to females. Furthermore it was also perceived that the maximum number of patients affected due to dental caries were in the age group of 21-30 years (48%) followed by 11-20 years (20%) and 31-above 50 years age group (32%) as shown in Fig. 1. A basic reason for this might be that they were mostly busy in their routine daily life and did not use proper toothpastes on regular basis so there were maximum chances of cavities. Another reason for this might be that most of the adults were willing to eat chocolates, sugar, candies etc. which facilitate the growth of microorganisms so that's why a high percentage was observed in a group of patients having age in the range of 21-30 years. The results of current study coincide with the results of different research group who divided the patients into different groups according to their age wise and observed that age factor was important parameter while studying dental cavities due to different daily routine of diets [18].

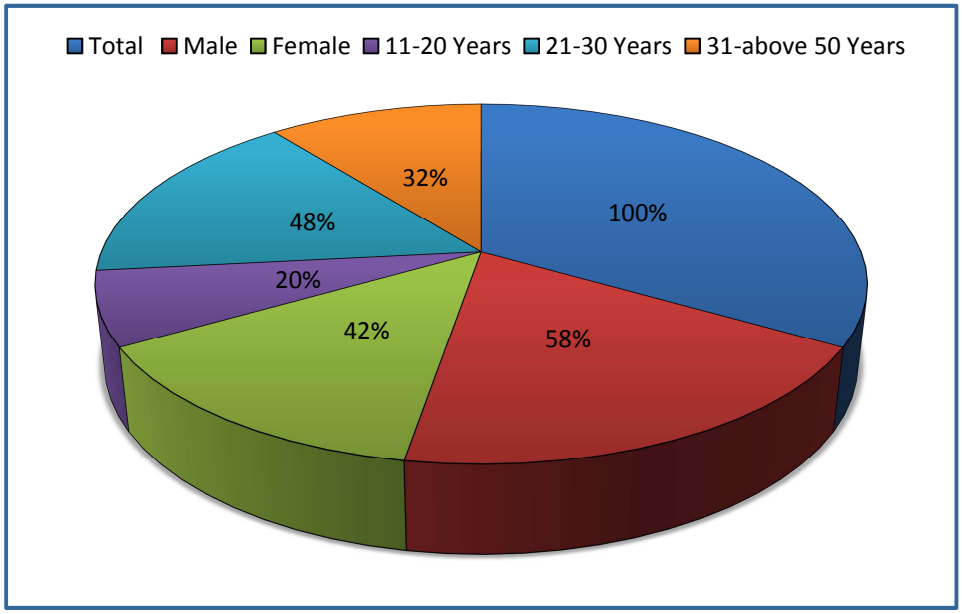

Fig. 1 Distribution of patients on the basis of Age and Gender Wise.

Moreover, Fig. 2 summarizes the data showing it clearly that the prevalence of dental caries were more common in illiterate peoples as they didn't aware about the oral health and the impact of oral hygiene on the quality of life. About 50% of the patients included in the study were illiterate, 10% were matriculate, 25% were intermediate and the remaining 15% of the patients were those having higher education level.

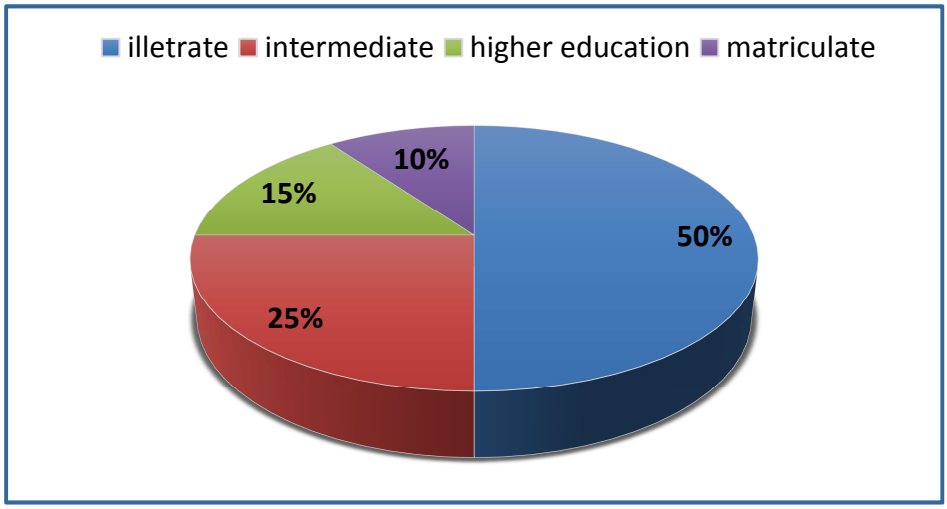

Fig. 2 Distribution of patients on the basis of Education.

3.1 Bacteriological Analysis of Dental Caries Samples

Isolated bacteria were characterized according to Bergey's Manual of Determinative Bacteriology (9th Edition). On the basis of microscopy and biochemical tests, 9 different bacterial isolates were identified in the present study, out of which, 4 were Gram positive and 5 were Gram negative. After microscopy, these bacterial isolates were sub-cultured on nutrient agar media plates and different cultural characteristics were observed and then complete identification of these bacterial isolates were carried out by performing biochemical tests. It was observed that the maximum number of occurrence was by *Salmonella spp* (26.13%), followed by *E. coli* (17.39%), *Proteus spp* (17.39%), *Shigella spp* (8.69%), *Pseudomonas spp* (8.69%), *Vibrio spp* (8.69%), *Enterobacter spp* (4.34%), *Yersinia spp* (4.34%) and *S. mutans* (4.34%). A group of researchers performed similar study to isolate different pathogenic organism from the dental caries and observed *S. mutans* and *Streptococcus sobrinus* as dominating bacteria in dental caries samples [2]. Another group of researchers also performed a similar study to isolate and characterized 96 strains of *S. mutans* from the dental plaques in Koreans using bacterial cultivation with selective media, molecular biological methods and biochemical tests [19]. Different researchers also isolated different bacterial strains from dental caries by using pure culture technique and observed that *Streptococcus salivarias* was dominating in healthy children while *Streptococcus sanguis* was leading strain among childrens who were disabled [20]. In addition to this, different researchers in different era isolates different bacterial species from dental caries by using conventional culturing technique [18, 21]. Table 1 gives us detailed description of microscopy, cultural and biochemical tests of all identified bacterial isolates with respect to Bergey's Manual (9th Edition) .

Table 1. Detailed description of microscopy, cultural and biochemical tests of bacterial isolates.

Isolate #	Cultural characteristics	Gram's Reaction	Citrate Test	Indole Test	Oxidase Test	Coagulase Test	Urease Test	Catalase Test	TSI test	Identified Organisms
1	Small, pale yellow, circular, translucent, smooth, flat	+	-	+	-	+	-	+	K/A	*Escherichia coli*
2	Large, off white, irregular, opaque, smooth, flat	-	-	-	-	+	-	+	-	*Salmonella spp.*
3	Large, off white, irregular, opaque, rough, lobed	+	+	+	-	+	+	+	K/NC	*Proteus spp.*
4	Pinpoint, yellow, circular, opaque, smooth, lobed	+	-	+	-	+	-	+	-	*Shigella spp.*
5	Small, white, circular, opaque, rough, raised	+	-	+	-	-	-	-	A/NC	*S. mutans*
6	Pinpoint , off white, circular, translucent, smooth	-	+	-	+	-	-	+	K/A, H_2S	*Pseudomonas spp.*
7	Large, off white, irregular, opaque, smooth, flat	-	+	-	-	-	-	+	K/A, H_2S	*Enterobacter spp.*
8	Small, off white, irregular, translucent, smooth, flat	-	-	+	+	+	-	+	A/NC	*Vibrio spp.*
9	Pinpoint, off white, regular, opaque, smooth, flat	-	-	-	-	-	+	+	A/A	*Yersinia spp.*

Key: + = Positive; - = Negative; A = Acid production; K = alkaline reaction; NC = No change; H_2S = Sulfur reduction; K/A = Red/yellow; K/NC = Red/no color change; K/A, H_2S = Red/yellow with bubble and black precipitate; A/NC = Acid/no color change; A/A = Yellow/yellow.

3.2 Cultural Sensitivity Pattern of Different Toothpastes against Bacterial Isolates

In the present research study, different toothpastes i.e. TP-1, TP-2, TP-3, TP-4 and TP-5 were used in a concentration of 20 mg/mL to evaluate their activity against bacterial isolates. It was observed all these toothpastes were highly effective against test organisms as shown in Fig. 3. Moreover, these toothpastes were suitable to antibiotic effectiveness and they also contained fluoride which supports to avoid tooth decay and promote minty fresh breath as similar to reports by Cheesbrough, 2002. A group of researchers also used agar well diffusion method to evaluate antiseptic activity of commercially available different fluoride toothpastes against *Streptococcus spp* and found that these toothpastes had tremendous antiseptic activity against *Streptococcus spp* [2]. Another group of researchers determined that the caries might be influenced by the anti-microbial effect of fluoride, as by the usage of fluoride toothpastes there might be high chance of reduction of the *S. mutans* count [22]. In addition to this, it was observed that TP-1 toothpaste showed maximum activity against *Yersinia spp* while the activity of TP-1 was also significant against other isolated bacteria because it contained fluoride as a major constituent which played a major role in the eradication of such type of pathogenic organisms. Another group of researchers reported in their study about different toothpastes that contained fluoride as major constituents to determine their activity against pathogenic bacterial isolates from the dental caries and in addition to this they also utilized other anticaries agents including mouthwash and teeth polishers [23]. They determined that fluoride had strong antibacterial activity and moreover, they also helped to give strengthened to the teeth by providing calcium phosphate to the teeth enamel. Another group of researchers described the mechanism that how fluoride toothpaste had anticaries activities and found that topical application of fluoride was more effective as compared to systemic use because within digestive tract it would be change into inert compounds by reacting with the gastric contents [24]. Systemically if we use fluoride contents they have no effect on such type of pathogenic organisms but topical application of such type of toothpaste had tremendous effects against the pathogenic isolates.

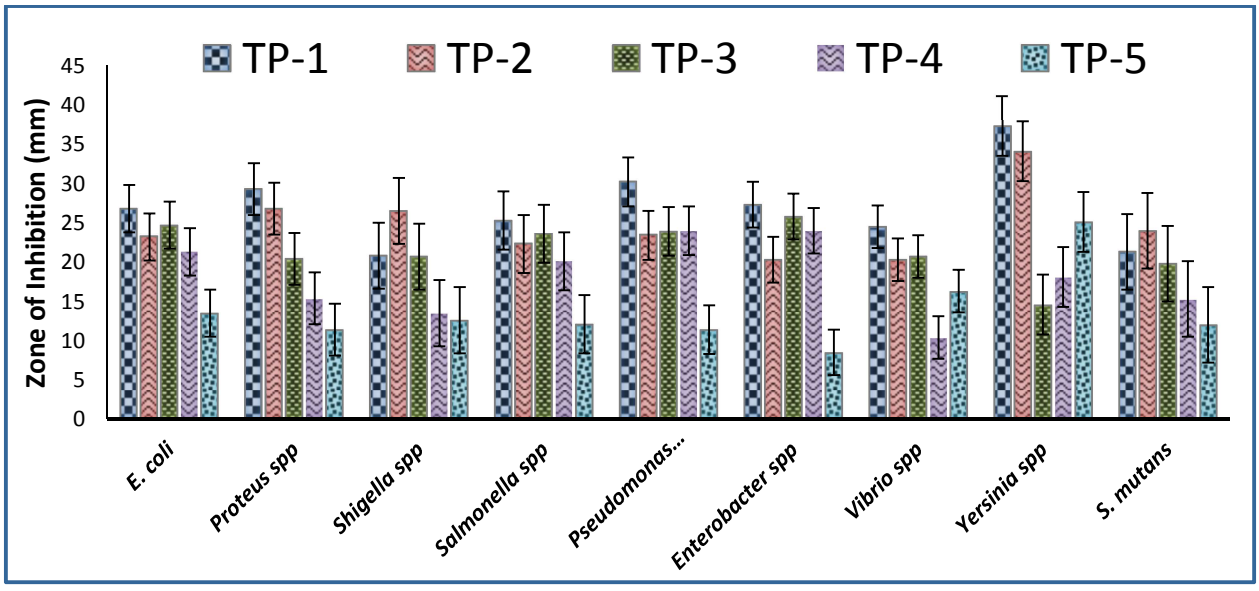

Fig. 3 Cultural sensitivity pattern of different toothpastes against bacterial isolates *(Bars indicate standard deviation)*.

3.3 Antibiogram Analysis of Bacterial Isolates against commercially available Antibiotics

A group of researchers investigated that most the organisms isolated from dental caries showed resistance to varieties of antibiotics and a basic reason for this resistance might be a needless use of antibiotic prescription in dentistry [25]. For the distribution of resistance among *Streptococci*, different therapies of antibiotics contribute great share while treating dental infections, the resistance of oral

bacteria towards antibiotics had become a great problem. A shift from narrow spectrum antibiotics to broad spectrum antibiotics prescriptions had been reported in the recent years due to increase in antibiotic resistance [26]. A possible reason for this might be that microorganisms showed genetic exchange as a result those organisms which initially did not showed resistance to antibiotics were now resistance strains.

In the present study Kirby Bauer disc diffusion method was used to study the antibiogram analysis of bacterial isolates against 9 different commercially available antibiotics i.e. Vancomycin (VA), Erythromycin (E), Clindamycin (DA), Amoxicillin (AML), Tetracycline (TE), Rifampicin (RD), Ampicillin (AMP), Ticarcillin (TIC), and Metronidazole/silver sulphadizine (MTZ). These antibiotics were stored at 2-8°C for the prevention of potency loss. In the Table 2, it was observed that vancomycin and rifampicin showed better activity against *Proteus spp* and *Salmonella spp* while tetracycline showed better activity against *E. coli*, *Proteus spp, Shigella spp, Salmonella spp* and *Pseudomonas spp*. Further, amoxicillin showed better activity against *E. coli*, *Proteus spp, Salmonella spp, Enterobacter spp* and *Vibrio spp,* while rifampicin and ticarcillin showed significant activity against *E. coli*, *Proteus spp, Shigella spp, Salmonella spp, Pseudomonas spp, Enterobacter spp* and *Vibrio spp*. On the other hand, metronidazole/silver sulphadizine and erythromycin showed better activity against *E. coli*, *Proteus spp, Salmonella spp, Enterobacter spp* and *Yersinia spp* while ampicillin showed substantial activity against *Proteus spp, Salmonella spp, Pseudomonas spp* and *Enterobacter spp* which coincide with the findings of previous results [2]. Moreover, different researchers evaluated antimicrobial susceptibility pattern of pathogenic bacteria isolated from dental plaque against commercially available antibiotics and found similar results [20]. Beside this, a group of researchers mentioned that the oral microflora had highest percentage of resistant to different antibiotics used in the study and also found that most of strains showed resistance to metronidazole/silver sulphadizine [27]. Another group of researchers determined the occurrence, proportions and identities of oral bacteria resistant to six antibiotics in 35 children (4-5 years old) who had not received antibiotics while 432 antibiotic-resistant isolates were recovered which included 18 genera and 47 species and among them most of organisms showed resistance to ampicillin and tetracycline antibiotics [28].

Table 2. Antibacterial susceptibility pattern of different antibiotics against test organisms.

	Zone of Inhibition (mm*)								
Antibiotics	***E. coli***	***Proteus***	***Shigella***	***Salmonella***	***Pseudomonas***	***Enterobacter***	***Vibrio***	***Yersinia***	***S. mutans***
Clindamycin (DA)	25	18	18	26	25	10	12	19	10
Vancomycin (VA)	15	21	14	19	14	15	14	12	13
Tetracycline (TE)	20	29	18	20	27	8	12	15	14
Amoxicillin (AML)	18	24	10	23	13	23	26	9	9
Rifampicin (RD)	14	25	11	35	17	8	11	11	11
Ticarcillin (TIC)	23	28	19	19	20	22	24	15	15
Metronidazole (MTZ)	10	14	7	8	7	8	8	9	8
Erythromycin (E)	25	28	15	29	11	26	10	15	9
Ampicillin (AMP)	10	30	10	25	18	20	13	9	14

Key: mm = millimeter

4. CONCLUSIONS

It was concluded from the present research study that among 9 different bacterial species isolated from dental caries through pure culturing technique, maximum number of occurrence was by *Salmonella spp* (26.13%) followed by *E. coli* (17.39%), *Proteus spp* (17.39%), *Shigella spp* (8.69%), *Pseudomonas spp* (8.69%), *Vibrio spp* (8.69%), *Enterobacter spp* (4.34%), *Yersinia spp* (4.34%) and *S. mutans* (4.34%). Furthermore, 9 different commercially available antibiotics were tested against bacterial isolates and all these antibiotics expressed significant activity against greater part of test organisms but few of them showed resistance. While, a greater percentage of the antibiotic resistant bacteria identified were members of the normal flora of oral cavity however, some pathogenic and opportunistic pathogenic species were also identified. The commonly misuse of antibiotics by the dentists lead to the prevalence of resistant bacteria. Moreover, eating of sweets, toffees, chocolates, and irregular use of toothbrush might also lead to the formation of dental cavities. Beside this, five different toothpastes (TP-1, TP-2, TP-3, TP-4 and TP-5) were evaluated in the present research study against test organisms and it was concluded that all these toothpastes showed significant activity against isolated bacteria but among these, TP1 showed better activity as compared to other toothpastes.

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CONFLICT OF INTEREST

All authors declare no conflict of interest regarding this article.

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