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Bacteriological Characterization of Pathogenic Bacteria Isolated from Dental Patients

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ABSTRACT

This study aimed to isolate and characterize bacterial dental caries and for this purpose, 60 samples were collected from people with caries Tooth decay for the period from the beginning of May 2018 to the end of September 2020, for both sexes, at different ages ranging from (7-60) years, Then they were collected and transported directly to the laboratory and the samples were planted by plotting on plates of tryptic soy blood agar anaerobic conditions, Only 13 specimen showed a positive result with (21.7%) percentage, the most common bacteria which responsible for dental cavities are Streptococcus mutans, Staphylococcus aureus and Enterococcus faecalis. Here, three pathogenic bacteria *S. aureus, S. mutans and E. faecalis* were identified by Bergey's Manual of Systematic Bacteriology, Antibiotic sensitivity patterns of the isolated pathogenic bacteria were assessed against the selection of antibiotics. these types of bacteria have been shown high resistant to different antibiotics like *Erythromycin, Methicillin, Oxacillin, Cephalexin, Aztreonam, Amikacin, Gatifloxacin, Chloramphenicol, Gentamicin, Norfloxacin, Pencillin-G* and *Ceftazidime* while showed sensitivity to some antibiotics like Trimethoprim-sulfamethoxazole, Ciprofloxacin, Amoxicillin, Levofloxacin, Nitrofurantoin and Vancomycin, and using antimicrobial agents, as a biocontrol agent, against certain antibiotic-resistant bacteria causing dental caries, To overcome this problem, by applying different strain of probiotics e.g., *Lactobacillus acidophilus, Bifidobacterium bifidum, Lactobacillus plantarrum, Lactobacillus rhamnosus, Enterococcus faecalis, Bacillus subtilis,* Yeast, and *Lacteolfort capsules* were applied against the three pathogenic bacteria which showed growth inhibition of these isolates , the zone of inhibition has high efficiency in case of *Lactobacillus* spp than *Bifidobacterium* spp even though against those with antibiotic-resistant pattern. There was another propagation used to overcome the bacterial dental caries which was mouthwashes propagation e.g., chlorohexidine, which showed high efficiency in the case of *S. aureus* then *S. mutans* and *E. faecalis*, this study refers that the isolated probiotics and mouthwashes are promising biocontrol agents that could challenge antibiotic-resistant dental caries bacteria to announce new successful alternatives to antibiotics.

INTRODUCTION

Dental caries is considered one of the most common diseases all over the planet (Deeley 2013). It is produced from an ecological imbalance of metabolic activities in the dominant oral microbiome (Motegi *et al.,* 2006). Dental caries comes when bacteria that colonize the mouth break down sugars in our food and result in organic acids that lead to damage to the surface of the teeth and give rise to small holes (Deeley 2013). Different microorganisms accountable for dental caries such as *Streptococcus mutans, Enterococcus fecalis, Staphylococcus aureus, Veillonella parvula, Nesseria spp, Actinomyces odontolyticus Fusobacterium necrophorum, Klebsiella pneumoniae, Pseudomonas fluorescence, Candida albicans,* and *Enterobacter aerogens* (Yadav 2016, Yadav and Prakash, 2015, Aas *et al*., 2000, Marsh and Bowden, 2000).

Multi-Drug resistant takes charge of the deaths of many people around the world (Chanishvili 2012). It results in 700,000 global deaths every year (Tagliabue and Rappuoli 2018, O'Neill 2014), and it is motivated that the death numbers will increase to 10 million deaths by 2050 (O'Neill 2014).

In the recent study streptococcus mutans, *Enterococcus faecalis* and *Staphylococcus* aureus that was insulated previously from patients with dental caries exhibited antibiotic-resistant features and promised to validate the efficiency of probiotics and mouth wash on multidrug-resistant dental caries pathogenic bacteria isolates from dental patients.

Probiotics have been defined as live microbial feed supplements that beneficially impact the host animal by improving its intestinal microbial balance (Fuller 1989). The mechanisms of probiotics actions have been considered as competitive inhibition of the multiplication of pathogenic bacteria by ultra the pH and reducing the oxygen availability leading to less favorable intestinal conditions (Schepper *et al.,* 2017), non-competitive inhibition which is described by (Lopetuso *et al.,* 2019) include producing bacteriocins, synthesis of essential micronutrients such as vitamins, amino acids and enzymes and enhancing the bioavailability of dietary nutrients (Pandey *et al.,* 2015), enhancing the metabolic activity of carbohydrates (Kerry *et al.,* 2018 and Rowland *et al.,* 2018). and stimulation of the host immune system (Kober and Bowe 2015).

Chlorhexidine is an antibacterial compound against most bacterial species found in the oral cavity (Subramaniam *et al.,* 2011) However, chlorhexidine can cause a change in taste and produce yellow or brown pigments on tooth surfaces. As a result, there is controversy on the use of chlorhexidine for caries prevention (Autio-Gold 2008).

MATERIALS AND METHODS

Ethical Aspects:

The Ethics Committee of Benha University Hospital gave its approval to the study protocol. All procedures have been performed following the Declaration of Helsinki.

Collection of specimens.

A total of 60 samples of dental caries pathogenic bacteria were purchased from different patients at Benha University, Qalyubiya Governorate. Specimens were collected under hygienic conditions using sterile cotton swabs, labeled, and transported immediately to the laboratory for microbiological investigation.

Isolation, Identification and Biochemical Tests of Dental Caries (**Cook and Brown 1960):**

Cotton swabs of all specimens were planted on selected plate agar media such as Tryptic soy agar, Nutrient Agar (NA) medium (Eaton *et al.,* 1995), MRS Broth (Sharpe *et al.,* 1966) and incubated at 37 °C for 48 h. The isolated bacteria were preserved on preservation media on a nutrient agar medium with 30% glycerol (Cruichshank *et al.,* 1975). The isolated bacteria were identified by using morphological, growth cultures characters and biochemical tests. The colonial appearance of suspected colonies on different

solid selective media was observed. Gram's stain according to (Cruichshank *et al.*, 1975).

Biochemical identification (Finegold *et al.*, 1982 and Quinn *et al.*, 2002):

Catalase Test:

Transmit a small amount of bacterial colony to a surface of a clean, dry glass slide using a loop or sterile wooden stick. Put a drop of 3% H_2O_2 onto the slide and mix. the rapid evolution of oxygen (within 5-10 s) as evidenced by bubbling is a positive result. no bubbles or only a few scattered bubbles are the negative results, this test is described according to (Varghese and Joy 2014).

Coagulase Test:

Procedure: Slide Coagulase Test. A drop of rabbit plasma was placed on a clean glass slide, then A colony of the organism was dipped in the rabbit plasma using a sterile inoculating loop and the slide rocked back and forth gently for one minute. The results: white clumps in plasma are a positive result while Negative one: no clumps (Cheesbrough 2000).

Antibiotic Susceptibility Testing (AST):

Antibiotics sensitivity testing was practised on triple sugar agar by the disc diffusion method, for the following antibiotics, Erythromycin (E, 15 µg), Methicillin(ME,30 µg), Oxacillin (OX, 25 µg), Cephalexin (CX,30 µg), Trimethoprim-Sulfamethoxazole, (TMP/SMX, 25 µg), Levofloxacin (LV, 5 µg), Gatifloxacin (GT, 10 µg), Nitrofurantoin (F, 10 µg), Amoxicillin (AX, 25 µg), Chloramphenicol (C, 30 µg), Ciprofloxacin (CIP, 5 µg), Gentamicin (GN, 10 µg), Norfloxacin (NOR, 10 µg), Penicillin-G (P, 10 µg), Vancomycin (V, 30 µg), Ceftazidime (CAZ, 30 µg), Aztreonam (ATM, 30 µg), Cefotaxime (CTX, 30 µg) and Amikacin (AK, 30 µg). (Wayne, 2019), in this technique, 1ml of TSB having a standard concentration of selected bacteria(10^8cfu/ml) was inoculated in molten soft TSB agar (7% agar) at 45 °C, then poured Mueller-Hinton agar plates and permitted to solidify. Next, disks of antibiotics (Nineteen antibiotics were used) of standard concentrations are applied to the plate's surface. The plates have been incubated at 37 °C for 24 hours and observed carefully Based on the inhibitory zone, the outcome was sorted as resistant, intermediate, or susceptible. Multidrug-resistant strains were those that have been clarified resistance to at least eight antibiotic classes. The results were interpreted conferring to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

Table 1: The antibiotic discs which have involved in our study.

Antimicrobial agent	Symbol	Disc potency(µg)
Erythromycin	E	15
Methicillin	ME	30
Trimethoprim-Sulfamethoxazole	TMP/SMX	25
Levofloxacin	LV	5
Norfloxacin	NOR	10
Gatifloxacin	GT	10
Nitrofurantoin	F	300
Chloramphenicol	C	30
Ciprofloxacin	CIP	5
Gentamicin	CN	10
Amikacin	AK	30
Cephalexin	CX	30
Penicilling	P	10
Oxacillin	OX	1
Vancomycin	VA	30
Aztreonam	ATM	30
Ceftazidime	CAZ	30
Cefotaxime	CTX	30
Amoxicillin	AMK	30

Antibacterial activity of the probiotic CFSM by agar well diffusion method

Agar well diffusion method was employed for the initial evaluation of antimicrobial properties of probiotic strains Table (2) against dental caries isolates (Wayne 2002). The tested isolates were inoculated into 10 mL of sterile nutrient broth and incubated at 37 ºC for 24 hours. The cultures have swabbed on the surface of sterile tryptic soy agar plates using a sterile cotton swab. Agar wells have been prepared with the help of a sterilized cork borer with a 10 mm diameter. Using a micropipette, 100ul of CFSM in MRS and skim milk were inserted into the wells in the plate. The plates have been incubated in an upright position at 37 °C for 24 hours. (Durairaj *et al.*, 2009), The diameter of inhibition zones has been calibrated in mm and the results have been recorded. Categorization of the antimicrobial activity of the tested CFSM has been made according to (Abdel-Daim *et al.*, 2013).

Table 2: Probiotic strains used in this study.

Bacterial strain	Source
1-*Lactobacillus acidophilus* EMCC 1324 (La)	Egypt Microbial Culture Collection, Microbiological Resources Centre, Ain-Shams University, Cairo, Egypt
2-*Lactobacillus rhamnosus* EMCC 1105 (Lr)	
3-*Bifidobacterium bifidum* EMCC 1334 (Bb)	
4-*Enterococcus.fecalis* ATCC 19433	Serology unit and Bacteriology unit, Animal Health Research Institute
5-*Lactobacillus.plantarum* ATCC 14917	
6- *Lacteolfort capsules*	From pharmacy at benha city
7- *Bactozyme*	From veterinary pharmacy
8- *yeast*	

Propagation of Mouth Wash (Chlorohexidine) Against the Three Isolates by Agar Well Disk Diffusion Methods:

Mouthwash solutions were obtained from the local pharmacy. explained the active ingredients, and pH of the mouthwash solutions tested, mouthwash that contains chlorohexidine has applied by agar well diffusion methods, 100 ml of chlorohexidine concentration was added to wells directly, which performed in duplicates plates then leave the plates standing for 10 minutes to allow the diffusion of the mouthwash into wells then incubate the plates at 37 °C for 24 h as described in (Subramaniam *et al*., 2011).

RESULTS

Dental Caries Bacteria:

In the current study, *Staphylococcus aureus, Streptococcus mutans* and *Enterococcus faecalis* were isolated previously from infected patients with dental decays and cavities from culture collection kindly taken from Botany and Microbiology Dept., Faculty of Science, Benha University, Egypt. The bacterial colonies were cultured on specific media, Blood agar, and MSA media. Gram-stained films were microscopically examined using a 100x oil immersed lens of B-150 OPTIKA microscope *S. aureus* was gram-positive coccus and appeared in clusters, *E. faecalis* were gram-positive cocci in single or in pairs and S. *mutans* were gram-positive cocci in short chains (Fig 3).

The isolates were known biochemically using conventional methods both of the isolates were Gram-positive, nonmotile and ferment mannitol (Fig 3), *S. aureus* causes beta hemolysis on blood agar *E. Faecalis and S. mutans* causes alpha hemolysis on blood agar (Fig 2), catalase test was positive for *S. aureus* and negative for both *E. faecalis and S. mutans (*Fig 4), coagulase test was positive for *S. aureus* and negative for *E. faecalis* and *S. mutans* (Table 4). The isolated bacteria were designed *as*

Staphylococcus aureas ME01 for *S. aureas* *Enterococcus faecalis* EF-01 for *E. faecalis* and *Sttreptococcus mut*ans STM01 for *St. mutans* (Table 4).

Isolation and Identification of Dental Caries spp:

First, the swabs had been taken from the oral dental caries by transporting media tryptic soy browth and nutrient browth then spreading each swab on the tryptic soy blood agar plates then incubating all samples at 37 °C for 48 h after that a collection of biochemical characterization tests were applied to all the isolates (Fig.1).

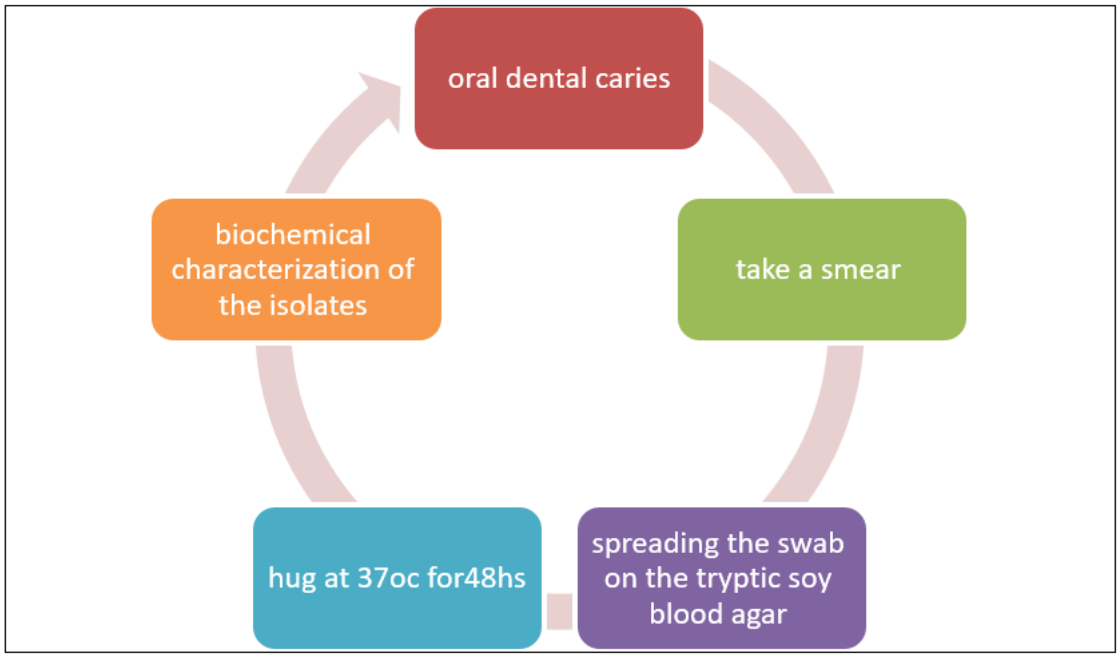

Fig. 1: Phases of bacterial isolation and diagnosis

Colonial Appearance:

On tryptic soy blood agar, *S. aureas* displays light to golden yellow pigment or white pigment, *S. mutans* appear white and transparent while *E. faecalis* appear pointed white shape (Fig. 2).

A B C

Fig. 2: morphological characterization of the isolates appeared on blood agar media (A) *S. mutans* which causes alpha haemolytic, while (B) *S. aureas* appear beta haemolytic and (C) *E. faecalis* appear alpha or gamma haemolytic.

Morphological Appearance:

Enterococcus faecalis appears monococci and sometimes diplococci. *Staphylococcus aureus* appears cocci in clusters. While the *Streptococcus mutans* appears monococci and short chains (Fig. 3).

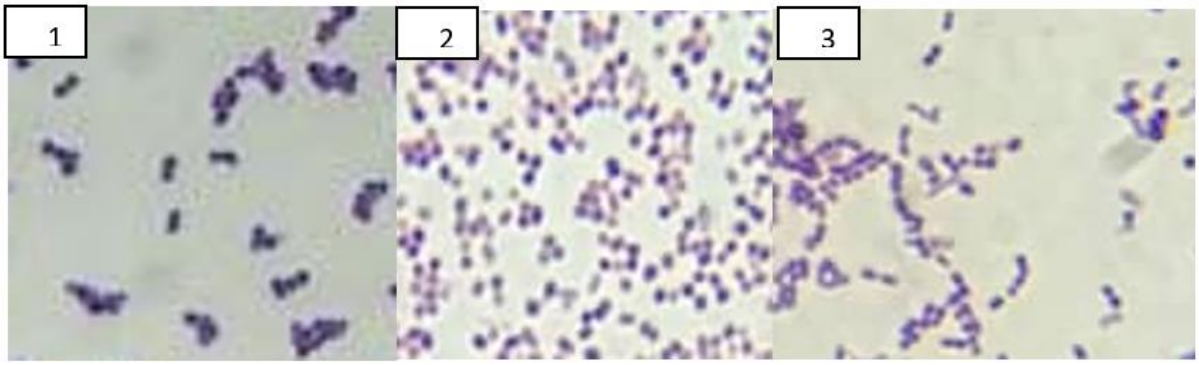

Fig. 3: Microscopic examination of (1) *E. faecalis*, (2) *S. aureus* and (3) *S. mutans* using 100X oil immersed lens of B-150 OPTIKA microscope.

Biochemical Identification:

Sixty specimens were collected from different age groups (less than 18 years (n=17), 18-45 years (n = 30), and >45 years (n = 13), gender (male (n =5 (8.33%) and female (n = 8 (13.33%) as shown in Table (3). thirteen specimens, out of 60, from patients with Dental caries, showed many symptoms that indicate many different diseases such as soft dental caries, dental plaque and root nerve decay associated with bacterial dental caries (BDC). Cultural and biochemical characteristics were used for the identification of the bacterial isolates that were obtained from patients with culture-positive bacterial dental caries (BDC) (Table 5). Using gram staining, the 13 bacterial isolates were gram-positive cocci as described in (Table 5, Fig. 3).

Regarding the biochemical identification of dental caries isolates by traditional methods, *St. mutans* and *E. faecalis* were catalase and coagulase-negative (Table 5, Fig. 4), while *S. aureas* was catalase and coagulase positive as shown in (Table 5).

Biochemical Characterization (Catalase Test):

Catalase test positive was detected by bubbling while catalase test negative was detected by no bubbles, where *S. aureas* were catalase positive which was noticed by bubbles on the slide (A), while both of the other isolates were catalase-negative, *S. mutans* (B), *E. faecalis* (C) (Fig. 4).

Fig. 4: Biochemical characterization of catalase test to catalase positive as in *S. aureas* (A*)*, and catalase negative in *S. mutans* (B) and *E. faecalis* strains (C).

Antibiotic Sensitivity Test of *S. aureus, E. faecalis* and *S. mutans*:

An antibiotic sensitivity test was performed for the three isolated bacteria against a selection of nineteen antibiotics. Qualitative data from the antibiograms showed that *S. aureus* ME01, *S. mutans* STM01 and *E. faecalis* EF01 were resistant to at least eight antibiotics against the tested antibiotics, respectively. *S. aureus* resisted Nitrofurantoin, Amoxoicillin, Gentamicin, Norfloxacin, Penicillin-G, Vancomycin, Ceftazidime, Cefotaxime, and Amikacin but was sensitive to Chloramphenicol and Aztreonam in Table (6) and Fig. (5).

E. faecalis EF01 resisted Chloramphenicol, Gentamicin, Norfloxacin, Penicillin-G, Ceftazidime, Aztreonam, Cefotaxime, and Amikacin. While this isolate was susceptible to Nitrofurantoin, Amoxicillin, Ciprofloxacin, and Vancomycin. Collectively the results showed that in Table (6) the isolated dental caries bacteria were considered as Multi-Drug Resistant (MDR).

S. mutans STM01 resisted Erythromycin, Oxacillin, Methicillin, Chloramphenicol, Gentamicin, Norfloxacin, Penicillin-G, Ceftazidime, Aztreonam, Cefotaxime, and Amikacin, On the other hand, all *S. mutans* isolates were sensitive to Trimethoprim-sulfamethoxazole, levofloxacin, gatifloxacin, Nitrofurantoin, Aztreonam, Amoxicillin and Vancomycin (Table 6).

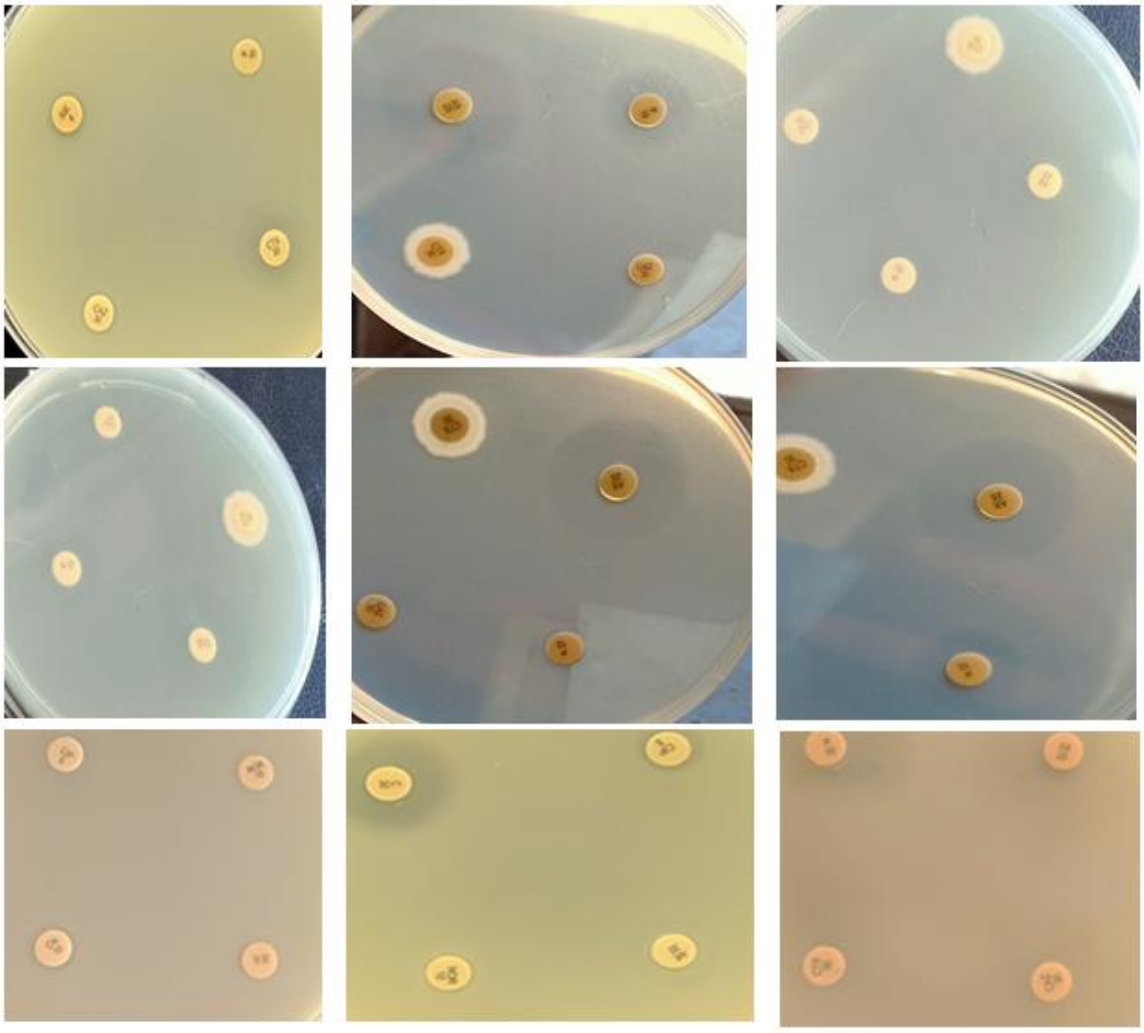

Fig. 5: Antibiotic susceptibility test by disk well diffusion methods against the pathogenic bacteria of dental caries

Furthermore, study the antibacterial activity of cell-free preparations of probiotics and mouth washing against dental caries isolates as described in Tables (7 &8).

Antibacterial Activity of The Probiotic CFSM:

Cell-free preparations of probiotic Lactobacillus and Bifidobacterium strains, grown in MRS medium, showed promising growth inhibition of *S. aureus, s. mutans and E. faecalies* isolates, even though against those with the antibiotic resistant pattern as shown in Table (7).

Effect of Mouthwash Propagation Against the Three Isolates by Agar Well Disk Diffusion Methods:

Mouthwash has high efficiency in inhibiting the growth of *Staphylococcus, streptococcus and Enterococcus* spp. associated with BDC noticed by the inhibition zone through the plates as shown in Table (8) which noticed the inhibition zone in case of *S. aureus* 22 mm was greater than the zone of *S. mutans* that showed 19 mm and *E. faecalis* has 17.5 mm inhibition zone.

Table 3: Risk factors in dental caries.

Gender	Age			No of (+) sample (%)
	Less than 18	18-45	more than 45	
Male	4	11	3	5 (8.33%)
Female	13	19	10	8 (13.33%)
Total isolates	60			13 (21.7%)

Data shows in Table 2 that the no of positive isolates has been found in females n=8 (13.33%) more than males n=5 (8.33%) in cases of 18-45 age which are associated with bacterial dental caries (BDC). Data shows in Table 3 that the diseases that appear on different specimens show diversity like (soft caries, dental plaque, and root nerve decay).

Table 4: The diseases appear on different specimen**.**

Specimen types of	Diseases
1-Twenty – four swabs	Soft caries
2- six samples	Saliva
3-nineteen swabs	Dental plaque
4-Seven samples	sputum
5-Four swabs	Root nerve decay

Table 4 showed that the thirteen isolates were ten *Staphylococcus spp* (ME01-ME10), two *Streptococcus spp* (STM01-STM02) and one *Enterococcus* spp. (EF01), all *staph*. spp. were cocci in clusters, beta-hemolytic on blood agar, catalase, and coagulase positive, while *Streptococcus spp* were cocci and found in short chains and *Enterococcus spp* were cocci in singles and sometimes in pairs, both *Streptococcus* and *Enterofaecalis spp* were catalase and coagulase-negative.

Table 5: Biochemical characterization of the isolates clinically isolated from patients with dental caries

No of Isolates	Morphology and arrange	Motility	Gram reactions	Blood hemolysis	Oxidase	Catalase	Gelatinase	Coagulase	Indole	Nitrate	Mannitol	glucose	Presumptive isolates
Staphylococcus. Spp *(ME01)*	Coccus in clusters	-	+	Beta	-	+	+	+	-	+	+	+	*S. aureus*
Staph. spp *(ME02)*	Coccus in clusters	-	+	Beta	-	+	+	+	-	+	+	+	*S. aureus*
Staph. spp*(ME03)*	Coccus in clusters	-	+	Beta	-	+	+	+	-	+	+	+	*S. aureus*
Staph. spp *(ME04)*	Coccus in clusters	-	+	Beta	-	+	+	+	-	+	+	+	*S. aureus*
Staph. spp *(ME05)*	Coccus in clusters	-	+	Beta	-	+	+	+	-	+	+	+	*S. aureus*
Staph. spp *(ME06)*	Coccus in clusters	-	+	Beta	-	+	+	+	-	+	+	+	*S. aureus*
Staph. spp*(ME07)*	Coccus in clusters	-	+	Beta	-	+	+	+	-	+	+	+	*S. aureus*
Staph. spp*(ME08)*	Coccus in clusters	-	+	Beta	-	+	+	+	-	+	+	+	*S. aureus*
Staph. spp*(ME09)*	Coccus in clusters	-	+	Beta	-	+	+	+	-	+	+	+	*S. aureus*
Staph. spp *(ME10)*	Coccus in clusters	-	+	Beta	-	+	+	+	-	+	+	+	*S. aureaus*
Streptococus. spp *(STM 01)*	Cocci and short chains	-	+	Alpha	-	-	-	-	-	-	+	+	*S. mutans*
*Streptococcus.*spp *(STM02)*	Cocci and short chains	-	+	Alpha	-	-	-	-	-	-	+	+	*S. mutans*
Enterococcus. spp *(EF01)*	Cocci in singles and sometimes in pairs	-	+	Gamma or alpha	-	-	-	-	-	-	+	+	*E. faecalis*

Table 6: Antibiotic sensitivity pattern of the isolated dental caries bacteria Against a selection of antibiotics

Bacteria	ER	OX	ME	TMP/SMX	LV	GT	CX	F	AX	C	CIP	GN	NOR	P	VA	CAZ	ATM	CTX	AK
S. Aureus ME01	*R*	*R*	*R*	*R*	*S*	*S*	*R*	R	R	S	I	R	R	R	S	R	S	R	R
S. Aureus ME02	I	R	R	S	S	S	R	R	R	S	R	R	R	R	S	R	R	R	R
S. Aureus ME03	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S. Aureus ME04	R	R	R	R	S	S	R	R	R	S	S	S	R	R	S	R	S	R	R
S. Aureus ME05	I	R	R	S	R	S	R	R	R	R	R	S	R	R	R	R	R	R	R
S. Aureus ME06	R	R	R	I	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R
S. Aureus ME07	R	R	R	S	S	S	R	R	R	S	R	S	R	R	S	R	R	R	R
S. Aureus ME08	R	R	R	I	S	S	R	R	R	R	S	R	R	R	S	R	S	R	R
S. Aureus ME09	R	R	R	R	R	R	R	R	R	R	S	R	R	R	S	R	S	R	R
S. Aureus ME10	R	R	R	R	R	R	R	R	R	R	S	S	R	R	S	R	S	R	R
Resistance (%)	80	100	100	40	50	40	100	100	80	60	50	60	100	100	30	100	40	100	70
S. mutans STM01	*R*	*R*	*R*	*S*	*S*	*S*	*R*	S	S	R	R	R	R	R	S	R	S	R	R
S. mutans STM02	R	R	R	S	S	S	R	S	S	S	S	R	R	R	S	R	S	R	R
Resistance (%)	100	100	100	0	0	0	100	0	0	50	50	100	100	100	100	100	0	100	100
E. faecalies EF01	R	R	R	S	S	R	R	S	S	R	S	R	R	R	S	R	R	R	R
Resistance (%)	100	100	100	0	0	100	100	0	0	100	0	100	100	100	0	100	100	100	100

Erythromycin (E, 15 µg), Methicillin (ME 30µg), Oxacillin (OX,1 µg), Cephalexin (CX, 30 µg), Trimethoprim-sulfamethoxazole (TMP/SMX 25 µg), levofloxacin (LV, 5 µg), Gatifloxacin (GT, 10 µg). Nitrofurantoin (F, 10 µg), Amoxicillin (AX, 25 µg), Chloramphenicol (C 30 µg), Ciprofloxacin (CIP 5 µg), Gentamicin (GN, 10 µg), Norfloxacin (NOR, 10 µg), Penicillin G (P, 10 µg), Vancomycin (V, 30 µg), Ceftazidime (CAZ, 30 µg), Aztreonam (ATM, 30 µg), Cefotaxime (CTX, 30 µg) and Amikacin (AK, 30 µg). Denotes for Resistant **(R),** Intermediate **(I),** and Susceptible **(S).**

The ten *S. aureus* was resistant to Erythromythin (80%), Methicillin, Ceftazidime, Cefotaxime, Nitrofurantoin, (100%), Norfloxacin, Penicillin G, Oxacillin Cephalexin (100%), Amoxicillin, (80%) Amikacin (70%), and Approximately, 40% of the isolates were shown resistant to Aztreonam, Trimethoprim-sulfamethoxazole and Gatifloxacin while 50% were shown resistant to levofloxacin and Ciprofloxacin and 60% Chloramphenicol and Gentamicin, 30% resistant to Vancomycin, while the two *S. mutans* were resistant to Erythromicin, Norfloxacin, Gentamicin, Amikacin, Ceftazidime, Cefotaxime, penicillin G, vancomycin and Methicillin with (100%), Chloramphenicol and Ciprofloxacin with (50%) and sensitive to Aztreonam, Amoxicillin, Nitrofurantoin, gatifloxacin and Trimethoprim-sulfamethoxazole with (100%). *E. faecalis* was resistant to Erythromycin, Methicillin, Norfloxacin, Gatifloxacin, Chloramphenicol, Ciprofloxacin, Gentamicin, Amikacin, Cephalexin, PenicillinG, Oxacillin, Aztreonam, Ceftazidime, Cefotaxime with (100%) and sensitive to Amoxicillin, Nitrofurantoin, levofloxacin, and Vancomycin with (100%).

Table 7: Antimicrobial activity of cell-free preparation of probiotic *Lactobacillus* and *Bifidobacterium* strains against *Staphylococcus*, *Streptococcus* and *Enterococcus* spp., associated with BDC.

Probiotic strain	*S. aureus*	*S. mutons*	*E. feacalis*
	Inhibition zoon(mm)		
Lactobacillus acidophilus EMCC 1324(La)	15.5	15	13
Bifidobacterium bifidum (Tissier 1900) EMCC 1334 (Bb)	14	12.5	13
Enterococcus fecalis ATCC 19433	R	R	R
Lactobacillus plantarum EMCC 1027(Lp)	13.5	14	.13
Lactobacillus rhamnosus EMCC 1105 (Lr)	13	12	13
yeast	R	R	R
Bacillus. Subtilis MBG874	11	12	11
Lacteolfort capsules	R	R	R
Bactozyme	R	R	R

Table (7) demonstrates that some probiotic strains were efficient to inhibit the growth of *Staphylococcus*, *Streptococcus* and *Enterococcus* spp. associated with BDC that appear to in case of *Lactobacillus* spp then *Bifidobacterium* spp.

Table 8: Effect of mouthwash on *S. aureus, S. mutans* and *E. faecalis* which cause dental caries (DC)**.**

Isolates	*S. aureus*	*S. mutans*	*E. feacalis*
	Inhibition zoon(mm)		
Mouthwash (chlorohexidine 125mg)	22	19	17.5

Data presented in Table (8) showed that mouthwashes of Chlorhexidine showed a greater mean diameter of inhibition zone more than 15 mm against *S. aureus* (22 mm) than *S. mutans* (19 mm) and *E. faecalis* (17.5 mm) that indicating mouth wash has a great efficiency against the isolates associated with bacterial dental caries (BDC).

DISCUSSION

Despite great improvements in the oral health of the population in several countries, it is claimed that poor oral health may have a deep impact on general health, several oral diseases related to chronic diseases as well as the value of life, (CDC 2016). Dental caries is known as one a hygiene-related disease caused by decay-causing bacteria that result in acid resulting in damage to tooth enamel. In the current study, three pathogenic bacteria, *S. aureus*, *S. mutans* and *E. faecalis* which are related to dental caries, were isolated previously from infected patients with dental decays and cavities, which agrees with (Wang *et al*., 2012, Ohara-Nemoto *et al.,* 2008). The isolated bacterial candidates were characterized microscopically (Nonhoff *et al*., 2005, Ligozzi *et al.*, 2000), and described biochemically using conventional methods and according to previous studies

The prevalence of *Streptococcus mutans* in patients with ID aged 6 to 30 years old was 96.6 %, which is in agreement with similar surveys conducted with school children in other parts of the world (Oda *et al*., 2015), while the prevalence of *Streptococcus mutans* in adults has been reported to be greater than 60 % and 82.7 % in subjects with Down syndrome aged 1–48 years old (de Castilho *et al.*, 2011) which disagree with the present study, the three isolates have been found in female n=8 (13.33%) with ID aged 18 to 45 more than male n=5(8.33%) with ID

aged 18 to 45 age which associated with bacterial dental caries (BDC).

In the last decade, several studies have focused on the relationship between periodontal diseases and oral bacteria. The investigation examined the prevalence of *Enterococci* in the oral cavity of Tunisian children using specific primers, twenty-one *Enterococc*i (33.9%) among 113 gram-positive cocci were isolated and identified from the oral cavity of 62 children. Nineteen *Enterococci* were isolated from the carious lesion (55.8%) and two from caries-free (7%). Similar results have been reported by (Gold *et al.,* 1975). suggesting that Enterococci were detected in 60% of oral samples collected from various school children (Kouidhi *et al.,* 2011) which disagrees with this study in which *one E. faecalis* (7.69%) among 60 g positive bacteria cocci were isolated from dental plaque.

Enterococcus spp is considered the third most common pathogen isolated from bloodstream infections and the most frequently isolated species in teeth with persistent infection after root canal treatment (Sedgley *et al.,* 2004). Different bacteriological studies have evaluated that *E. faecalis* is present in 29-46% of root-filled teeth with periapical lesions, which agrees with (Sedgley *et al.,* 2004).

In this study, one sample isolated from root decay was *E. faecalis* which agrees with (Kouidhi *et al.,* 2011). The results of the study have been showing Variable response of Gram-positive bacteria isolates to a number of antibiotics used in this study which included (Pencillin –G, Metronidazole, (Amoxicillin as described in Table (6). So, bacterial isolates showed *S. aureus* resistant to all species antibiotics and this result agreed with work of Gousia *et al.,* 2011. While the result of this study varies with what the researcher found it (Seifi *et al.,* 2012).

Recently, most bacteria had the potential to develop resistance against different classes of antibiotics (CDC 2019). Antibiotic resistance was one of the top concerns that threaten global health. Egypt is one of the countries that have less severe restrictions on antibiotic remedies (Esmael *et al.,* 2020, Esmat *et al.,* 2017, Awad *et al.,* 2016, Sabry *et al.,* 2014), which increases the chance for bacteria to resist antibiotics. In the current study, antibiotic sensitivity testing of *S. aureus, S. mutans* and *E. faecalis* against a selection of nineteen antibiotics showed that the three isolates resisted at least eight of the tested antibiotics. (Guo *et al.,* 2020, Miller *et al.,* 2014), Resistance mechanisms against antibiotics by *S. aureus, S, mutans and E. faecalis* were reported.

Antibiotic resistance can be developed through mutations in chromosomal genes or by mobile genetic elements (horizontally acquired resistance) as described in (Foster 2017). In that view, resistance is acquired through mutation, (Miller *et al.,* 2014, Jensen and Lyon 2009). mechanism of horizontally acquired resistance or overexpression of the drug efflux was discussed previously.

Conclusion

Dental caries is considered as one a hygiene-related disease produced by decay-causing bacteria that cause acid resulting in damage to the tooth, Samples from patients with dental caries have been raised in sterile swabs and cultivated. From the morphological and biochemical characteristics, the isolated bacteria were found to be, *Staphylococcus aureus*, Streptococcus *mutans* and *E. faecalis* were prepared using the selective media. The antibacterial activity was determined against the isolated *Streptococcus mutans* and other isolates. most bacteria could develop resistance against different groups of antibiotics. Antibiotic resistance was known as one of the top concerns that threaten global health.

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