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Distribution and Antibiotic Resistance of Streptococci and Enterococci Isolated from Dental Caries and Healthy People

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ABSTRACT

Introduction: Antimicrobial resistance is one of the most significant challenges globally that threatens our ability to treat infectious diseases. This study investigated Streptococci and Enterococci in dental caries and healthy individuals and determined antibiotic resistance in the recovered bacteria. **Methods:** One hundred and twelve samples were collected, 56 from patients with dental caries and 56 samples from the teeth and saliva of healthy people. The samples were cultured on blood agar and purified on Mitis Salivarius agar. All isolates were identified by biochemical tests and Vitek 2 system and then examined for antibiotic susceptibility by disc diffusion method. **Results:** Streptococci and enterococci were the most isolated agents from dental caries and the teeth and saliva of healthy people. *Streptococcus* spp. comprised 48.61% of bacteria in dental caries and 28.40% in healthy individuals, while *Enteroccus* spp. was 22.22% in dental caries and 30.84% in healthy individuals. *Streptococcus mutans* and *Streptococcus salivarius* were more prevalent in dental caries and healthy individuals, respectively, while *Enterococcus faecium* was detected in both dental caries and healthy individuals. Also, there were significant differences between the number of streptococci isolated from healthy and caries people ($P<0.05$). In isolates recovered from healthy people, the streptococcal spp. exhibited high resistance to azithromycin (82.6%), cefixime and tetracycline (91.3%), and amoxicillin (60.8%). In contrast, enterococci were resistant to tetracycline (92%) and cefixime (76%). In dental caries isolates, the streptococcal and enterococcal spp. showed the highest resistance to amoxicillin (> 68%). **Conclusions:** The streptococcal and enterococcal spp. comprised the most isolated bacteria from healthy individuals and dental caries and exhibited multidrug resistance.

INTRODUCTION

Worldwide, there is an increasing trend in using antibiotics to treat and prevent dental infections [1]. Antibiotic resistance has emerged due to inappropriate prescription and practice of antibiotic use [2]. The oral cavity contains a complex and diverse microbial community in which oral microorganisms are colonized in biofilms as a complex structure that adheres to various oral surfaces. In biofilms, microorganisms can adapt to changes in their environment, while the biofilm structure protects bacteria from antibiotics [3].

Tooth decay is a predominant oral disease affecting children and adults [4] and remains a significant health problem globally [5]. Studies indicate that dental caries is a multifactorial illness. Three main factors contribute to them: 1) acidogenic and acidophilic microorganisms, 2) sugar-containing foods, and 3) host factors, such as salivary flow or oral cleanliness, which lead to a dysbiotic state [6]. *Streptococcus* spp. are the primary bacterial species associated with dental caries. These bacteria produce high amounts of lactic acid during sugar fermentation, resulting in a lowered pH and forming plaques around teeth.

A plaque is a biofilm, an aggregation of non-cariogenic and cariogenic bacteria embedded in a matrix containing bacterial enzymes and other secreted compounds, polysaccharides, and proteins. It provides attachment sites for colonization and bacteria growth and plays an essential role in antibiotic resistance [7]. Among streptococci, *S. mutans* is the primary species associated

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with dental plaques in humans [8]. Other tooth decay-associated pathogens include non-mutans streptococci, *Actinomyces* spp., *Veillonella* spp., *Lactobacillus* spp., and *Bifidobacterium* spp. [9].

The members of *Enterococcus* are transient constituents of the oral microbiota, causing several oral and systemic infections. Among enterococci, *E. faecalis* is the predominant species associated with oral diseases, such as endodontic infections, caries, peri-implantitis, and periodontitis [10]. This agent is frequently implicated in endodontic treatment failure due to high resistance to antibiotics [11]. Most of the available data on the oral carriage of *E. faecalis* is mainly in patients undergoing endodontic therapy. It is considered one of the 25 most abundant pathogens that cause persistent endodontic infections. Also, a study evaluated the virulence attributes of *E. faecalis* isolates from the oral cavity, food, and clinical specimens and reported that oral isolates had the highest percentages of virulence genes and extracellular enzymes and a capacity to form biofilms [12]. Therefore, the oral cavity may constitute a critical reservoir for the virulent antibiotic-resistant *E. faecalis* strains.

The present study aimed to isolate the *Streptococcus* spp. and *Enterococcus* spp. from healthy individuals and dental caries and evaluated their antimicrobial resistance.

MATERIAL AND METHODS

Samples collection. The plaque samples were collected from patients attending the dental clinic of Al-Shaheed Al-Sader Hospital in Baghdad Province, Iraq. Samples from healthy individuals with no oral disease, including hospital staff and patients' companions, were collected. The samples were collected from November 2019 to January 2020. The patients were informed about the study, and patients' consent and approvals were obtained prior to collecting the samples.

The ethical committee of the College of Science for Women, University of Baghdad, Baghdad, Iraq, approved this study.

One hundred twelve samples were collected from 56 persons with dental caries and 56 without oral diseases after being diagnosed as healthy by a dentist. Samples were taken from plaque by a sterile cotton swab after drying the teeth with an air spray to prevent contamination from saliva. Samples from healthy individuals were collected from the teeth surface and saliva with cotton swabs. The samples were transferred to transport media, then immediately transported to the laboratory.

Isolation and identification of bacteria. The collected samples were cultured on a blood agar medium incubated aerobically in a jar with a gas pack (Oxoid, England) at 37˚C for 24 h. The colonies were then sub-cultured and purified on Mitis Salivarius Agar (MSA) (HIMedia, India) for 24 h under anaerobic conditions at 37˚C. Isolates were identified based on many characteristics such as colony morphology on MSA, Gram stain, catalase test, growth in 6.5% NaCl, growth in 45˚C, and optochin sensitivity. Furthermore, the isolates were identified by Vitek 2 system using Vitek 2 Gram-positive identification card (GP) (bioMerieux, France).

Antibiotic susceptibility test (AST). The AST was performed against clinically recommended antibiotics, amoxicillin (10 µg), azithromycin (15 µg), cefixime (5 µg), tetracycline (30 µg), vancomycin (30 µg), and ciprofloxacin (10 µg) (Bioanalyse, Turkey) using the disc diffusion method. Isolate suspensions were prepared at 0.5 McFarland turbidity (1.5×10^{8} CFU/mL), and 0.1 ml was plated onto Mueller Hinton agar plates. Antibiotic discs were placed on each plate, followed by incubation at 37°C for 18 h. The inhibition zone around the disc was measured in millimeters by roller [13]. The sensitivity patterns of isolates against antibiotics were interpreted according to the Clinical and Laboratory Standard Institute (CLSI 2020).

Detection of multidrug-resistant (MDR) bacteria. Defining MDR isolates was done according to a new standardized international document [14] using the results of the antimicrobial susceptibility of isolates to all antimicrobial agents used in the current study. The isolates that exhibited resistance to at least one agent in ≥3 antimicrobial categories were considered MDR.

Statistical analysis. The statistical analysis system (SAS, 2012) software was used to analyze the results. A comparison of numerical variables between the study groups was made using the Chi-square test for categorical data. A P-value < 0.05 was considered statistically significant.

RESULTS

Bacterial isolates. 72 Gram-positive and 81 Gram-positive isolates were obtained from dental caries and healthy individuals. A high diversity of bacterial species was found among all samples (Table 1). At least two different bacterial genera were identified in each sample. The most identified bacteria in dental caries and healthy individuals comprised *Streptococcus* spp. (48.61% and 28.40%), followed by *Enterococcus* spp. (22.22% and 30.86%).

Other Gram-positive bacteria were *Lactococcus* spp., *Leuconostoc* spp., *Staphylococcus* spp., *Propionibacterium* spp., *Gemella* spp., *Kocuria* spp., *Pediococcus* spp., *Granulicatella* spp., and *Actinomyces* spp.). There were significant differences (P <0.05) between the number of streptococci, enterococci, and other Gram-positive bacteria in dental caries and healthy individuals.

The most common isolates among *Streptococcus* species were *S. salivarius* in healthy individuals and *S. mutans* in dental caries. Seven *Streptococcus* species, *S. mutans*, *S. salivarius*, *S. oralis*, *S. mitis*, *S. sanguinis*, *S. parasanguinis*, and *S. sobrinus*. In the genus *Enterococcus*, *E. faecium*, *E. faecalis*, and *E. gallinarum*

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were identified, with *E. faecium* being the most isolated bacteria (Table 2). All streptococci and enterococci were catalase-negative, all enterococci grew at 45˚C and NaCl 6.5%, and all streptococci were optochin resistant.

Table 1. Significant differences between enterococci and streptococci.

Source	Streptococci	Enterococci	Other bacteria	*P*-value
			N (%)	
Dental caries	35 (48.61)	16 (22.22)	21 (29.2)	0.0075*
Healthy individuals	23 (28.40)	25 (30.86)	33 (40.74)	0.0402 *
P- value	0.0086*	0.0811Ns	0.049*	-

* ($P \leq 0.05$) , Ns: Non-significant

Table 2. The numbers and ratio of *Streptococcus* spp. and *Enterococcus* spp. isolated from healthy individuals and dental caries.

Bacterial species	Dental caries	Healthy people	*P*-Value
		N (%)	
***Streptococcus* spp.**	35 (48.61)	23 (28.40)	-
S. mutans	14 (40)	4 (17.40)	0.0048*
S. salivarius	0 (0.0)	15 (65.22)	0.0001*
S. sobrinus	7(20)	0 (0.0)	0.0084*
S. mitis	3 (8.6)	0 (0.0)	0.078NS
S. oralis	5 (14.3)	3 (13.03)	0.894NS
S. sanguinis	2 (5.7)	1 (4.35)	0.804 NS
S. parasanguinis	4 (11.4)	0 (0.0)	0.0493*
***Enterococcus* spp.**	16 (22.22)	25 (30.86)	-
E. faecium	11 (68.75)	17 (68)	0.988 NS
E. faecalis	5 (31.25)	2 (8)	0.0071*
E. gallinarum	0 (0.0)	6 (24)	0.0068*

*($P \leq 0.05$), Ns: Non-significant.

Table 3. Antibiotics resistance of streptococci and enterococci isolates from healthy and dental caries people.

Antibiotics	*Streptococcus* spp. Dental caries	*Streptococcus* spp. Healthy individuals	*P*-value	*Enterococcus* spp. Caries	*Enterococcus* spp. Healthy	*P*-value
	No. (%)			No. (%)		
Amoxicillin	28 (80.4)	14(60.8)	0.0084 *	11 (68.7)	14 (56)	0.0372 *
Azithromycin	11 (31)	19 (82.6)	0.0001 *	9 (56.2)	15 (60)	0.755 Ns
Cefixime	24 (68.5)	21 (91.3)	0.0075 *	8 (50)	19 (76)	0.0052 *
Ciprofloxacin	7 (20)	1 (4.3)	0.0216 *	10 (62.5)	4 (16)	0.0001 *
Tetracycline	16 (45.7)	21 (91.3)	0.0001 *	8 (50)	23 (92)	0.0001 *
Vancomycin	7 (20)	10 (43.4)	0.0089 *	9 (56.2)	16 (64)	0.091 Ns
P-value	0.0001 **		---	0.0001 *		---

* ($P \leq 0.05$), Ns: Non-Significant.

Antibiotics resistance. The resistance patterns of streptococcal and enterococcal isolates to various antibiotics are presented in table 3. A high resistance level was observed for amoxicillin (> 68%) in streptococci and enterococci from caries. While in isolates from healthy individuals, the highest resistance (> 91%) was recorded for tetracycline and cefixime. Substantially, lower ciprofloxacin resistance was observed against streptococci and enterococci isolates from healthy people. There were significant differences ($P \leq 0.05$) in resistance between enterococci and streptococci from healthy and caries.

The MDR patterns for all isolated bacteria are presented in Fig. 1. In our study, 50 ≤ of the isolates were resistant to ≥ 3 classes of antibiotics.

DISCUSSION

This study investigated streptococcal and enterococcal bacteria in dental caries and healthy individuals and determined antibiotic resistance patterns in the recovered bacteria. The current results showed that the most frequently isolated bacteria belonged to the *Streptococcus* species, with 48.61% in dental caries and 28.40% in healthy individuals. Similar results were obtained by others [7], where *Streptococcus* spp. were the primary bacteria associated with plaque. Also, a study in Florida, USA (2017) concluded that streptococci were natural inhabitants of the oral cavity but are involved in forming biofilms on the tooth, leading to tooth decay known as dental caries [15].

In our study, the primary streptococcal species in dental caries was *S. mutans* (40%). In contrast, *S. salivarius* (65.22%) was the dominant species in healthy individuals. Similar reports also indicated that *S. mutans* was the most prevalent in dental caries [16, 17]. A study (18) in Mexico (2018) also confirmed that *S. mutans* was the most dominant species and directly associated with the development of dental caries [18]. The properties that allow *S. mutans* to colonize the oral cavity include the

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production of acid, interaction with other bacterial species colonizing this ecosystem, and the ability to survive in an acidic environment [19] and produce glucan matrix from sucrose [20]. *S. salivarius* is typically an inhabitant of the soft surfaces of the mouth and is thought to have a limited ability to colonize the tooth surface and directly compete with *S. mutans* [21].

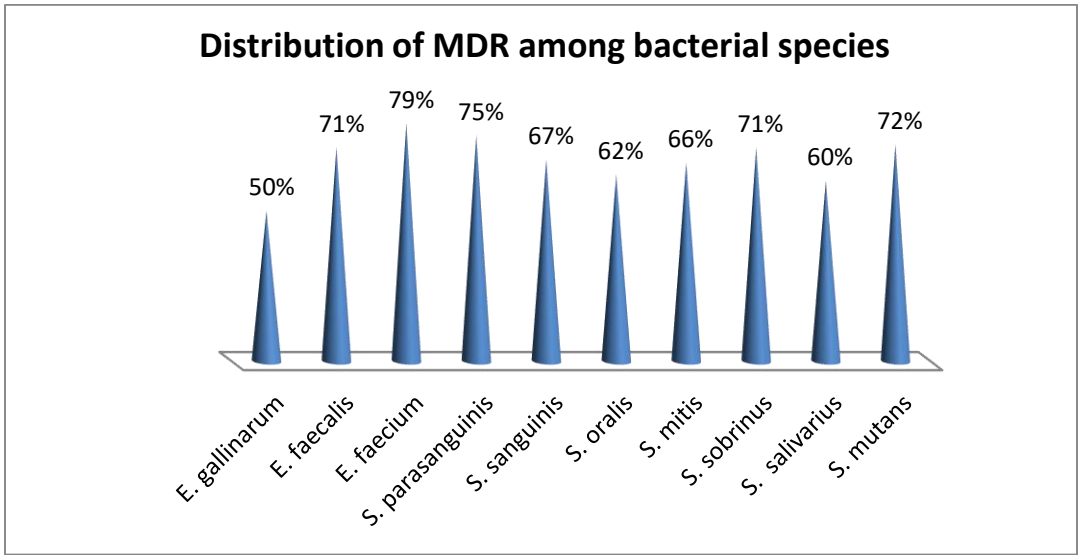

Fig. 1. The MDR pattern in the bacterial species isolated from dental caries and healthy individuals.

Enterococcal species were isolated and identified in healthy individuals and dental caries in our study. Although enterococcal spp. is not considered a microflora in the mouth, it occurs in high percentages. Compared with other enterococci, *E. faecium* was the most dominant bacteria in healthy individuals and dental caries, with 68.75% in dental caries and 68% in healthy individuals. Different results were obtained by Durgesh *et al.* (2016), who reported *E. faecalis* (72%) as the most isolated species compared to *E. faecium* (28%) [22].

In the present study work, *Streptococcus* spp. isolated from dental caries were resistant to azithromycin (31%), tetracycline (45.7%), amoxicillin (80.4%), cefixime (64.5%), ciprofloxacin, and vancomycin (20%). A similar study in India on patients experiencing dental extraction (2015) reported that among *Streptococcus* spp. isolates, 17.7% were resistant to tetracycline, 42.7% to azithromycin, and 100% were sensitive to vancomycin. [23]. In Yemen, 2.3% of isolates were resistant to vancomycin and 14.9% to amoxicillin [24]. In another study in Iraq, ~80% of streptococcal isolates were resistant to ciprofloxacin, 35% to tetracycline, and 5% to vancomycin [25].

The current study indicated high resistance levels in *Enterococcus* spp. isolated from dental caries to amoxicillin (68.7 %), vancomycin (56.2%), and tetracycline (50%). In contrast, more than 95% of enterococcal isolates from the oral cavity were susceptible to vancomycin and amoxicillin in Saudi Arabia [22]. In Philadelphia, USA, 53.2% of *E. faecalis* isolates from subgingival were resistant to tetracycline and 100% sensitive to vancomycin [10]. In another study on *E. faecalis* from dental calculus in Iran, the resistance rate to vancomycin, tetracycline, and ciprofloxacin was estimated as 4.6%, 11.6%, and 6.9%, respectively [26]. The differences in results of our study and previous studies are probably due to geographical areas.

Nowadays, MDR bacterial infections constitute a significant health problem and significantly burden effective therapeutic strategies [21]. Our study showed high-level MDR (> 50%) in all species, which is nearly comparable with a report from Russia [27] and disagrees with another one in Iran [28], where only 7.8% were MDR.

Bacterial resistance may depend on the complexity of the bacterial community in the biofilm involved. A study showed lower bacterial resistance in planktonic bacteria, while dental infections are organized as biofilms, which are more tolerant to antibiotics [29].

A study on the relationship between biofilm formation by different streptococci from the oral cavity and the antibiotic sensitivity indicated 71% viridans streptococci. These better biofilm producers were more resistant to amoxicillin and erythromycin [30].

The excessive and improper use of antibiotics contributes to the emergence of resistant strains even in normal oral flora, making the mouth a harbor for bacteria with multiple antibiotic resistance genes.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest associated with this manuscript.

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