Antibiotic Resistance of Strong Biofilm Forming Bacteria Isolated from Different Oral Samples of Patients with Dental Problems

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ABSTRACT

Globally, the wide use of antibiotics in human treatments, animal food supplements, and agriculture practice was the main cause of the emergence of multi antibiotics resistant strains of bacteria which led to a critical health problem. The purpose of this study was to reviewing the antibiotics resistance ability of strong biofilm former isolates isolated from saliva, teeth and necrotic roots canals. Biofilm formation assay was done by using Microtiter plate method (MTP), among 267 bacterial isolates (137 from saliva, 88 from teeth and 42 from necrotic roots canal), 104 isolates (55 from saliva, 32 from teeth and 17 from necrotic roots canals) were considered as strong biofilm former and were screened according to its antibiotics resistance ability. The results showed that almost all the isolates can form visible growing biofilms when compared to the controls, though with difference in the degree of adhesions (strong, moderate and weak). *Enterococcus* sp. isolated from saliva, teeth and necrotic roots canals was the strongest biofilm former bacteria and as well as the most antibiotic resistant isolates. Further, the study revealed that antimicrobial resistance of bacteria isolated from saliva, teeth and necrotic roots canals of patients with dental caries conditions is associated with biofilm formation.

Keywords: Biofilm formation, Antibiotic resistance, *Enterococcus*, Saliva, Teeth, Necrotic roots canals

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INTRODUCTION

The oral microbiota has been reported to serve as a reservoir for numerous antimicrobial resistance genes such as those encoding resistance to commonly utilized classes of antimicrobials such as tetracyclines, beta-lactams, and macrolides, which have been extensively used in treating oral infectious conditions, including those of endodontic origin

[1]. During the conventional resistance against antimicrobial agents by planktonic bacteria, it normally involves the inactivation of the antimicrobial agent, alteration of the targets, and exclusion of the antimicrobial agent [2]. These typical actions involve the acquisition of distinct genetic factors, like genes for β -lactamase or efflux pumps. One of the most vital characteristics of bacterial biofilm formation is the increased rate of resistance by the constituent microbes to antimicrobial agents as well as other stressors. The nature of the structural of biofilms and the characteristics of the sessile cells, generate resistance towards the antimicrobials, giving rise to an environment that is protected against adverse conditions including the host's defenses [3]. Biofilms may serve as physical diffusion barriers that are capable of preventing antimicrobial agents from getting to their targets. Antimicrobial agents were shown to be capable of penetrating these structures through a thick mixture of exopolysaccharide, DNA, and protein in order to get to their targets [4]. Nevertheless, this mixture was unable to achieve an effective concentration in some parts as a result of the physical and/or chemical properties of the matrix, thereby giving rise to an apparent increase in the rate of resistance. Certainly, recent mathematical modeling has suggested that while limited antimicrobial diffusion may give rise to the death of the outer layer of bacteria, it also stimulates subpopulations of bacteria buried deeper within the biofilm to enact adaptive changes, thereby countering the effect of the antimicrobials [5]. However, limited antimicrobial agent diffusion seems not to be a universal trait shared by all biofilms. Hence there is conflicting data about whether the biofilm matrix is a major contributing factor influencing biofilm resistance [2]. A reduction in penetration and diffusion of the antimicrobial agent via the biofilm matrix has been shown to affect biofilm survival in some instances. For instance, at sub-MIC concentrations of β -lactam antimicrobials, increased alginate production in *Pseudomonas aeruginosa* biofilms was induced, and the biofilm matrix of some slime-producing coagulase-negative staphylococci was enhanced. Moreover, even though it was originally suggested that tolerance of biofilms to aminoglycoside was due to transport limitation owing to the binding of these positively charged antimicrobial agents to the negatively charged exopolysaccharide matrix, repeated dosing of antimicrobial agent during treatment may possibly result in saturation of the binding sites [6,7,8,9]. This study aimed to perform phenotypic screening of the strong biofilm former bacterial isolates from saliva, teeth, and necrotic root canals according to their multi-antibiotic resistance ability.

MATERIALS AND METHODS

Bacterial isolates

This study was carried out in the department of Microbiology, school of bioscience and biotechnology, faculty of science and technology, National University Malaysia. All bacterial isolates isolated from saliva, teeth and necrotic roots canals samples by employing standard bacteriologic techniques. Preliminary tests were done to classify the isolates according to the morphological and cultural characteristics. For further identification, biochemical tests were used. As a result, 267 isolates (137 from saliva, 88 from teeth and 42 from necrotic roots canals) were identified to the genus level.

Biofilm formation assay by using Microtiter plate method (MTP)

This technique of quantitative detection of biofilm using 96 well microtitre plate employing the ability of the isolates to form a biofilm on abiotic surfaces was used. This method was used as mentioned by [10]. All tests were carried out in triplicate, and the strains were divided into the following categories: no biofilm producer, weak biofilm producer, moderate biofilm producer, or strong biofilm producer [11]. For this, it was necessary to establish the cutoff value (OD_c). The OD_c was defined as three standard deviations (SD) above the mean

OD of the negative control (uninoculated medium): OD_c = average OD of negative control + (3×SD of negative control). Based on the OD values:

OD ≤ OD_c = non biofilm producers;

OD_c < OD ≤ 2 × OD_c = weak biofilm producer;

2 × OD_c < OD ≤ 4 × OD_c = moderate biofilm producers;

3 × OD_c < OD = strong biofilm producers

Antibiotics Susceptibility Assay

Antibiotic resistance of the strong biofilm forming isolates (saliva n=55, teeth n= 32 and necrotic roots canals n= 17) were determined on Muller & Hinton Agar (Oxoid) using Kirby-Bauer disk diffusion method [12]. The antibiotics selected for the study comprised of: kanamycin K (30μg), vancomycin VA (30μg), amoxycillin AML (10μg), erythromycin E (30μg), ampicillin AMP (10μg), cephalothin KF (30μg), chloramphenicol C (30μg), tetracycline TE (30μg), ciprofloxacin CIP (10μg), nalidixic acid NA (30μg), gentamicin CN (10μg), novobiocin NV (30μg), carbenicillin CAR (100μg), oxacillin OX (5μg), penicillin G (10 UI) and streptomycin S (10μg). All the antibiotic discs were procured from Oxoid (UK). *E. faecalis* ATCC 33186 was used as control strains.

RESULTS AND DISCUSSION

Table 1 biofilm formation ability among bacterial isolates from saliva

Genus	Isolates number	Strong	Moderate	Weak
<i>Enterococcus</i> sp.	20	16	4	0
<i>Streptococcus</i> sp.	18	10	6	2
<i>Staphylococcus</i> sp.	16	10	4	2
<i>Bacillus</i> sp.	9	5	2	2
<i>Actinomyces</i> sp.	3	0	2	1
<i>Escherichia coli</i>	11	6	1	4
<i>Salmonella</i> sp.	4	0	3	1
<i>Shigella</i> sp.	2	0	2	0
<i>Pseudomonas</i> sp.	5	3	2	0
<i>Klebsiella</i> sp.	4	0	3	1
<i>Clostridium</i> sp.	8	0	6	2
<i>Micrococcs</i> sp.	4	0	4	0
<i>Enterobacter</i> sp.	6	0	5	1
<i>Proteus</i> sp.	7	5	1	1
<i>Peptostreptococcus</i> sp.	4	0	2	2
<i>Lactobacillus</i> sp.	3	0	3	0
<i>Fusobacterium</i> sp.	3	0	0	3
<i>Eubacterium</i> sp.	2	0	2	0
<i>Prevotella</i> sp.	2	0	1	1
<i>Bacteroides</i> sp.	4	0	3	1
<i>Aerococcus</i> sp.	2	0	1	1
Total	137	55	57	25

Microtiter Plate Method (Crystal Violet Method)

The findings from this screening technique showed that almost all the isolates can form visible growing biofilms when compared to the controls, though with difference in the degree

of adhesions. The results showed that 16 (80%) of the *Enterococcus* sp. isolates from saliva considered as a strong biofilm former while 4 (20%) showed moderate biofilm formation. For *Streptococcus* sp., *Staphylococcus* sp., *Bacillus* sp., *E. coli*, *Pseudomonas* sp., *Proteus* sp. the numbers of the strong biofilm former isolates were 10 (55.5%), 10 (62.5%), 5 (55.5%), 6 (54.5%), 3 (60%), and 5 (71.4%) respectively (Table 1).

The results obtained from this method showed that 8 (57.1%) of the *Enterococcus* sp. isolates from teeth can be considered as a strong biofilm former, while 4 (28.6%) isolates were considered moderate and 2 (14.3%) isolates were considered as weak biofilm formers. Also the numbers of the strong isolates were 5 (29.4%), 7 (43.7%), 1 (33.3%), 4 (44.4%), 2 (28.5%), 1 (33.3%), 2 (22.2%) and 2 (40%) for *Streptococcus* sp., *Staphylococcus* sp., *Bacillus* sp., *E. coli*, *Pseudomonas* sp., *Klebsiella* sp., *Enterobacter* sp. and *Proteus* sp. respectively (Table 2).

Table 2 biofilm formation ability among bacterial isolates from teeth

Genus	Isolates numbers	Strong	Moderate	Weak
<i>Enterococcus</i> sp.	14	8	4	2
<i>Streptococcus</i> sp.	17	5	9	3
<i>Staphylococcus</i> sp.	16	7	6	3
<i>Bacillus</i> sp.	3	1	2	0
<i>Actinomyces</i> sp.	2	0	2	0
<i>E. coli</i>	9	4	3	2
<i>Pseudomonas</i> sp.	7	2	3	2
<i>Klebsiella</i> sp.	3	1	2	0
<i>Enterobacter</i> sp.	9	2	6	1
<i>Proteus</i> sp.	5	2	2	1
<i>Lactobacillus</i> sp.	3	0	2	1
Total	88	32	41	15

One of the important results that observed during the employment of the MTP method was the high percentage of the strong biofilm formers among the *Enterococcus* sp. isolates, isolated from necrotic roots canals which the number was 8 (88.9%) followed by *Streptococcus* sp. 5 (71.4%), *Clostridium* sp. 2 (66.6%), *Staphylococcus* sp. 1 (25%) and *Bacillus* sp. 1 (20%). (Table 3). In this study, it was noted that the ability of *Enterococcus* sp. isolates to produce strong biofilm formation was higher than the others, and this was due to many reasons. Enterococci have been reported as important organisms in periodontal infection [13]. The adherence and production of a biofilm by *E. faecalis* on different biomaterials have been demonstrated, and the capacity of enterococci to bind to various medical devices has been associated with the ability of enterococci to produce biofilms [14]. The number of genetic factors known to be involved in biofilm production has increased in recent years, due to the availability of genomic and proteomic approaches, but much more research is needed to allow a better understanding of the regulation of biofilm production [15]. These genetic factors such as the presence of *esp* gene (Enterococcal surface protein), *gelE* gene (Secretory metalloprotease gelatinase E) and *bee* gene (Biofilm enhancer in

Enterococcus/a putative cell wall-anchored protein) [15]. A complete understanding of the role of genetic and environmental factors in the development of biofilm may lead to improved strategies for biofilm control among enterococci.

Table 3 biofilm formation ability among bacterial isolates from necrotic roots canals

Genus	Isolates number	Strong	Moderate	Weak
<i>Enterococcus sp.</i>	9	8	1	0
<i>Streptococcus sp.</i>	7	5	2	0
<i>Staphylococcus sp.</i>	4	1	2	1
<i>Bacillus sp.</i>	5	1	3	1
<i>Actinomyces sp.</i>	3	0	1	2
<i>Clostridium sp.</i>	3	2	1	0
<i>Peptostreptococcus sp.</i>	4	0	2	2
<i>Fusobacterium sp.</i>	2	0	2	0
<i>Prevotella sp.</i>	1	0	1	0
<i>Bacteroides sp.</i>	3	0	3	0
<i>Chromobacterium sp.</i>	1	0	0	1
Total	42	17	18	7

Antibiotic resistance among saliva isolates

Fifty-five strong biofilm producing isolates were subjected to screening its ability to resist sixteen type of antibiotics. Figure (1) showed the percentage of antibiotics resistance among the gram-positive strong biofilm former isolates of saliva (n=41). These isolates identified to four genera as *Enterococcus sp.*, *Streptococcus sp.*, *Staphylococcus sp.* and *Bacillus sp.* It is apparent from this figure that all the isolates were resistant to the nalidixic acid and penicillin G, also all the isolates resistant to streptomycin except one isolate of *Bacillus sp.* (S96). The results also indicated that all the *Enterococcus sp.* isolates were resistance to the all antibiotics except ampicillin and amoxicillin, which two isolates (S88 and S106) were sensitive to ampicillin and five isolates (S52, S88, S98, S106 and S131) were sensitive to amoxicillin (Table 4). The observed increase in antibiotic resistance among *Enterococcus sp.* isolates could be attributed to the strong biofilm formation ability of these isolates since many studies reported the number of genetic factors known to be involved in biofilm production [15], in addition to that these organisms have developed resistance to virtually all antimicrobials currently used in clinical practice using a diverse number of genetic strategies [16]. Due to this ability to recruit antibiotic resistance determinants, MDR enterococci display a wide repertoire of antibiotic resistance mechanisms including modification of drug targets, inactivation of therapeutic agents, overexpression of efflux pumps and a sophisticated cell envelope adaptive response that promotes survival in the human host and the nosocomial environment [16]. Figure (2) presents an overview of the antibiotics resistance among gram-negative strong biofilm former isolates from saliva (n=14). The results showed that all the isolates have the ability to resist vancomycin, novobiocin, erythromycin, penicillin, oxacillin and cephalothin, while this resistance decreased with other types of antibiotics (ciprofloxacin, gentamycin, carbenicillin and streptomycin). The results indicate that all *E. coli* isolates (n=6) were sensitive to the tetracycline, gentamycin, carbenicillin, nalidixic acid and chloramphenicol, while *Proteus sp.* isolates (n=5) were sensitive to gentamycin and

chloramphenicol only. For *Pseudomonas* sp. isolates (n=3), the results showed that all the isolates (S3, S33 and S97) sensitive only to carbenicillin while the resistance to the other types of antibiotics were varied. Resistance rates are increasing among several problematic gram-negative pathogens that are often responsible for serious nosocomial infections [17].

Fig 1 Antibiotics resistance of gram-positive isolates of saliva samples

Fig 2 Antibiotics resistance of gram-negative isolates of saliva samples

Table (4) Antibigram pattern of the strong biofilm former isolates from saliva

Isolates codes	Antibiogram
S1, S2, S16, S22, S31, S40, S46, S98, S124, S131, S133 (<i>Enterococcus</i> sp.)	CIP,TE,VA,CN,NV,CAR,NA,AMP,AML,E,K,C,P,S,OX,KF
S3, S33 (<i>Pseudomonas</i> sp.)	TE,VA,CN,NV,NA,AMP,AML,E,K,C,P,S,OX,KF
S4, S41(<i>Escherichia coli</i>)	CIP,VA,NV,E,P,OX,KF
S12 (<i>Streptococcus</i> sp.)	CN,NV,CAR,NA,AMP,AML,P,S,KF
S14 (<i>Proteus</i> sp.)	CIP,TE,VA,NV,AMP,AML,E,C,P,S,OX,KF
S18 (<i>Staphylococcus</i> sp.)	CIP,TE,VA,NA,AMP,AML,K,P,S,KF
S30, S43 (<i>Proteus</i> sp.)	TE,VA,NV,NA,AMP,AML,E,C,P,OX,KF
S34 (<i>Staphylococcus</i> sp.)	CIP,TE,VA,CN,NA,AMP,AML,P,S,KF
S36 (<i>Bacillus</i> sp.)	CIP,TE,VA,CN,NV,NA,AMP,AML,E,C,P,S,OX,KF
S44 (<i>Bacillus</i> sp.)	CAR,NA,AMP,E,C,P,OX,KF
S49 (<i>Staphylococcus</i> sp.)	CIP,VA,CN,NA,AMP,AML,K,C,P,S,KF
S52, S56 (<i>Enterococcus</i> sp.)	CIP,TE,VA,CN,NV,CAR,NA,E,K,C,P,S,OX,KF
S55, S86 (<i>Proteus</i> sp.)	TE,VA,NV,CAR,NA,AML,E,C,P,OX,KF
S58 S91 (<i>Staphylococcus</i> sp.)	VA,NA,AMP,AML,C,P,S
S64 (<i>Streptococcus</i> sp.)	CN,NV,CAR,NA,AMP,AML,P,S,OX,KF
S66(<i>Escherichia coli</i>)	CIP,VA,NV,CAR,NA,AMP,E,P,S,OX,KF
S67, S70 (<i>Staphylococcus</i> sp.)	VA,CAR,NA,AMP,AML,E,K,C,P,S,OX,KF
S73, S82 (<i>Streptococcus</i> sp.)	TE,CN,NV,CAR,NA,AMP,P,S,OX,KF
S76 (<i>Escherichia coli</i>)	VA,NV,AMP,AML,E,P,OX,KF
S78 (<i>Bacillus</i> sp.)	CAR,NA,AMP,AML,E,C,P,OX,KF
S83 (<i>Streptococcus</i> sp.)	VA,NV,CAR,NA,AMP,P,S,OX,KF
S88 (<i>Enterococcus</i> sp.)	CIP,TE,CN,NV,CAR,NA,AMP,E,K,C,P,S,OX,KF
S89, S130 (<i>Escherichia coli</i>)	VA,NV,AMP,AML,E,C,P,OX,KF
S93, S96 (<i>Bacillus</i> sp.)	CAR,NA,AMP,C,P
S97 (<i>Pseudomonas</i> sp.)	CIP,TE,VA,NV,NA,AML,E,K,C,P,OX,KF
S100, S106 (<i>Enterococcus</i> sp.)	CIP,TE,VA,CN,NV,CAR,NA,AMP,E,K,C,P,S,OX,KF
S108 (<i>Streptococcus</i> sp.)	CIP,NV,CAR,NA,AMP,P,S,KF
S112 (<i>Streptococcus</i> sp.)	CIP,NV,NA,AMP,P,S,KF
S114 (<i>Staphylococcus</i> sp.)	VA,CAR,NA,AMP,C,P,S
S118 (<i>Streptococcus</i> sp.)	CIP,NV,NA,AMP,C,P,S,KF
S125, S128 (<i>Streptococcus</i> sp.)	CIP,NV,NA,C,P,S,OX,KF
S132, S137 (<i>Staphylococcus</i> sp.)	VA,NV,CAR,NA,AMP,E,C,P,S,

Antibiotic resistance among teeth isolates

The antibacterial susceptibility test of bacterial isolates is important for choosing appropriate therapy for infections in critically ill patients. Figure (3) showed the clear trend of increasing in the antibiotic resistance ability among the strong biofilm former isolates from teeth (n=32). The high percentages of resistance were 100%, 97% and 94% for streptomycin, novobiocin and nalidixic acid respectively, followed by kanamycin (75%), penicillin (75%), tetracycline (72%), chloramphenicol (69%), erythromycin (69%), oxacillin (66%), carbenicillin (66%), amoxicillin (63%), cephalexin (59%), ampicillin (59%), gentamicin (59%), vancomycin (59%) and ciprofloxacin (56%). Erythromycin has been recommended as an alternative option for patients who are allergic to penicillin and are also widely used for antibiotic prophylaxis of endocarditis associated with dental procedures [18], in our results the isolates have been showed high resistance to erythromycin whereas 22 from 32 isolates were resistant to erythromycin. Another study recommended that amoxicillin and penicillin G are the most effective antibacterial drugs for the treatment of dental caries [19], in this study the isolates from teeth showed high resistance to both antibiotics recommended by the previously mentioned study. The results of the antibiotic resistance ability of the strong biofilm former isolates from teeth are shown in the table (5). The significant outcome of the results was the high resistance of *Enterococcus* sp. isolates to all types of antibiotics that used. As mentioned in the literature review, apart from their important role in nosocomial infections, enterococci also play significant roles in dissemination and persistence of antibiotic resistance [20]. The results showed that all the isolates were resistant to the nalidixic acid and this is also documented by another study which mentioned that enterococci possess intrinsic resistance to nalidixic acid [21]. Also, this increasing resistance was observed among the *Enterococcus* sp. isolates against kanamycin where all the eight isolates were resistant to this antibiotic. High-level kanamycin resistance is a well-documented phenomenon among enterococci species [21]. High resistance to novobiocin as observed in the study do not support the results of the previous research of [22]. Table (6) list the antibiogram pattern of the strong biofilm former isolates from teeth.

Fig 3 Levels (%) of antibiotic resistance demonstrated by Strong biofilm producing isolates of Teeth

Table 5 Numbers of antibiotics resistance isolates from teeth according to genera

Antibiotics	<i>Enterococcus</i> sp. (n=8)	<i>Streptococcus</i> sp. (n=5)	<i>Staphylococcus</i> sp. (n=7)	<i>Bacillus</i> sp. (n=1)	<i>E. coli</i> (n=4)	<i>Pseudomonas</i> sp. (n=2)	<i>Klebsiella</i> sp. (n=1)	<i>Enterobacter</i> sp. (n=2)	<i>Proteus</i> sp. (n=2)
CIP	8	3	4	0	1	0	0	2	0
T	8	4	5	0	1	2	0	1	2
V	6	0	2	0	4	2	1	2	2
CN	8	2	5	1	0	2	0	0	1
NV	8	5	7	0	4	2	1	2	2
CAR	6	4	5	0	3	1	0	1	1
NA	8	5	7	0	3	2	1	2	2
AMP	6	1	1	1	3	2	1	2	2
AML	6	1	3	0	4	2	1	1	2
E	8	1	2	0	4	2	1	2	2
K	8	5	7	1	0	2	1	0	0
C	8	2	3	1	1	2	1	2	2
P	6	3	3	1	4	2	1	2	2
S	8	5	7	1	4	2	1	2	2
OX	6	2	2	1	4	2	1	2	2
KF	6	5	4	0	1	1	0	1	1

Table 6 Antibigram pattern of the strong biofilm former isolates from teeth

Isolates	Antibiogram
H1 (<i>Streptococcus</i> sp.)	CIP,TE,NV,CAR,NA,K,S,KF
H2 (<i>Staphylococcus</i> sp.)	CIP,TE,CN,NV,CAR,NA,AMP,AML,K,P,S
H3 (<i>Bacillus</i> sp.)	CN,AMP,K,C,P,S,OX
H4 (<i>Escherichia coli</i>)	CIP,VA,NV,CAR,AMP,AML,E,P,S,OX
H5, H14, H79, H85 (<i>Enterococcus</i> sp.)	CIP,TE,VA,CN,NV,CAR,NA,AMP,AML,E,K,C,P,S,OX,KF
H6 (<i>Escherichia coli</i>)	VA,NV,NA,AML,E,P,S,OX
H7 (<i>Staphylococcus</i> sp.)	CIP,NV,NA,K,C,S,KF
H8 (<i>Pseudomonas</i> sp.)	TE,VA,CN,NV,CAR,NA,AMP,AML,E,K,C,P,SOX,KF
H10 (<i>Staphylococcus</i> sp.)	TE,VA,CN,NV,CAR,NA,AML,K,S

H11 (<i>Enterococcus</i> sp.)	CIP,TE,CN,NV,CAR,NA,E,K,C,S
H12 (<i>Streptococcus</i> sp.)	CIP,TE,CN,NV,CAR,NA,AMP,AML,K,C,S,KF
H19 (<i>Pseudomonas</i> sp.)	TE,VA,CN,NV,NA,AMP,AML,E,K,C,P,SOX
H27 (<i>Enterococcus</i> sp.)	CIP,TE,VA,CN,NV,CAR,NA,E,K,C,P,S,OX,KF
H30 (<i>Proteus</i> sp.)	TE,VA,NV,CAR,NA,AMP,AML,E,C,P,S,OX
H35 (<i>Enterobacter</i> sp.)	CIP,VA,NV,NA,AMP,E,C,P,S,OX
H40 (<i>Escherichia coli</i>)	TE,VA,NV,CAR,NA,AMP,AML,E,P,S,OX
H43 (<i>Proteus</i> sp.)	TE,VA,CN,NV,NA,AMP,AML,E,C,P,S,OX,KF
H50 (<i>Streptococcus</i> sp.)	CIP,CN,NV,NA,E,K,P,S,KF
H57 (<i>Enterococcus</i> sp.)	CIP,TE,CN,NV,NA,AMP,AML,E,K,C,S
H59 (<i>Enterobacter</i> sp.)	CIP,TE,VA,NV,CAR,NA,AMP,AML,E,C,P,S,OX,KF
H61 (<i>Staphylococcus</i> sp.)	CIP,NV,CAR,NA,K,P,S
H63 (<i>Staphylococcus</i> sp.)	TE,CN,NV,CAR,NA,E,K,C,S,OX
H67 (<i>Staphylococcus</i> sp.)	TE,CN,NV,CAR,NA,E,K,C,S,OX,KF
H70 (<i>Staphylococcus</i> sp.)	CIP,TE,VA,CN,NV,NA,AML,K,P,S
H72 (<i>Enterococcus</i> sp.)	CIP,TE,VA,CN,NV,NA,AMP,AML,E,K,C,P,S,OX,KF
H77 (<i>Streptococcus</i> sp.)	TE,NV,CAR,NA,K,C,P,S,OX,KF
H80 (<i>Klebsiella</i> sp.)	VA,NV,NA,AMP,AML,E,K,C,P,S,OX
H83 (<i>Streptococcus</i> sp.)	TE,NV,CAR,NA,K,P,S,OX,KF
H88 (<i>Escherichia coli</i>)	VA,NV,CAR,NA,AMP,AML,E,C,P,S,OX,KF

Antibiotic resistance among necrotic roots canals isolates

The enucleation of infection from the root canal system by means of mechanical and chemical cleansing presents a major hindrance to combat in endodontic treatment. Unfortunately, because of the complex nature of the root canal anatomy, a complete cleansing and disinfection are almost impossible, leaving residual organic and inorganic matter. Pulpal tissue remnants and exudate from the periapical region serve as a substrate for micro-organisms invading the root canal system via either coronal leakage or residing despite treatment [23]. Antibiotic resistance has increased substantially in the recent years and is posing an ever-increasing therapeutic problem [24,25]. It's important to study the ability of the antibiotics resistance of the most prevalence and strong biofilm former isolates present inside the root canal, to present a good understanding of the causes of root canal filling failure or the recurrence of the infection after filling it. After collecting the data of the antibiotic susceptibility test among the strong biofilm former isolates (n=17) from necrotic roots canals, the results showed the increasing ability of these isolates to resist the various

typed of antibiotics used in this study. The results summarized in figure (4) showed that the percentage of resistance to the kanamycin and streptomycin were 100%, followed by novobiocin (94%), ciprofloxacin (88%), cephalothin (88%), tetracycline (82%), gentamicin (82%), carbenicillin (82%), nalidixic acid (82%), chloramphenicol (82%), penicillin (82%). Also, the percentages of resistance were (76%) for both of ampicillin, amoxicillin and erythromycin, and (71%) for oxacillin. The lowest percentage of antibiotic resistance was against vancomycin (53%). Table (7) shows the number of strong biofilm former isolates resistant to each antibiotic used in this study. For *Enterococcus* sp., all the isolates (n=8) resisted to the ciprofloxacin, tetracycline, vancomycin, gentamicin, novobiocin, nalidixic acid, ampicillin, amoxicillin, erythromycin, kanamycin, chloramphenicol, streptomycin. Enterococci have emerged as important nosocomial pathogens and emergence of resistance to many of the antimicrobials used for Gram-positive organisms has made the management of infections due to *Enterococcus* species difficult [26]. The propensity of *Enterococcus* species to easily acquire resistance genes and the presence of some unique mechanisms conferring resistance to antibiotics like aminoglycosides and glycopeptide has severely limited the choices available for treating serious infections due to these organisms [27]. The emergence of multi-drug resistant enterococci has led to a scenario which is almost as bad as the pre-antibiotic era since many of these multi-drug resistant (MDR) strains have developed resistance to practically all available antibiotics [27]. Vancomycin resistance among *Enterococcus* isolates a major problem in most of the western world, especially in the United States where according to the National Nosocomial Infections Surveillance (NNIS) data, more than 28 percent of all nosocomial enterococcal strains are vancomycin resistant [25]. Table (8) showed the antibiogram of the isolates from necrotic roots canals.

Fig4 Levels (%) of antibiotic resistance demonstrated by Strong biofilm producing isolates of necrotic root canals

Table (7) Numbers of antibiotics resistance isolates from necrotic roots canals according to genera

Antibiotics	<i>Enterococcus</i> sp. (n=8)	<i>Streptococcus</i> sp. (n=5)	<i>Staphylococcus</i> sp. (n=1)	<i>Bacillus</i> sp. (n=1)	<i>Clostridium</i> sp. (n=2)
Ciprofloxacin	8	3	1	1	2
Tetracycline	8	3	1	0	2
Vancomycin	8	0	1	0	0
Gentamicin	8	2	1	1	2
Novobiocin	8	5	1	0	2
Carbenicillin	6	4	1	1	2
Nalidixic acid	8	5	1	0	0
Ampicillin	8	2	0	1	2
Amoxicillin	8	2	0	1	2
Erythromycin	8	1	1	1	2
Kanamycin	8	5	1	1	2
Chloramphenicol	8	2	1	1	2
Penicillin	7	3	1	1	2
Streptomycin	8	5	1	1	2
Oxacillin	7	2	0	1	2
Cephalothin	7	5	1	0	2

Table 8 Antibigram pattern of the strong biofilm former isolates from teeth

Isolates codes	Antibiogram
M1, M2, M10, M20, M39, M40 (<i>Enterococcus</i> sp.)	CIP,TE,VA,CN,NV,CAR,NA,AMP,AML,E,K,C,P,S,OX,KF
M4, M22 (<i>Clostridium</i> sp.)	CIP,TE,CN,NV,CAR,AMP,AML,E,K,C,P,S,OX,KF
M6 (<i>Streptococcus</i> sp.)	CIP,NV,CAR,NA,AMP,AML,K,C,P,S,KF
M12 (<i>Streptococcus</i> sp.)	CIP,CN,NV,CAR,NA,K,P,S,KF
M18 (<i>Streptococcus</i> sp.)	CIP,TE,CN,NV,CAR,NA,AMP,AML,E,K,P,S,KF
M28 (<i>Bacillus</i> sp.)	CIP,CN,CAR,AMP,AML,E,K,C,P,S,OX
M29 (<i>Enterococcus</i> sp.)	CIP,TE,VA,CN,NV,NA,AMP,AML,E,K,C,S,OX,KF
M31 (<i>Streptococcus</i> sp.)	TE,NV,CAR,NA,K,C,S,OX,KF
M34 (<i>Enterococcus</i> sp.)	CIP,TE,VA,CN,NV,NA,AMP,AML,E,K,C,P,S
M37 (<i>Streptococcus</i> sp.)	TE,NV,NA,K,S,OX,KF
M41 (<i>Staphylococcus</i> sp.)	CIP,TE,VA,CN,NV,CAR,NA,E,K,C,P,S,KF

CONCLUSION

It was concluded that almost all the bacterial isolates in this study can form visible growing biofilms when compared to the controls, though with difference in the degree of adhesions (strong, moderate and weak). The findings revealed that *Enterococcus* sp. isolated from saliva, teeth and necrotic roots canals was the strongest biofilm former bacteria. Further, the

study revealed that antimicrobial resistance of bacteria isolated from saliva, teeth and necrotic roots canals of patients with dental caries conditions is associated with biofilm formation.

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