International Journal of Medical Toxicology & Legal Medicine Volume 27, No. 4, 2024
e-ISSN: 0974-4614
p-ISSN: 0972-0448

Streptococcus Mutans and Enterococcus Gallinarum Isolated from Dental Abutments for Patients with Periodontitis

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Received: 14.08.2024 Revised: 12.09.2024 Accepted: 06.10.2024

ABSTRACT

Background: Periodontitis impacts a sizable proportion of the population. As the condition progresses, periodontal abscesses, tooth loss, and pain are all serious problems. It has also been shown that periodontitis is connected to health concerns outside of the mouth.

Material and methods: This study included 230 samples ranging in age from 40 to 71 years (200 patients and 30 controls). From July 2022 to October 2022, patients in Kirkuk were referred to three key facilities: the dental specialist center, maxillofacial surgical center, and department of periodontics in Azadi Teaching Hospital, and the specialist consulting clinic. The genomic DNA was obtained using the Wizar genomic DNA purification kit (Promega, Madison, WI, USA), which is intended to recover bacterial DNA from both species with various gram counts. On 5 samples of DNA isolated from Enterococcus gallinarum, PCR was done using primers. Five DNA samples from various strains of Streptococcus mutans were subjected to polymerase chain reaction amplification of the van C-1 and ermB genes.

Results: The outcomes of genetic testing Vancomycin resistance gene vanC-1 for Enterococcus gallinarum (80%) and Erythromycin resistance gene ermB for Streptococcus mutans (100%). The convergence of the nitrogen base of the two bacteria with the NCBI was observed through the analysis of the Phylogenetic tree for four genes, where they were identical to the proportion of (96.92%) for van C-1 gene for Enterococcus gallinarum and (100%) for ermB gene for Streptococcus mutans.

Conclusions
Vancomycin resistance gene vanC-1 for Enterococcus gallinarum and Erythromycin resistance gene ermB for Streptococcus mutans (both genes were found in NCBI)

Keywords: Periodontitis, ermB, and vanC-1

1. INTRODUCTION

A condition known as periodontitis affects the gums, which surround the tooth roots. Both regional and systemic etiological factors contribute to the disease[(1)]. More than 700 distinct species of bacteria can be found in the human oral cavity[(2)]. A tiny number of harmful bacteria exist in the human oral cavity, but the bulk of the bacteria there are commensals. [(3)]. Periodontitis, one of the most common disorders that affect teeth, destroys the tooth's supporting and encircling tissue. [(4)].

The word "periodontitis" is made up of the two words "periodont-" and "itis," where "periodont-" stands for "structure surrounding the teeth" and "itis" stands for "inflammation." If

left untreated, periodontitis, a disorder that first affects the gingival tissue, extends to deeper tissues, disrupting the natural homeostasis of the bone and ultimately resulting in tooth loss[4]. Periodontal disease has a complex etiology[5]. It has been determined that the bacterial biofilm growing on tooth surfaces is the main factor causing periodontitis [6, 7]. Genetics, environmental variables, the patient's systemic health, lifestyle decisions, and different socioeconomic determinants all contribute to the evolution of the illness while local elements like plaque and calculus and the host response all play a role[5,8,9]. It has been demonstrated that periodontopathogens negatively affect patients' general health in addition to having deleterious effects that extend beyond the periodontium. [10,11].

Periodontitis risk is increased by unchecked hyperglycemia. Uncontrolled diabetes affects the immune system, resulting in neutrophil malfunction or macrophages that are overly sensitive and generate cytokines that promote inflammation. Uncontrolled hyperglycemia alters the metabolism of connective tissue, which has an impact on the resorptive and formative phases of periodontal disease[12,13].

Aim of the study: The purpose of the study was to use PCR to find the vancomycin resistant *vanC-1* for Enterococcus gallinarum and Erythromycin resistant ermB for Streptococcus mutans.

Materials and methods

1-Samples collection:

In this study, 230 samples with ages ranging from (40 to 70) years old were examined: 200 patients and 30 controls. Three major facilities received referrals for the patients, Dental specialist center, maxillofacial surgery center and department of periodontics in Azadi Teaching Hospital and the specialist consulting clinic from July 2022 to October 2022. Four groups were created from the participants in this study: (G1), the initial group was for patients suffering from periodontitis and have diabetes mellitus with Rheumatoid n 80(40%). The second group (G2) was suffering from periodontitis and have diabetes mellitus n 65(32.5%). The third group (G3) was patients suffering from periodontitis and have Rheumatoid n 55(27.5%). The fourth group (G4) was healthy persons volunteers with no gum disease referred as control n (30). A brief questionnaire form (Appendix I) was used to gather data on age, chronic conditions, and treatment methods.

Gingival Crevicular Fluid (GCF) Specimens taken.

Following teeth scaling, periodontal probe was used to measure the depth of the dental pockets. Gingival crevicular fluid (GCF) sample was then collected using sterile absorbent paper points (size 30-45 mm), periodontal pockets with depths of 3 to 8 mm were selected, and two paper points were inserted inside them until mild resistance was felt and was left. In order to use two paper points as a reducing transport medium for anaerobic bacteria, five millilitres of thioglycolate broth were added to sterilised Appandorf tube vials. and culture in (cve agar, Blood agar, and chocolate agar plates were kept in an incubator with 5% CO2 at 35-37°C for up to 48 hours.

DNA extraction and PCR:

DNA from isolates' genomes of Enterococcus gallinarum and Erythromycin (ermB) for Streptococcus mutans were extracted using the Wizar genomic DNA purification kit as directed by the manufacturer (Promega, Madison, WI, USA). The PCR reaction was conducted to amplify vanC-1 and ermB gene using specific primers in the table (1). In 2 ml of brain heart infusion broth that was supplemented with 1% glucose and stirred, isolates of Enterococcus gallinarum and Streptococcus mutans were cultured in anaerobic conditions.[14] .DNA was

extracted using the PCR reagent kit promega, Madison, WI, USA with final elusion into 50µl elusion buffer. A 822 base pair region of the vanC-1 gene sequences was targeted by PCR primers ,forward 5` TGAAGTTGGAGAAGCCGAGT 3` and reverse primer5` CTGCCAAGCTTCGCCTATAC 3, while the base pair region was 219 of ermB gene for Streptococcus mutans, PCR primers ,forward 5` CGTGTCACTTTAATTCACCAAGA 3`and reverse primer was 5` TTGAGTGTGCAAGAGCAACC 3`.

Table(1): The primers that used to detect genes

Gene	Sequence	Temperature	Bp	Reference
Enterococcus gallinarum vanC-1	F 5` TGAAGTTGGAGAAGCCGAGT 3`	51	822	All primers were designed in this study using NCBI
	R 5` CTGCCAAGCTTCGCCTATAC 3`			
	R 5` AAAGCCCCATTGCACAG 3`			
Streptococcus mutans ermB	F 5` CGTGTCACTTTAATTCACCAAGA 3`	57	219	
	R 5` TTGAGTGTGCAAGAGCAACC 3`			

Result:

Table(2):Isolation and Identification of anaerobic and facultative anaerobic bacteria according to source of isolation

Bacterial isolates(aerobia facultative anaerobic)	Group 1	Group 2	Group 3	Group 4 Healthy
Peptostreptococcus prevotii (71)	32(16%)	19 (9.5%)	20(10%)	0
Clostridium spp(23)	9(4.5%)	7(3.5%)	7(3.5%)	0
Fusobacterium nucleatum. (10)	4(2%)	3(1.5%)	3(1.5)	0
Enterococcus gallinaum (79)	34(17%)	25(12.5%)	20(10%)	2(6.6%)
Streptococcus mutans(65)	20(10%)	34(17)%	11(5.5%)	2(6.6%)
Total (248)				
p- value	0.312			0.02

As shown in Table (2), there are no differences among 3 groups for patients suffering from Periodontitis group, group 2, and group 3) for anaerobic bacterial isolation, 32(16%), 19 (9.5%), 20(10%) were positive isolated for peptostreptococcus prevotii respectively. 9(4.5%),7(3.5%),7(3.5%) were positively isolated for Clostridium spp respectively, 4(2%),3(1.5%),3(1.5) were positive isolated for Fusobacterium nucleatum respectively at P-value 0.312

Amplification of the vanC-1 gene

Using primers created for this purpose, the results showed out of (5) presumptive isolates ,(4) of them were positive for target gene vanC-1. Electrophoresis on the agarose gel of the PCR products showed appearance of bands of 822bp bases in all the amplified samples, which

represents 80% of the studied samples (Fig. 2).

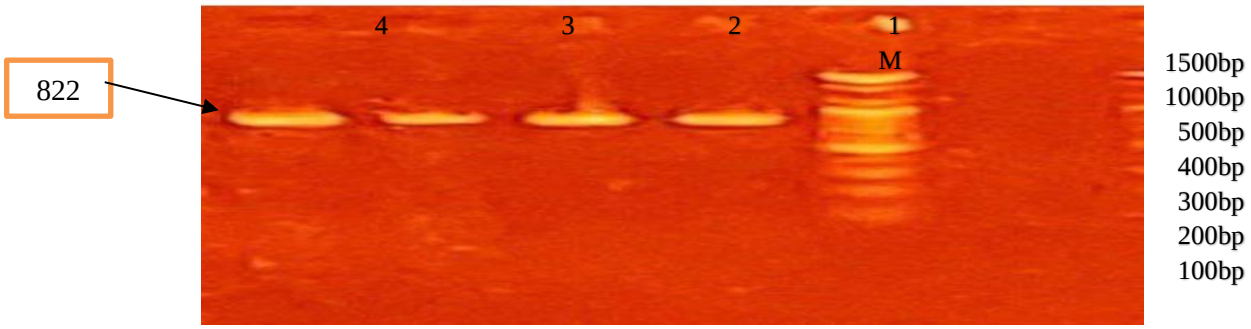

Figure (1) Agarose gel electrophoresis of PCR amplification produce(vanC-1), expected amplification products are 822bp (lanes1-5:samples, lane M: marker 100bp,1.5%agrose gel and 70 Volt for 1 hr. in TBE buffers.

Amplification of the ermB gene

erthromycin-resistant streptococcus mutans by using target gene (ermB) the results showed all of (5) presumptive isolates were positive for target gene (ermB), Electrophoresis on the agarose gel of the PCR products showed appearance of bands of **219bp** bases in all the amplified samples, which represents 100% of the studied samples

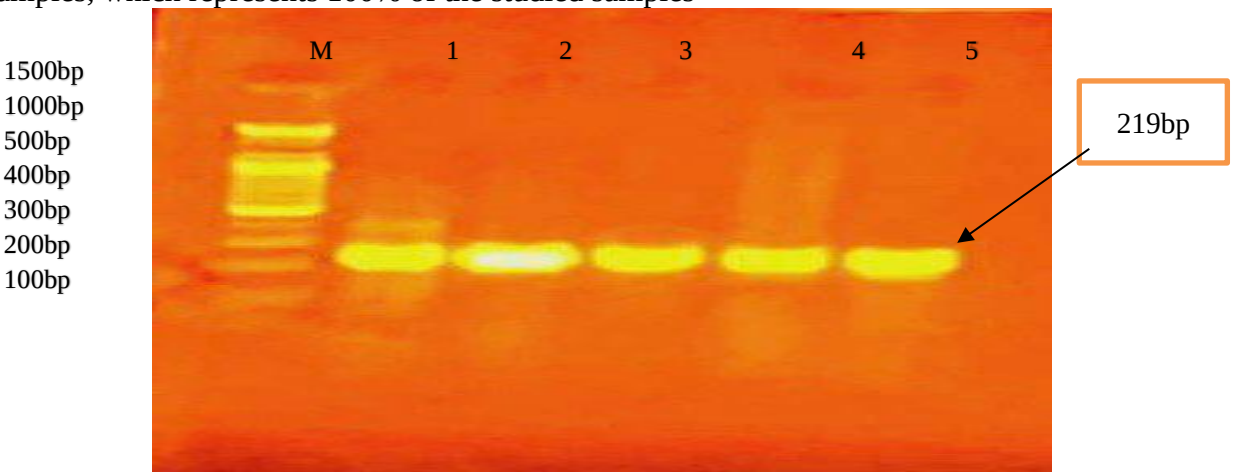

Figure (2) Agarose gel electrophoresis of PCR amplification produce(ermB) , expected amplification products are 219bp (lanes1-6:samples, lane M: marker 100bp,1.5%agrose gel and 70 Volt for 1 hr. in TBE buffers.

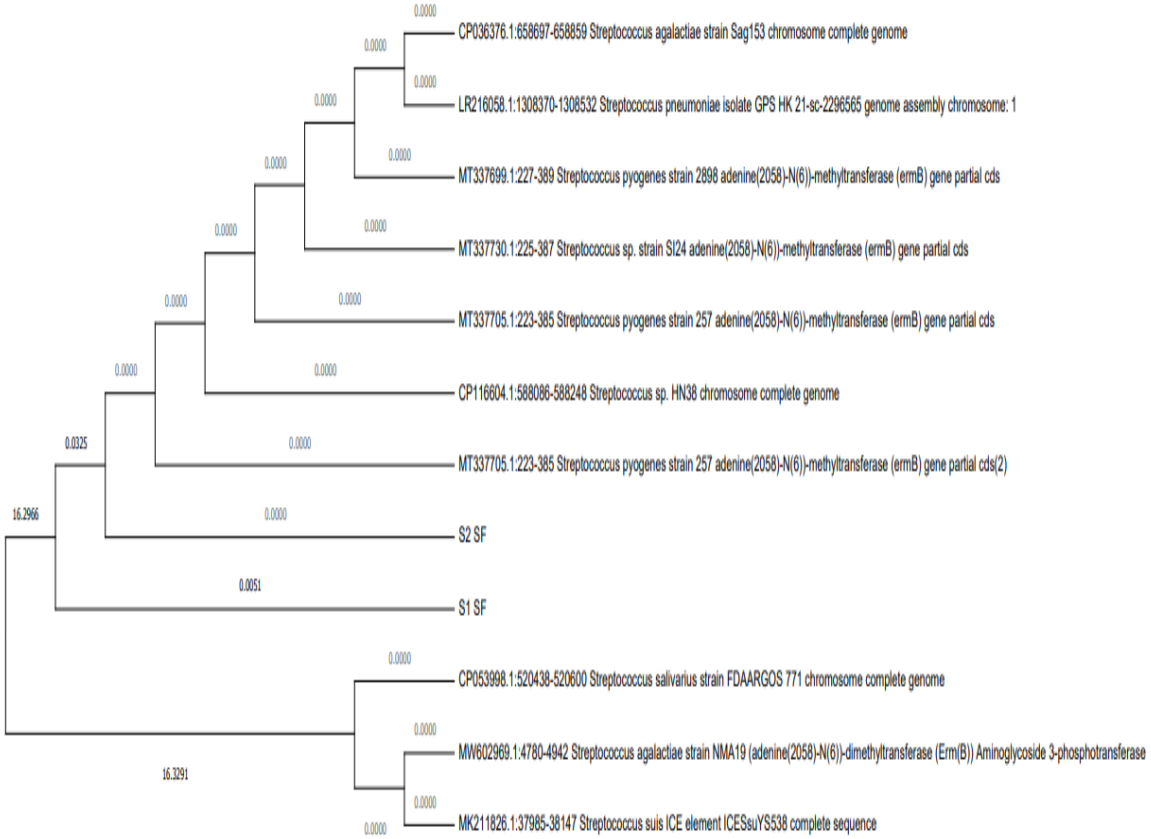

Figure (3): Phylogenetic tree of Streptococcus mutans show the degree difference Streptococcus mutans for gene(erm B) resistansc for erythromycin the degrees With the universal gene ,so we made a genetic tree and this strain was explained in genetic tree.where this gene was registered in NCBI the name (AAAS)

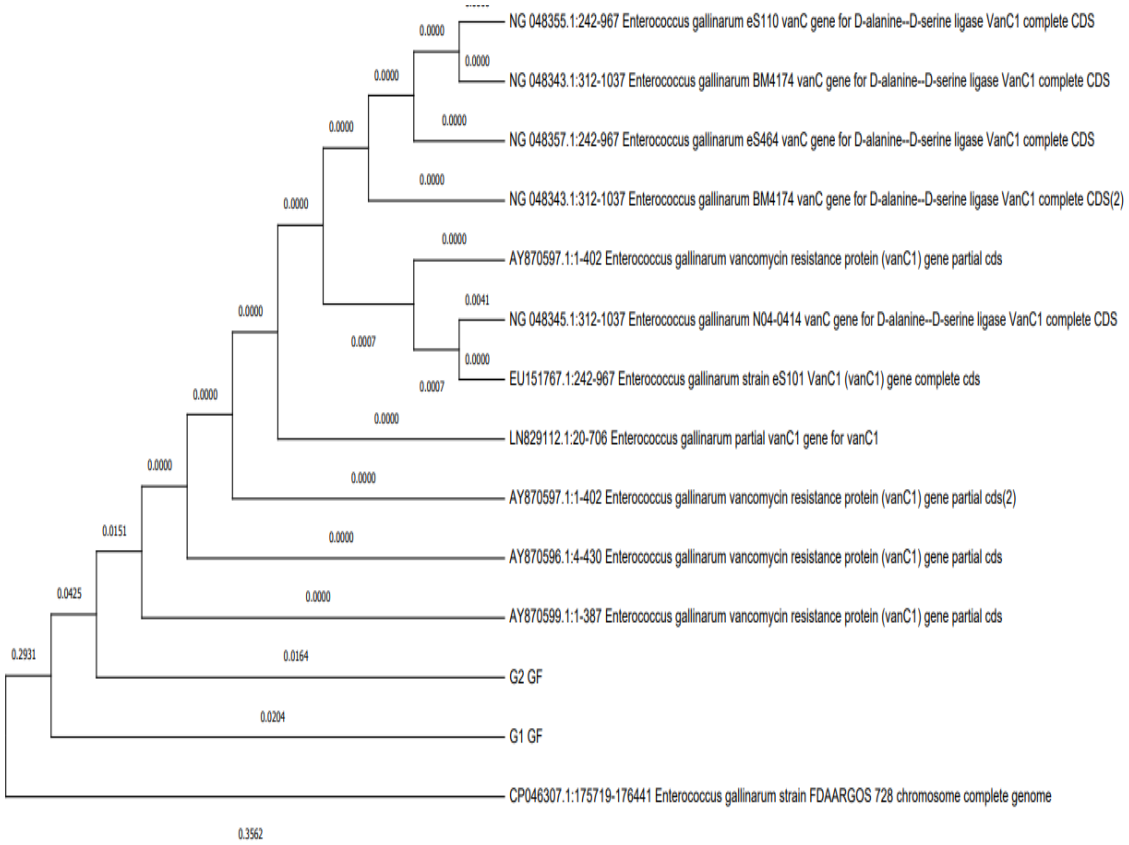

Figure (4):Phylogenetic tree of vancomycin resistant *van* C-1

Disscusion

Gram-positive, spherical or ovoid cells organised in pairs or chains, non-sporulated, and non-capsulated Enterococcus gallinarum is isolated from patients with periodontitis[15].

There are numerous forms of vancomycin resistance in enterococci, which is becoming a more prevalent clinical issue. An altered pathway of peptidoglycan production and hydrolysis of the typical vancomycin-sensitive precursors are involved in the resistance mechanism in the constitutively vancomycin-resistant Enterococcus gallinarum BM4174. D-Ala-D-Ser is synthesised by a ligase catalysed by the vanC-1 gene, and it is substituted for D-Ala-D-Ala in peptidoglycan precursors. [16]. The 42 strains of E. gallinarum evaluated included vanC-1-related sequences, and it is likely that this species' decreased susceptibility to vancomycin is due to the production of peptidoglycan precursors with lower vancomycin affinity[17].

ErmB-encoding genes change the bacterial ribosome's shape, which decreases the binding of the antibiotics macrolide, lincosamide, and streptogramin B (MLSB) to the target site of the 50S ribosomal subunit. [18]**.** Target modification by 23S RNA methylases, which are encoded by erm genes, was the distinctive mechanism of macrolide resistance discovered in streptococci. [19]**.** The degree difference *Streptococcus mutans* for gene(*erm* B) resistansc for erythromycin the degrees With the universal gene ,so we made a genetic tree and this strain was explained in genetic tree.where this gene was registered in NCBI the name (AAAS) .the sample under the current study were observed identical(96.25%) to the samples with the serial number(CP128779.1 Klebsiella pneumonia strain ARLG-4803),(AP026931.1 Streptococcus pneumonia PZ900701541 (,(AP026924.1 Streptococcus pneumonia PZ900700063)**.**

Our result show the gene resistant for vancomycin *van* C-*1*gene in *Enterococcus gallinarum (LC769350.1)* was identical in different proportions (93%-95%) it had a genetic tree that was identical to the two samples sent. Each sample was different. The two sample were recorded in NCBI.the sample under the current study were observed identical(99.38%) to the samples with the serial number(CP046307.1Enterococcus gallinarum strain FDAARGOS),(MN151325.1 Mixed culture bacterium),(CP040461.1 Enterococcus sp. M190262).

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