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Enterococcus faecalis an Emerging Microbial Menace in Dentistry-An Insight into the *In-Silico* Detection of Drug Resistant Genes and Its Protein Diversity

Microbial Genetics Section

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ABSTRACT

Introduction: Antimicrobial drug resistance is evolving as a serious threat to mankind due to indiscriminate use of antibiotics and lack of awareness about the mechanisms involved in drug resistance. *Enterococcus faecalis*, a common pathogen of the oral cavity has gained drug resistance over a period of years, making treatments refractory and ineffective.

Aim: To detect the antimicrobial resistance encoding genes of *Enterococcus faecalis* employing computational tools.

Materials and Methods: Antibiotic resistance genes were detected by retrieving sixteen genome sequences of *Enterococcus faecalis* from NCBI database which were further analysed using *ResFinder*. *PlasmidFinder* identified resistance encoding plasmids from recruited genomes under study. Protein sequences of most common phenotypes were subjected to protein BLAST and non-duplicate isolates showing 95-100% identity were selected for multiple sequence alignment using MEGA v7.0. Additionally, reconstruction of phylogenetic tree was performed to ascertain the diversity of these proteins among different genus and species.

Results: *In silico* analysis of genomes revealed that almost all the probed isolates exhibited resistance towards glycopeptides and macrolides. The genes *lsa(A)* were found in 100% of the isolates, followed by *tet (M)* and *erm (B)* exhibiting a frequency of 37.5% and 25% respectively. Apart from major antibiotics, the isolates also demonstrated resistance towards aminoglycoside, phenicol, tetracycline and trimethoprim class of antimicrobials.

Conclusion: The present investigation has emphasised the novelty in the application of in-silico tools in the understanding of the antibiotic resistance profile explored by the dreadful endodontic pathogen viz., *E. faecalis*. Further, this approach will aid in the implementation of improved treatment strategies and will facilitate to combat the dissemination of resistant gene cassettes to other oral pathogens or commensals residing in the vicinity.

Keywords: Drug resistance, Glycopeptides, Macrolides, Phylogenetic analysis, Plasmids

INTRODUCTION

Enterococcus faecalis is a commensal and opportunistic pathogen commonly observed in oral diseases. It has been grouped as one among the 25 pathogens listed to be linked to persistent endodontic infections [1]. Interestingly, the frequency of oral carriage of *E. faecalis* remains inconsistent when considering different clinical conditions for example; individuals with gingivitis and periodontitis exhibit a prevalence of 3.7-35% [2], whereas it is 60% in diabetes patients and 6.6% in normal control group [3]. One of the studies on age and gender based carriage of *E. faecalis* showed 94.1% prevalence in children, 89.5% in adults and 81.6% in elderly Brazilian population. The study also reported that the predominant enterococcal species isolated from oral rinse was *E. faecalis* (88.7%) [4].

The pathogen is considered to be a microbial menace due to its frequent association with failure of treatments precipitated by resistance to antimicrobials [5]. They possess intrinsic resistant mechanisms and also have the ability to acquire gene cassettes coding for drug resistance through horizontal gene transfer. *E. faecalis* has been found to be associated with carious lesions, chronic periodontitis [6], persistent [7] and primary endodontic infections [8] along with a polymicrobial cosmos. Recalcitrant biofilm formation is often observed in both treated and untreated root canals [9]. Deep seated infections involve a polymicrobial community and anaerobic environment. Hence, drugs targeted against these pathogens may also pose a selective pressure on other organisms at the vicinity including *E. faecalis* which compels them to resist drugs and propagate in this stringent environment by modifying their genetic make-up or acquisition of drug resistant genes [10]. Several drug resistant isolates have been recovered from root canal infections [11,12].

The presence of such antibiotic resistant bacterial population does not only hamper the treatment process, but also acts as a reservoir for drug resistant genes which can be acquired by other organisms or even commensals residing in the vicinity of these pathogens. The transfer of such genes is enabled by mobile elements viz., plasmids and transposons [13].

The rationale behind this study is to project those interesting and rare drug resistant phenotypes exhibited by the pathogen. Hence, the basic drug resistant profile of *E. faecalis* needs to be validated to arrive at a conclusion about the novel mechanisms underlying resistance phenotype exhibited by this organism. The present study was aimed to probe into their genome for novel drug resistant genes and diversity of protein encoded by the predominant genes. To the best of our knowledge, this is the first study which reports acquired drug resistant genes in *E. faecalis* by in silico analysis.

MATERIALS AND METHODS

Strain: A cross sectional study was designed to analyse sixteen whole genome sequences of *Enterococcus faecalis* retrieved from the NCBI database [14] as of Feb' 2018, scaffolds and contigs were excluded. Also, the size of the genome, replicons, GC percentage, number of genes and proteins encoded were derived from the same database [14] [Table/Fig-1]. Assembled sequences were submitted to MLST (Multilocus sequence typing) 1.8 algorithm from Center for Genomic Epidemiology (CGE) [15]. MLST was performed including seven housekeeping genes (*aroE, gdh, gki, gyd, pstS, xpt, yqiL*) of *E. faecalis* to classify the strains based on the sequence types.

Strain name/ number	Sequence type	Size (Mb)	GC %	Replicons	Genes	Pro-teins
E.faecalis V583	ST-6	3.36	37.3	Chromosome Plasmids pTEF1, 2	3412	3264
E.faecalis OG1RF	ST-1	2.74	37.8	Chromosome	2710	2602
E.faecalis 62	ST-66	3.13	37.4	Chromosome Plasmid EP62pA	3157	3075
E.faecalis D32	ST-40	3.06	37.4	Chromosome Plasmids EFD32pA, B	3174	2973
E.faecalis-symbioflor 1	ST-248	2.81	37.7	Chromosome	2885	2733
E.faecalis DENG1	ST-191	2.96	37.5	Chromosome	3050	2881
E.faecalis ATCC 2912	ST-30	3.04	37.3	Chromosome Plasmids p1, p2	3128	2922
E.faecalis LD33	ST-25	2.80	37.6	Chromosome	2867	2695
E.faecalis KB1	ST-9	3.03	37.2	Chromosome	3014	2815
E.faecalis L9	ST-29	2.69	37.7	Chromosome	2706	2578
E.faecalis L12	ST-711	2.67	37.8	Chromosome	2660	2543
E.faecalis CLB21560	ST-28	3.24	37.8	Chromosome Plasmids pA, pB	3404	3211
E.faecalissoralis	ST-65	3.05	37.2	Chromosome Plasmids p1, p2	3059	2886
E.faecalis W11	Unknown	2.70	37.7	Chromosome	2699	2577
E.faecalis AR01/ DG	ST-108	2.88	37.5	Chromosome Plasmids ARO1.1, ARO1.2	2929	2788
E.faecalis FDA ARGOS_338	ST-19	2.86	37.6	Chromosome	2939	2572

[Table/Fig-1]: Strains of *Enterococcus faecalis* genome selected for the present study.

Resistant Gene Profiling: *Resfinder 3.0* is a user-friendly, computational tool [16] from the CGE. The whole genome sequence in the FASTA format was used as an input file to obtain facts about drug resistant genes present in the pathogen. The test was conducted for acquired antimicrobial resistance including all available antimicrobial drugs of six major classes such as aminoglycoside, beta-lactam, colistin, fluoroquinolone, fosfomycin, fusidic acid, glycopeptide, macrolide, lincosamide, streptogramin, nitroimidazole, oxazolidinone, phenicol, rifampicin, sulphonamide, rifampicin, trimethoprim and run using default parameters [Table/Fig-2].

Plasmid Profiling: *PlasmidFinder 1.3* is yet another effective tool from CGE to probe the presence of plasmids in the genomes selected [17]. The algorithm ran using default parameters and the reference database selected was that of *Enterococcus*, *Streptococcus* and *Staphylococcus*. The data obtained from the study was used to correlate between the presence of plasmids and the drug resistant phenotypes recorded earlier [Table/Fig-3].

Multiple Sequence Alignment (MSA): The protein sequences of predominant drug resistant phenotypes coding for *lsa(A)*, *tet(M)* and *erm(B)* were further analysed across several genus and species. Since *lsa(A)* was mostly present in *Enterococcus* spp. alone MSA was performed using FASTA sequence of different strains of *E. faecalis*. However, *tet(M)* and *erm(B)* were found to occur in diverse microbial population and hence, was subjected to MSA using MUSCLE programme of MEGA v.7.0 to ascertain the diversity of these proteins [18]. UPGMB was the clustering method applied with default parameters. Non-duplicate isolates exhibiting 100% query coverage with 95-100% identity were selected for the analysis [Table/Fig-4]. The subcellular localisation of the protein was deduced by PSORTb v3.0 [19].

Phylogenetic Analysis of Erm(B) AND Tet(M) Protein: The diversity in course of evolution of LsaA, Erm(B) and Tet(M) proteins [Table/Fig-5] were inferred by using the Maximum Likelihood method based on the Poisson correction model [20]. Evolutionary analyses were conducted in MEGA v7.0 [21]. The bootstrap consensus tree inferred from 500 replicates was taken to represent the evolutionary history of the protein analysed [22].

RESULTS

The principal objective of the present study was to acquire a basic understanding on the drug resistant profiles exhibited by *E. faecalis* strains submitted in public domain from across the globe. Sixteen whole genome sequences of *E. faecalis* returned resistance profiles as detected by *ResFinder 3.0* corresponding to: 1) aminoglycoside; 2) glycopeptides; 3) macrolide, lincosamide streptogramin B (MLS); 4) tetracycline; 5) phenicol; and (6) trimethoprim. Among the six antimicrobial drug groups gene *lsa(A)* encoding MLS resistance was found to be the most predominant (n=16; 100%), followed by *tet(M)* (n=6; 37.5%) and *erm(B)* (n=4; 25%). Three strains out of sixteen harboured more than four resistance encoding genes (25%) [Table/

Strain name/number	Aminoglycoside	Glycopeptide	Macrolide, Lincosamide, Streptogramin B	Tetracycline	Phenicol	Trimethoprim
E.faecalis V583	*aac(6') – aph (2")*	*van X-B, van B, van H-B, van W-B,van Y-B, van S-B,van R-B*	*lsa(A)* *erm(B)*	-	-	-
E.faecalis OG1RF	-	-	*lsa(A)*	-	-	-
E.faecalis 62	-	-	*lsa(A)*	*tet(M)*	-	-
E.faecalis D32	*ant(6)Ia*	-	*lsa(A)* *erm(B)*		-	-
E.faecalis-symbioflor 1	-	-	*lsa(A)*		-	-
E.faecalis DENG1	-	-	*lsa(A)*	*tet*(M)	-	-
E.faecalis ATCC 29212	-	-	*lsa(A)*	*tet(M)*	-	-
E.faecalis LD33	-	-	*lsa(A)*		-	-
E.faecalis KB1	-	-	*lsa(A)*		-	-
E.faecalis L9	-	-	*lsa(A)*		-	-
E.faecalis L12	-	-	*lsa(A)*		*fex(A)*	
E.faecalis CLB21560	*aac(6') – aph (2")* *ant(6)Ia, aph(3')-III*	-	*lsa(A)* *erm(B)*	*tet(M)*	-	*dfr(G)*
E.faecalissoralis	-	-	*lsa(A)*		-	-
E.faecalis W11	-	-	*lsa(A)*		-	-
E.faecalis AR01/DG	-	*van Z-A, van Y-A,van X-A, van A,vanH-A, vans A, vanR-A*	*lsa(A)* *erm(B)*	*tet(L)* *tet(M)*	-	-
E.faecalis FDA ARGOS_338	-	-	*lsa(A)*	*tet(M)*	-	-

[Table/Fig-2]: Acquired antimicrobial drug resistant genes present in *E.faecalis* strains as detected by *ResFinder*.
E.faecalis FDA ARGOS_338

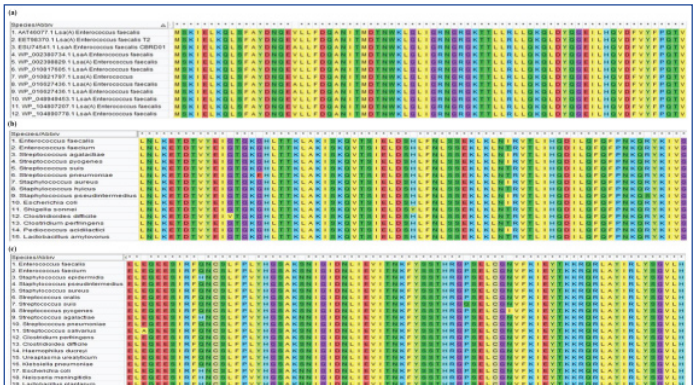
Fig-6]. The multi-locus sequence typing showed that each of the strains belongs to a specific sequence type and they may not be clustered into the same sequence type.

Plasmid profiling of the sequences retrieved were consistent with the data acquired from NCBI database. The plasmid type and % identity to the known plasmid sequences are provided in [Table/Fig-3]. Interestingly, aminoglycoside, glycopeptide and trimethoprim resistant phenotypes were found to be occurring only in strains harbouring plasmids, whilst the other phenotypes like tetracycline, phenicol and MLS were found in both plasmid bearing and non-plasmid containing strains [Table/Fig-3].

Strain name/number	Plasmids	% Identity	Resistant Phenotypes
E.faecalis V583	pTEF1, 2, 3, pAD1	100	Aminoglycoside, Glycopeptide, MLS
E.faecalis OG1RF	-	-	MLS
E.faecalis 62	p703/5, pTEF2, pCF10	100 99.5 96.4	Tetracycline MLS
E.faecalis D32	pGB354	96.6	Aminoglycoside MLS
E.faecalissymbioflor 1	-	-	MLS
E.faecalis DENG1	-	-	Tetracycline MLS
E.faecalis ATCC 29212	pTEF3, pAD1	95.5 95.9	Tetracycline MLS
E.faecalis LD33	-	-	MLS
E.faecalis KB1	-	-	MLS
E.faecalis L9	-	-	MLS
E.faecalis L12	-	-	Phenicol, MLS
E.faecalis CLB21560	pAD1, pTEF2	100 99.5	Aminoglycoside, Tetracycline, Trimethoprim, MLS
E.faecalissoralis	pTEF2	96.7	MLS
E.faecalis W11	-	-	MLS
E.faecalis AR01/DG	p703/5, pAD1, pTEF3	100 97.0 95.6	Glycopeptide, Tetracycline, MLS
E.faecalis FDA ARGOS_338	-	-	Tetracycline, MLS

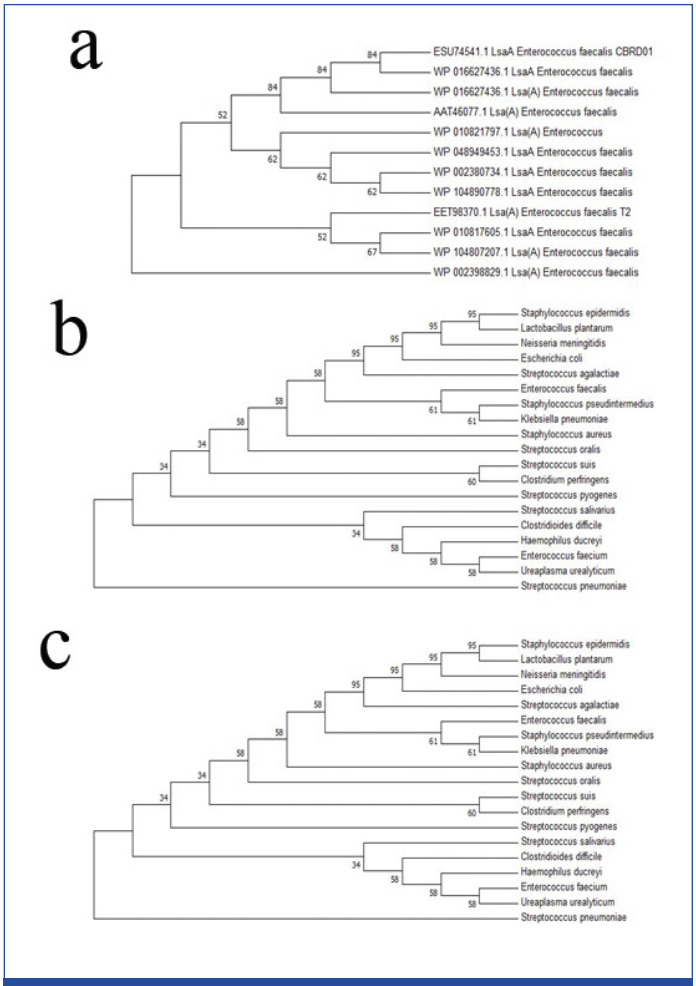
[Table/Fig-3]: Association between plasmids and resistant phenotypes. MLS: Macrolide; Lincosamide; Streptogramin B

Multiple sequence alignment of Tet(M) and Erm(B) proteins showed conserved sequences among a diverse group of organisms tested [Table/Fig-4]. Single amino acid variations were dispersed along the protein sequences which indicates the process of evolution of genes encoding these proteins. The MSA for Lsa(A) protein which was species specific and conserved among *Enterococci* with variations dispersed throughout the protein sequence [Table/Fig-4]. The subcellular localisation of proteins as deduced by PSORTb v3.0



[Table/Fig-4]: Multiple sequence alignment demonstrating diversity of (a) Lsa(A), (b) Tet (M) protein and (c) Erm (B) protein among different bacterial genus and species.

showed Lsa(A) to be in the transmembrane region and Tet(M) and Erm(B) proteins in the cytoplasm. Phylogenetic analysis performed using the protein sequences of Tet(M) and Erm(B) showed the evolution and diversification of protein among several genus and species analysed. Phylogenetic analysis of Lsa(A) protein showed diversity among the *E.faecalis* species studied, demonstrating rapid evolution of this protein at the species level [Table/Fig-5]. Thus, the present study provides an insight about the genetic basis of drug resistance exhibited by the strains of *E. faecalis* investigated.



[Table/Fig-5]: Phylogenetic tree demonstrating the diversity of (a) Lsa (A), (b) Tet (M) and (c) Erm (B) protein among different bacterial genus and species.

Gene	Frequency (N=16)	Percentage (%)
lsa (A)	16	100
tet (M)	6	37.5
erm (B)	4	25
van group of genes	2	12.5
aac(6')-aph (2")	2	12.5
ant(6)Ia	2	12.5
aph(3')-III	1	6.25
fex (A)	1	6.25
dfr (G)	1	6.25
tet (L)	1	6.25

[Table/Fig-6]: Frequency of occurrence of antibiotic resistant genes in the strains of *E.faecalis*.

DISCUSSION

Antimicrobials are an integral part of the health care system and find its use in various fields of medicine including dentistry. The emergence of drug resistant pathogens has turned the treatment options towards the dark side. The World Health Organization (WHO) has reiterated the fact that treatment failure due to resurgence of drug resistant organisms will be the challenge faced

in this millennium [23]. The commonly used drugs in dentistry are erythromycin, vancomycin, tetracycline, doxycycline, metronidazole etc., Although, local and systemic modes of delivery are used, local administration of antibiotics is preferred since the target tissue can be reached easily. This method also creates a selective pressure on the organisms infecting tissues, thus paving way for acquisition of drug resistant genes from similar pathogens. Since most of the drug resistant genes are carried on mobile elements like plasmids, transposons and integrons, the horizontal dissemination of drug resistant genes becomes undemanding.

The present study unravels the antibiotic resistant profiles of *E. faecalis* which is considered to be one of the vital pathogens related to endodontic and periodontal infections. Resistance towards vancomycin and tetracycline have been extensively studied in this pathogen. Interestingly, we could observe a few rare genes such as *fex(A)* and *dfr(G)* in two of the strains of *E. faecalis* (*E.faecalis* L12, *E.faecalis* CLB21560). Most of the Gram positive organisms exhibit resistance towards MLS class of drugs via two major mechanisms *viz.*, drug efflux and demethylation of 23S rRNA. The gene *lsa(A)* is responsible for intrinsic resistance to lincosamides and streptogramins A in *E. faecalis* [24]. BLAST analysis and several other reports confirmed the species specificity of this protein. Although the role of Lsa remains ill-defined it may be involved in drug efflux pathway, as the protein resembles ATP-Binding Cassette (ABC)-efflux pumps. Clindamycin and quinupristin-dalfopristin resistance was reported in 100% of the strains exhibiting this phenotype. Targeted inactivation of *lsa(A)* gene produced susceptible phenotypes, while recombinant plasmid carrying *lsa* gene mediated complementation restored the resistant phenotype [25].

Another best understood mechanism of streptogramin resistance is through the demethylation of 23S rRNA [26]. The Erm proteins disrupt the binding between macrolides and rRNA by the transfer of methyl group at A2058 of 23S rRNA rendering the strains resistant to MLS. *erm(A)* and *erm(B)* which confers MLS(A) and MLS(B) phenotypes are carried on broad host range plasmid such as pAMβ1 [27]. This report is in agreement with the present study where we could observe *erm (B)* gene in plasmid bearing strains of *E. faecalis*. Hospital strains of *E. faecalis* have recorded highest prevalence of resistance to erythromycin (34.1%) which is mostly encoded by *erm(B)* gene (70.9%) [28]. Erm type of protein shows diversity across different genus and species, thus elucidating an underlying genetic evolution of these species. Since erythromycin is routine drug of choice in dental clinics, periodical monitoring and investigations into resistance genes encoding Erm protein should be performed in *E. faecalis* isolated from tissue or oral site which is non-compliant with treatment. This may reduce further complications by resistant species and also prevent horizontal gene transfer among other organisms in oral cavity.

Tetracycline resistance in *Enterococci* dates back to early 1960, soon after its introduction in clinical settings. The most prevalent resistant genes isolated from asymptomatic apical periodontitis were reported to be *tet(M)* (42%), *tet(W)* (29%) and *erm(C)* (24%) [29]. *In vitro* resistance studies on plaque samples demonstrated multidrug resistance in *E. faecalis* strains. The resistance profile in decreasing order was clindamycin and metronidazole (100%), erythromycin (80.8%) and tetracycline (53.2%) [30]. The Tet(M) protein mediates resistance via ribosomal protection while, Tet(L) confers resistance through drug efflux mechanisms which are energy dependent [31]. The present study also reports an overall prevalence of 43.8% of tetracycline encoded genes which is in close association with other *in vitro* studies.

Gentamycin resistance is generally mediated by Aminoglycoside Modifying Enzymes (AME).

The *aac(6') – aph (2")* possess both adenyltransferase and phosphotransferase activities, which confers resistance to gentamycin, amikacin, tobramycin and kanamycin. The encoded enzyme is carried on transposons accommodated within the plasmid or the chromosomal DNA. Interestingly, *aac(6')-aph (2")* was also observed in strains harbouring plasmids in the present study, which validates the location of this gene in the pathogen. These mobile elements also facilitate transfer of gene clusters to other anaerobic pathogens found in deep seated wounds. An investigation on the distribution of AME phenotype conducted in Japan detected *aac(6')-aph (2")* in about 42.5% of *E. faecalis* strains [32]. Strains with *aac(6')-aph (2") +ant(6)-Ia + aph(3')*-IIIa phenotype showed high level resistance to gentamycin and streptomycin [33]. The genome of *E. faecalis* CLB21560 also demonstrated a similar combination of genes, hence confirming the AME phenotype of this strain.

Vancomycin resistant *E. faecalis* (VREF) has emerged as major pathogen in nosocomial infections. Rengaraj R et al., reported highest prevalence of *van A* and *B* genes in VREF isolated from clinical specimens [34]. The vancomycin resistant phenotype is aided by the presence of a cluster of genes encoding cell wall modification precursors that exhibits poor affinity towards vancomycin [35]. Here, the normal peptidoglycan precursor with D-alanyl-D-alanine is replaced with D-alanyl-D-lactate, which binds to vancomycin with a lower affinity of 0.001 times when compared to the normal precursors. Several van proteins act synergistically to establish the phenotype [36]. A recent study testing efficacy of genetically engineered bacteriophage φEf11/φFL1C(Δ36)PnisA in treatment of vancomycin resistant strain proved to be a success with vancomycinR strain (*E. faecalis* V583) exhibiting 99% susceptibility in comparison to 18% in sensitive strains (*E. faecalis* JH2-2) [37]. The VRE phenotype was observed in two strains analysed in the present study. Additionally, these phenotypes were found to co-occur along with MLSR encoding genes and found in plasmid bearing strains.

The *fex(A)* gene encodes chloramphenicol resistance which was first identified on the transposon Tn558 in *Staphylococcus lentus* plasmid. This gene is carried on non-conjugative plasmids in *E. faecalis*. The gene encodes a transmembrane efflux proteincontaining 475 amino acids forming fourteen transmembrane domains [38,39]. Resistance to trimethoprim-sulfamethoxazole is mediated by *dfrG*, which encodes the enzyme trimethoprim insensitive dihydrofolate reductase [40]. The *dfr* genes are mostly found in the plasmids and only a few transmissible *dfr* genes have been marked in Gram positive organisms, *dfrG* is one among them and is reported to be detected in *Streptococcus pyogenes* [41]. The present study also reports the presence of *dfrG* gene in a strain *E. faecalis* CLB21560 harboring plasmids which substantiates earlier reports. The prevalence of this gene in *E. faecalis* was also found to be low (n=1; 6.25%). The present study reiterates the fact that acquired drug resistance should not be ignored, as pathogens assimilating novel genes from the environment might complicate the treatment options and recovery in susceptible patients.

LIMITATION

Although the present study has certain limitations such as: a) lack of information about the antimicrobial drug resistance exhibited by strains *in vitro*; b) study restricted towards intrinsic and acquired resistance pertaining to gene clusters excluding point mutations/ substitutions; and c) antimicrobial resistance of clinical isolates of *E. faecalis* from different geographical locations. The present data provides a clear insight about the panel of genes to be detected primarily in case of a treatment failure in suspected *E. faecalis* infections. A better therapeutic strategy may be adopted for combating such refractory strains.

CONCLUSION

The trend of antimicrobial drug resistance is creeping into opportunistic and commensal organisms which may in future turn into resistant pathogens making treatments refractory or futile.

Hence, meticulous efforts are to be employed to identify, isolate and treat diseases involving resistant species. Alternate treatment modalities such as usage of combinatorial drugs, phage therapy, and probiotics can reduce the incidence of resistant forms. One of the interesting results derived from the study is that *E. faecalis* harbours one or more resistance encoding genes and that none of the strains were deprived of resistant genotype. To conclude, a vigilant surveillance and focused treatment will help to reduce the load of resistant gene pools in the oral cavity.

REFERENCES

[1] Anderson AC, Jonas D, Huber I, Karygianni L, Wölber J, Hellwig E, et al. *Enterococcus faecalis* from food, clinical specimens, and oral sites: Prevalence of Virulence Factors in Association with Biofilm Formation. Front Microbiol. 2016;11:1534.

[2] Sun J, Sundsfjord A, Song X. *Enterococcus faecalis* from patients with chronic periodontitis: virulence and antimicrobial resistance traits and determinants. Eur J Clin Microbiol Infect Dis. 2012;31:267–72.

[3] Chomicz L, Szubinska D, Piekarczyk J, Wojtowicz A, Piekarczyk B, Starosciak B, et al. Occurrence of subclinical infections of the oral cavity in the insulin treated diabetics. Wiad Parazytol. 2004;50(2):177–80.

[4] Komiyama EY, Lepesqueur LS, Yassuda CG, Samaranayake LP, Parahitiyawa NB, Balducci I, et al. Enterococcus species in the oral cavity: prevalence, virulence factors and antimicrobial susceptibility. PLoS One. 2016;11:e0163001.

[5] Sedgley C, Molander A, Flannagan SE, Nagel AC, Appelbe OK, Clewell DB, et al. Virulence, phenotype and genotype characteristics of endodontic *Enterococcus* spp. Oral Microbiol Immunol. 2005;20:10–19.

[6] Zhu X, Wang Q, Zhang C, Cheung GS, Shen Y. Prevalence, phenotype, and genotype of *Enterococcus faecalis* isolated from saliva and root canals in patients with persistent apical periodontitis. J Endod. 2010;36:1950–55.

[7] Pinheiro ET, Gomes BPFA, Ferraz CCR, Teixeira FB, Zaia AA, Souza-Filho FJ. Evaluation of root canal microorganisms isolated from teeth with endodontic failure and their antimicrobial susceptibility. Oral Microbiol Immunol. 2003;18:100–03.

[8] Duggan JM, Sedgley CM. Biofilm formation of oral and endodontic *Enterococcus faecalis*. J Endod. 2007;33:815–18.

[9] Sassone L, Fidel R, Figueiredo L, Fidel S, Faveri M, Feres M. Evaluation of the microbiota of primary endodontic infections using checkerboard DNA–DNA hybridization. Oral Microbiol Immunol. 2007;22:390–97.

[10] Pinheiro ET, Gomes BP, Drucker DB, Zaia AA, Ferraz CC, Souza-Filho FJ. Antimicrobial susceptibility of *Enterococcus faecalis* isolated from canals of root filled teeth with periapical lesions. Int Endod J. 2004; 37:756–63.

[11] Reynaud afGeijersstam AH, Ellington MJ, Warner M, Woodford N, Haapasalo M. Antimicrobial susceptibility and molecular analysis of *Enterococcus faecalis* originating from endodontic infections in Finland and Lithuania. Oral Microbiol Immunol. 2006;21:164–68.

[12] Jungermann GB, Burns K, Nandakumar R, Tolba M, Venezia RA, Fouad AF. Antibiotic resistance in primary and persistent endodontic infections. J Endod. 2011;37:1337–44.

[13] Flannagan SE, Clewell DB, Sedgley CM. A retrocidal plasmid in *Enterococcus faecalis*: passage and protection. Plasmid. 2008;59:217–30.

[14] https://www.ncbi.nlm.nih.gov/genome/?term=Enterococcus+faecalis.

[15] Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, et al. Multilocus sequence typing of total genome sequenced bacteria. J Clin Micobiol. 2012;50:1355-61.

[16] Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O,et al. Identification of acquired antimicrobial resistance genes. J Antimicrob Chemother. 2012;67:2640-44.

[17] Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, et al. Plasmid Finder and pMLST: in silico detection and typing of plasmids. Antimicrob Agents Chemother. 2014;58:3895-903.

[18] Robert E. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research. 2004;32:1792-97.

[19] Yu NY, Wagner JR, Laird MR, Melli G, Rey S, Lo R, et al. PSORTb 3.0: Improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. Bioinformatics. 2010;26:1608-15.

[20] Zuckerkandl E, Pauling L. Evolutionary divergence and convergence in proteins, in Evolving Genes and Proteins, edited by V. Bryson and H.J. Vogel. Academic Press, New York. 1965;97-166.

[21] Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33:1870-74.

[22] Felsenstein J. Confidence Limits on phylogenies: An approach using the bootstrap. Evolution. 1985;39:783-91.

[23] Harbarth S, Kahlmeter G, Kluytmans J, Mendelson M, Hospital GS, Burkert F, et al. Implementation of the global action plan on antimicrobial resistance. 2017;32:1–4.

[24] Bozdogan B, Leclercq R. Effects of genes encoding resistance to streptogramins A and B on the activity of quinupristin-dalfopristin against *Enterococcus faecium*. Antimicrob Agents Chemother. 1999;43:2720–25.

[25] Singh KV, Weinstock GM, Murray BE. An *Enterococcus faecalis* ABC homologue (Lsa) is required for the resistance of this species to clindamycin and quinupristin-dalfopristin. Antimicrob Agents Chemother. 2002;46:1845-50.

[26] Portillo A, Ruiz-Larrea F, Zarazaga M, Alonso A, Martinez JL, Torres C. Macrolide resistance genes in *Enterococcus* spp. Antimicrob Agents Chemother. 2000;44:967-71.

[27] Hollenbeck BL, Rice LB. Intrinsic and acquired resistance mechanisms in Enterococcus. Virulence. 2012;15:421-33.

[28] Quiñones Pérez D, Abreu Capote M, Marrero D, Alvarez AB, Ortiz C, Salomé F, et al. Antimicrobial susceptibility and genetic bases for resistance of infection-causing Enterococcus strains in Cuba. Rev Panam Salud Publica. 2011;30:549-54.

[29] Rôças IN, Siqueira JF Jr. Detection of antibiotic resistance genes in samples from acute and chronic endodontic infections and after treatment. Arch Oral Biol. 2013;58:1123-28.

[30] Thomas ER, Diane F, Joel EM, John ED, Arie JW. Antibiotic susceptibility of periodontal *Enterococcus faecalis*. J Periodontol. 2013;84:1026-33.

[31] Rathnayake IU, Hargreaves M, Huygens F. Antibiotic resistance and virulence traits in clinical and environmental *Enterococcus faecalis* and *Enterococcus faecium* isolates. Syst Appl Microbiol. 2012;35:326-33.

[32] Watanabe S, Kobayashi N, Quiñones D, Nagashima S, Uehara N, Watanabe N. Genetic diversity of enterococci harboring the high-level gentamycin resistance gene aac(6')-Ie-aph(2'')-Ia or aph(2'')-Ie in a Japanese hospital. Microb Drug Resist. 2009;15:185-94.

[33] Kobayashi N, Alam M, Nishimoto Y, Urasawa S, Uehara N, Watanabe N. Distribution of aminoglycoside resistance genes in recent clinical isolates of *Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus avium*. Epidemiol Infect. 2001;126:197-204.

[34] Rengaraj R, Mariappan S, Sekar U, Kamalanadhan A. Detection of vancomycin resistance among *Enterococcus faecalis* and *Staphylococcus aureus*. J Clin Diagn Res. 2016;10(2):DC04-6.

[35] Gold HS. Vancomycin-resistant enterococci: mechanisms and clinical observations. Clin Infect Dis. 2001;33:210-19.

[36] Bugg TD, Wright GD, Dutka-Malen S, Arthur M, Courvalin P, Walsh CT. Molecularbasis for vancomycin resistance in *Enterococcus faecium* BM4147: biosynthesis of a depsipeptidepeptidogly can precursor by vancomycin resistance proteins VanH and VanA. Biochemistry. 1991;29:10408-15.

[37] Tinoco JM, Liss N, Zhang H, Nissan R, Gordon W, Tinoco E, et al. Antibacterial effect of genetically-engineered bacteriophage φEf11/φFL1C(Δ36) PnisA on dentin infected with antibiotic-resistant *Enterococcus faecalis*. Arch Oral Biol. 2017;82:166-70.

[38] Lang KS, Anderson JM, Schwarz S, Williamson L, Handelsman J, Singer RS. Novel florfenicol and chloramphenicol resistance gene dis¬covered in Alaskan soil by using functional metagenomics. Appl Environ Microbiol. 2010;76:5321–26.

[39] Kehrenberg C, Schwarz S. *fexA*, a novel *Staphylococcus lentus* gene encoding resistance to florfenicol and chloramphenicol. Antimicrob Agents Chemother. 2004;48:615-18.

[40] Woods SE, Lieberman MT, Lebreton F, Trowel E, de la Fuente-Núñez C, Dzink-Fox J, Gilmore MS, Fox JG. Characterization of Multi-Drug Resistant *Enterococcusfaecalis* Isolated from Cephalic Recording Chambers in Research Macaques (Macacaspp.). PLoS One. 2017;12:e0169293.

[41] Bergmann R, Sagar V, Nitsche-Schmitz DP, Chhatwal GS. First detection of trimethoprim resistance determinant *dfrG* in Streptococcuspyogenes clinical isolates in India. Antimicrob Agents Chemother. 2012;56:5424-25.

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