Polymorphism

REVIEW

Comparative evaluation of microbiome of healthy and ailing dental implants- a systematic review

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ABSTRACT

Aim- To review and compare the microbiome associated with healthy dental implant and peri-implantitis sites.

Methods- Electronic search was conducted on PubMed, MEDLINE, Embase, Cochrane Library, Wiley Online Library databases to select studies assessing microbiome of healthy implants and peri-implantitis sites. Only original studies evaluating microbial profiles published in the last ten years in English were eligible.

Results: Polymerase Chain Reaction (PCR) detection frequency varied in healthy and peri- implantitis sites for *A. actinomycetemcomitans* (3-8 for healthy; 4-42 for peri-implantitis), *P.gingivalis* (9-2 for healthy; 8 to 93 for per-implantitis), *P.intermedia* (4-22 for healthy; 7-32 for peri-implantitis), T.forsythia (8 for healthy; 9-40 for peri-implantitis), *T.denticola* (7-10 for healthy; 7 to 54 for peri-implantitis) and *C.rectus* (0-44 for healthy; 0 to 70 for peri-implantitis). *Candida albicans (C.albicans*), Epstein Bar Virus 2 (EBV2) and Human Cytomegalovirus 1 (HCMV1) were found at both healthy and diseased peri-implant sites while HCMV 2 and EBV 1 were present at peri-implantitis sites only with the PCR technique. Prevalence percentage at peri-implant sites with Hybridization technique varied in healthy and peri-implantitis sites for *A.actinomycetemcomitans* (17%-23.1% for healthy; 23.1%-38% for peri-implantitis, *P.gingivalis* (27.7%-30.8% for healthy; 53.8%-56% for peri-implantitis), *P.intermedia* (21.3%-30.8% for healthy; 30.8%-45.8% for peri-implantitis), *T.denticola* (7.7%-14.9% for healthy; 8.3%-45.2% for peri-implantitis), *T.forsythia* (5.5%-46.1% for healthy; 61.5% for peri-implantitis), *P.endodontalis* (7.7% for healthy; 15.4% for peri-implantitis), *P.nigrescorens* (15.4% for healthy; 23.1% for peri-implantitis), *C.rectus* (00-27.7% for healthy; 15.4%-61.4% for peri-implantitis), and *F.nucleatum* (40.4%-61.5% for healthy; 38.5%-58.4% for peri-implantitis).

Conclusion- There was change in detection frequency of A.actionomycetemcomitans, P.gingivalis, P.intermedia, T.denticola, T.forsythia and C.rectus (not established statistically) at peri-implantitis sites. EBV 1 and HCMV 2 were associated with peri-implantitis sites only while C.albicans, EBV 2 and HCMV 1 were found present at both healthy and diseased implant sites.

KEYWORDS: Dental implant; Microbiome; Peri-implantitis; dental microbiome; Pyrosequencing .

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INTRODUCTION

Revolution came in prosthetic dentistry with the introduction of dental implants. At present, it is a major treatment option for replacing missing teeth. But many factors can cause implant failure. Implant failures have been classified as Early Implant Failure- improper patient selection, inferior surgical technique, overheating of the bone, too much torque during implant placement, a contaminated implant, contaminated osteotomy, and poor bone quality (Misch et al, 2008). Late Failure causes include- excessive masticatory forces, lateral forces, poor restoration, periodontal problems, and broken components (Misch et al, 2008). Micro-ecological disturbance around implants may also cause diseases leading to implant mobility and in severe cases, implant failure. Peri implant diseases present in two forms: Peri-Implant Mucositis (PM) –soft tissue inflammation around dental implant without an additional bone loss (Charalampkis et al, 2015) and Peri-Implantitis (PI) – an inflammatory process that causes inflammation of the soft tissues and bone structure around dental implants (Charalampkis et al, 2015). Chronic inflammation causes bone loss, which can eventually lead to implant loss. A high percentage of coccoid cells, low ratio of anaerobic/aerobic species, a smaller number of gram-negative species & a lower frequency of periodontal pathogens have been reported at healthy peri-implant sites (Lekholm et al, 1986; Bower et al, 1989; Ong et al, 1992; Mombelli et al, 2002).

Peri-implantitis may have an infectious etiology (Lang et al, 2011). Recent work indicates that peri-implant diseases present polymicrobial etiology, rather than a single pathogen (Maruyama et al, 2014, Shiba et al, 2016). It has been found that species like *P.gingivalis, Tannerella forsythia, Treponema denticola, and Aggregatibater actinomycetemcomitans* may be more commonly associated with peri-implantitis (Ebadian et al, 2012; Cortelli et al, 2013; Martin et al, 2017). Some studies also found EBV, Human Cytomegalovirus (HCMV) and Candida species to be associated with peri-implantitis (Jankovic et al, 2011; Kumar et al, 2012; Kato et al, 2017). However, it is still unclear whether there is a particular group of bacteria related to peri-implantitis. It has also been postulated that there are differences between the microbiome around healthy and diseased implants which directly or indirectly increase the risk of peri-implant disease (Kumar et al, 2012; Apatzidou et al, 2017; Martin et al, 2017).

Microbiome is the genome of all microorganisms (bacteria, fungi, protozoa, and viruses) living on and inside the human body. The oral microbiome was first recognized by Antonie Van Leeuwenhoek, using his microscope on dental plaque samples from himself and others (Yamashita et al, 2017). He reported differences in the individual oral microbiome and realized that these differences possibly influenced the oral health of an individual (Yamashita et al, 2017). The oral microbiome consists of various microbial niches with different virulence (the ability of a microbe or pathogen to infect or damage the host).

Dabdoub et al, 2013 found that the implant microbiome may be distinct from the periodontal microbiome. Socransky et al, 1998 described the role of the 5 main microbial species in the subgingival biofilm. It is reported that microorganisms play an important role in the occurrence of peri-implantitis (Mombelli et al, 1998) and almost 26% to 56% of the subjects with implant-supported prosthesis suffer from peri-implantitis at some point in life (Lindhe et al, 2008; Zitmann et al, 2008; Derks et al, 2016). Thus, the determination of the 'Microbiome' of peri-implantitis and healthy implants should be a major concern to improve the success rate of implant prosthesis. The aim of the present review, therefore, was to systematically evaluate microbial species present at healthy and diseased peri-implant sites and compare the respective microbiomes.

In the above background, we hypothesized that distinct microbial flora is found around peri-implantitis sites and healthy implant sites.

MATERIALS & METHODS

Objectives

This systematic review aimed to answer the following questions:

1. What is the Microbiome of healthy and failing implants?
2. Are there any similarities in the microbiome of healthy and failing implants?
3. What are the differences between the microbiomes of healthy and failing implants?

Although failing implants also include peri-implant mucositis, but this review only includes peri-implantitis.

Following PECO (Population, Exposure, Comparison, and Outcome) measures were considered:

Population- Studies on systemically healthy individuals with at least one healthy and/ or diseased implant; with microbiological findings from implant sites.

Exposure- Peri-implantitis.

Comparison- Differences between peri-implantitis and healthy implant tissue.

Outcome measures- Microbiological and microbiome status; total flora, specific species related to implant health or disease.

Inclusion Criteria: Studies assessing microbial species or microbiome in systemically healthy patients with healthy implants, peri-implantitis or both.

Only original research studies were included.

Studies published only after 2010 were included (published in the last 10 years).

Exclusion Criteria: Systematic and narrative reviews, Case reports, and Case series were not included.

Studies assessing microbial species in animals, and in-vitro studies were excluded.

Studies published before 2010 were not included (more than 10 years old were excluded).

Studies assessing microbial profile in peri-implant mucositis cases only.

Studies assessing peri-implant microbial profiles in patients with-

1) Uncontrolled systemic disease
2) In immune-compromised patients
3) History of the head or neck radiotherapy, undergoing radiotherapy or chemotherapy
4) Oral mucosal lesions (candidiasis, ulcerations, leukoplakia, oral cancer)
5) Drug, nicotine, or alcohol abuse
6) Pregnancy or lactation
7) Antibiotic usage in past 3 months for any systemic or dental procedure
8) Regular medication for any disorder
9) Maxillo-facial defects.

Online Search

Electronic search was conducted on Pub Med, MEDLINE, Embase, The Cochrane Library, Wiley Online Library databases. Boolean operators (OR, AND) were used to combine searches. Detailed search strategies were developed, for example, microbiome OR peri-implantitis OR peri-implantitis microbial assessment OR healthy implants microbiota OR oral biofilm OR biota. Online search was conducted till 30 June 2020 and relevant studies were included.

Study Selection

After initial screening, selected studies were further evaluated by full-text reading to be finally included in this review.

Data Compilation

Information from all studies was collected based on the following points: authors, year of publication, journal name, study type, and design, sample size and type, microbial analysis technique, microbial species evaluated, the microbiome of healthy implant sites, and peri-implantitis sites (Table 1).

Data Quality Evaluation

Following aspects were used for evaluating the studies: 1) study group selection (calculation of

sample size, methods used for assessing microbial species and peri-implant surroundings, standardization of outcome assessors, using clear inclusion/exclusion criteria); 2) comparison (implant site comparison based on study design/confounder analysis and management); 3) outcome (microbiologic outcome assessment, data collection measures and appropriateness of patient follow-up); and 4) statistical analysis (appropriateness/validity and unit of analysis).

Table 1: Tabulation of Implant groups, Study Design and Microbial Analysis Technique.

I- Based on PCR Microbial Analysis Technique						
S. No	Study	Author	Year	Journal Name	Implant Group	Study Design
1.	Correlation between different genotypes of human cytomegalovirus and Epstein-Barr virus and peri-implant tissue status.	Jankovic. S, Aleksic.Z, Dimitrijevic. B, Lekovic.V, Milinkovic. I, Kenney.B	2011	Australian Dental Journal	Healthy Implants, Peri-implant mucositis, Peri-implantitis	Cross-sectional Study
2.	Identification of Periodontal Pathogens in Healthy Peri-implant Sites	Casado.P.L, Otazu.I.B, Balduino. A, DeMello.W, Barboza.E.P, Durate.M.E.L	2011	Implant Dentistry	Healthy Implants, Peri-implant mucositis, Peri-implantitis	Cross-sectional Study
3.	The evaluation of bacterial flora in progress of peri-implant disease	Sato.J, Gomi.K, Makino.T, Kawasaki.F, Yashima.A, Ozawa.T, Maeda.N, Arai.T	2011	Australian Dental Journal	Peri-implant mucositis, Peri-implantitis	Cross-sectional Study
4.	Frequency of Periodontal Pathogens in equivalent Peri-implant and Periodontal Clinical Statuses	Cortelli S.C, Cortelli J.R, Romeiro R.L, Costa F.O, Aquino D.R, Orzechowski P.R, Araujo V.C, Duarte P.M.	2013	Archives of Oral Biology	Periodontal Health, Peri-implant Health, Peri-implant mucositis, Gingivitis Peri-implantitis	Cross-sectional Study

5.	Intra-individual variation in core microbiota in peri-implantitis and periodontitis	Maruyama.N, Maruyama.F., Takeuchi.Y, Aikawa.C, Izumi.Y, Nakagawa.I.	2014	Scientific Reports	Periodontitis, Peri-implantitis	Cross-sectional Study
6.	Real Time PCR Analysis of Fungal Organisms and Bacterial Species at Peri-implantitis Sites	Schwarz.F, Becker.K, Rahn.S, Hegewald.A, Pfeffer.K, Henrich.B	2015	International Journal of Implant Dentistry	Peri-implantitis	Cross-sectional Study
7.	Clinical, Radiographic and Microbiological Evaluation of High Level Laser Therapy, a New Photodynamic Therapy Protocol, in Peri-Implantitis Treatment; a Pilot Experience	Cacciangia.G, Rey.G, Baldoni.M, Paiusc.A	2016	Biomed Research international	Peri-implantitis	Longitudinal Study
8.	Genetic-Relatedness od Peri-implants and Buccal Candida Albicans Determined by RAPD- PCR	Bertone.A.M, Rosa.A.C, Nastri.N, Santillan.H.D, Ariza.Y, Iovannitti.C.A, Jewtuchowicz.V.M	2016	Acta Odontologica Latinoamericana	Periodontitis, peri-implantitis	Cross-sectional Study
9.	Prevalence of Epstein-Barr virus DNA and *Porphyromonas gingivalis* in Japanese periimplantitis patients	Kato.A, Imai.K, Sato.H, Ogata.Y	2017	BMC Oral Health	Healthy teeth, Healthy Implants, Peri-implantitis	Cross-sectional Study
II- Based on Hybridization Microbial Analysis Technique						
10.	Bacterial Analysis of Peri-implantitis and Chronic Periodontitis in Iranian Subjects	Ebadian.A.R, Kadkhodazadeh.M, Zarnegarni.P and dahle.G	2012	Acta Medica Iranica	Periodontal Health, Chronic Periodontitis, Healthy Implants,	Cross-sectional Study

					Peri-implantitis	
11.	Clinical and microbiological characteristics of peri-implantitis cases: a retrospective multicentre study	Charalampakis. G, Leonhardt.A, Rabe.P, Dahlen.G	2012	Clinical Oral Implants Research	Peri-implantitis	Cross-sectional Study
12.	Cluster of Bacteria Associated with Peri-Implantitis	Persson.G.R., renvert.S.	2014	Clinical Implant Dentistry and Related Research	Healthy Implants, Peri-implantitis	Cross-sectional Study
III- Based on Pyrosequencing Microbial Analysis Technique						
13.	Pyrosequencing reveals unique microbial signatures associated with healthy and failing dental implants	Kumar.P.S, Mason MR, Brooker.MR, O'Brien.K	2012	Journal of Clinical Periodontology	Periodontal Health, Periodontitis, Healthy Implants, Peri-implantitis	Cross-sectional Study
14.	Patient-specific Analysis of Periodontal and Peri-implant Microbiomes	Dabdoub.S.M., Tsigarida.A.A., Kumar.P.S,	2013	Journal of Dental Research	Healthy implants, Peri-implantitis	Cross-sectional Study
15.	Microbiological diversity of peri-implantitis biofilm by Sanger sequencing	Da Silva. E.S.C., Luciene. M.F, Jamil. C.F., Fernanda.A.S, Ramiro.S., Faveri.M.	2014	Clinical Oral Implants Research	Healthy Implants, Peri-implantitis	Cross-sectional Study
16.	Subgingival microbiome in Patients with Healthy and Ailing Implants	Zheng.H., Lixin. X., Wang.Z., Li.L, Zhang. J., Zhang.Q, Chen. T, Lin.J., Chen.F.	2015	Scientific Reports	Healthy Implants, Peri-implantitis, Periodontitis	Cross-sectional Study
IV- Based on Illumina Sequencing Microbial Analysis Technique						

17.	Exploring the microbiome of healthy and diseased peri-implant sites using Illumina Sequencing	Sanz-Martin.I, Doolittle-Hall.J, Teles.R.P, Pate.M, Belibasakis.G.N, Hammerle C.H et.al	2017	Journal of Clinical Periodontology	Healthy implants, peri-implantitis	Cross-sectional Study
18.	Microbiome of peri-implantitis affected and healthy dental sites in patients with a history of chronic periodontitis	Apatzidou.D, Lappin.d.F, Hamilton.G, Papadopoulo.C.A, Konstantinids.A, Riggio.M.P.	2017	Archives of Oral Biology	Healthy implant, Peri-implant disease	Cross-sectional Study
19.	Subgingival Microbiome Colonization and Cytokine Production during Early Dental Implant Healing	Payne.J.B, Johnson.P.G, Kok.C.R, Gomes-Neto. J.C, Ramer-Tait.A.E, Schmid.M.J, Hutkins.R.W.	2017	American Society for Microbiology	Healthy implant, Peri-implant disease	Longitudinal Study
V- Based Meta-transcriptomic Analysis Technique for Microbial Analysis						
20.	Distinct interacting core taxa in co-occurrence networks enable discrimination of polymicrobial oral diseases with similar symptoms	Shiba.T, Watanabe.T, Kachi.H, Koyanagi.T, Maruyama.N, Murase.K et.al	2016	Scientific Reports	Periodontitis, peri-implantitis	Cross-sectional Study

Data Synthesis and Normalization

The selected studies include metagenomes coming from different pipelines like pyrosequencing, Illumina, hybridization, etc., which generates a wide range of data as far as the total microbiome is concerned, which will affect the diversity and species richness. Therefore, to identify the microbiome consistency around healthy and diseased peri-implant sites, microbial species identified in all of the studies were compared and segregated based on the technique used for microbial evaluation.

RESULTS

A total of 580 studies were retrieved for possible inclusion after the removal of duplicate studies. Out of these 112 were included for full-text study and further 92 were excluded due to different reasons like not assessing the microbial species of healthy or failing implants, results were not quantified. Twenty full-text studies were included for the review (Fig 1) (Casado et al, 2011; Jankovic et al, 2011; Sato et al, 2011; Charalampakis et al, 2012; Ebadian et al, 2012; Kumar et al, 2012; Cortelli et al, 2013; Dabdoub

et al, 2013, Silva et al, 2014; Maruyama et al, 2014; Persson et al, 2014; Schwarz et al, 2015; Zheng et al, 2015; Bertone et al, 2016; Caccianiga et al, 2016, Shiba et al, 2016; Apatzidou et al, 2017; Kato et al, 2017; Martin et al, 2017; Payne et al, 2017) (Fig 2). Screening and quality assessment of the studies was based on study group selection, comparison of implant site, outcome measures and statistical analysis. Fourteen studies included in this review compared the microbiome around healthy implant sites and peri-implantitis sites while remaining six studies evaluated the microbiome around only failing implant sites. Quality assessment was done based on modified Newcastle–Ottawa Scale and it revealed 6 studies of high assessment, 8 studies of medium assessment and 6 studies of low assessment.

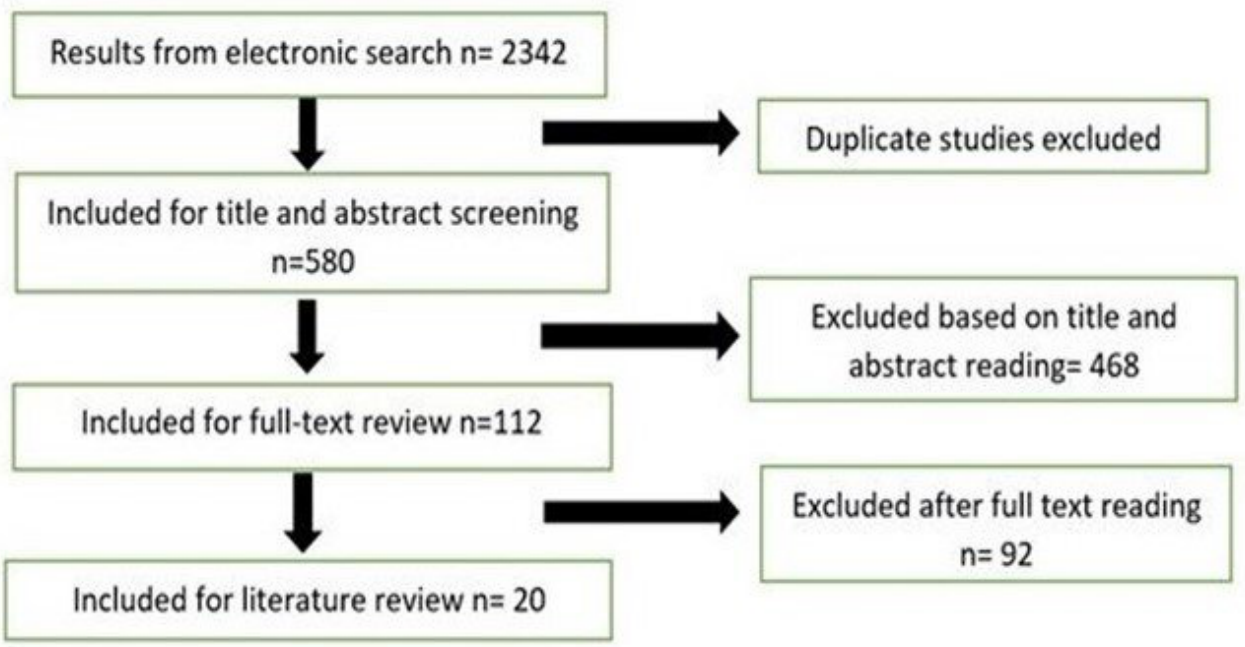

Figure 1. Flowchart of search strategy.

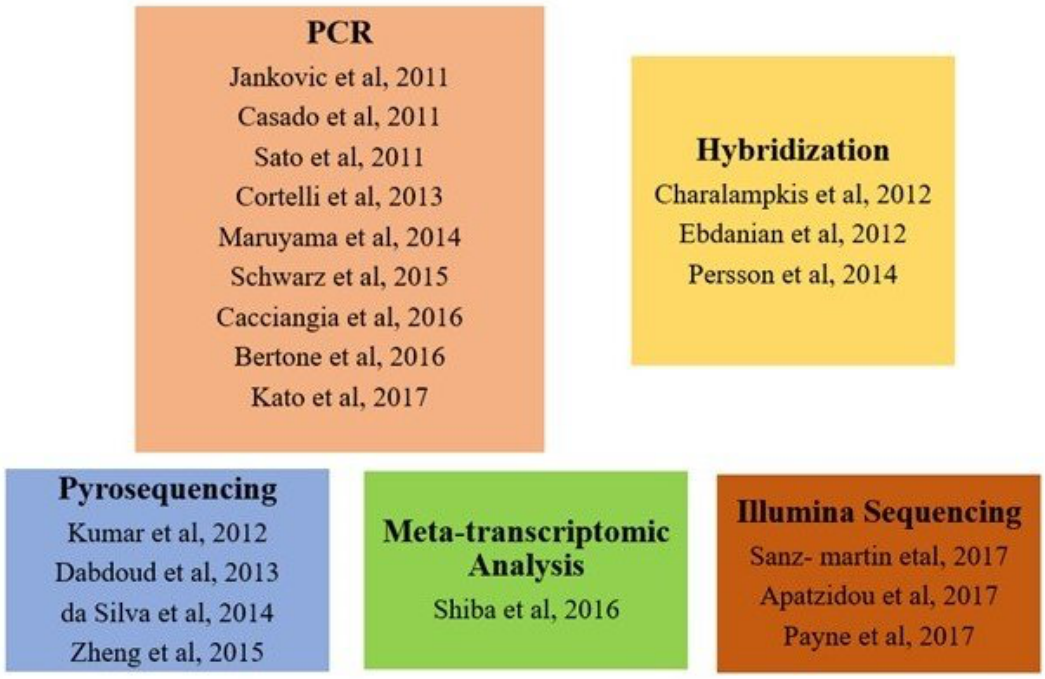

Figure 2. Studies based on the microbial analysis technique.

Details of the implant groups, study design, and microbial analysis technique for every study are mentioned (Table 1). These studies used one of the following methods for microbial assessment: Pyrotag sequencing (n= 4) (Kumar et al, 2012, Dabdoub et al, 2013, Silva et al, 2014, Zheng et al, 2015), hybridization method (n= 3) (Charalampakis et al, 2012; Ebadian et al, 2012; Persson et al, 2014), Polymerase Chain Reaction (PCR) based methods (n= 9) (Casado et al, 2011; Jankovic et al, 2011; Sato et al, 2011; Cortelli et al, 2013; Maruyama et al, 2014; Schwarz et al, 2015; Bertone et al, 2016; Caccianiga et al, 2016; Kato et al, 2017), Meta-transcriptomic analysis (n= 1) (Shiba et al, 2016) and Illumina based sequencing (n=3) (Apatzidou et al, 2017; Martin et al, 2017; Payne et al, 2017).

Studies assessing different species based on PCR technique

9 studies that used the PCR technique were included in this review for assessment. Total 7 studies compared the microbiome around healthy and failing peri-implant sites while 2 studies assessed the microbiome around peri-implantitis sites only. Data for *P.gingivalis, T.forsythia, T.denticola, Prevotella.intermedia, Parvimonas.micra, Prevotella.nigrescens, Fusobacterium. nucleatum, Campylobacter.rectus, A.actinomycetemcomitans, Eikenella. corrodens*, were assessed in five of the retrieved studies. These microorganisms were found around failing peri-implant as well as healthy peri-implant sites. *Canidida albicans* was also found at healthy and failing per-implant sites.

One study assessed the presence of viral microorganisms (HCMV 1 and 2 & EBV 1 and 2) at peri-implant sites. The only difference observed was that HCMV 2 was not found at healthy peri-implant sites while HCMV 1 and 2 & EBV 1 and 2 were present at peri-implantitis sites. Except for *P.nigrescens* and *Eubacterium nodatum*, which slightly prevailed in peri-implantitis samples, the prevalence of *P.gingivalis, T.forsythia, T.denticola, P.intermedia, P.micra, F.nucleatum, C.rectus, A.actinomycetemcomitans, E.corrodens, and*

C.albicans was similar in health and disease (Table 2 and Fig 3).

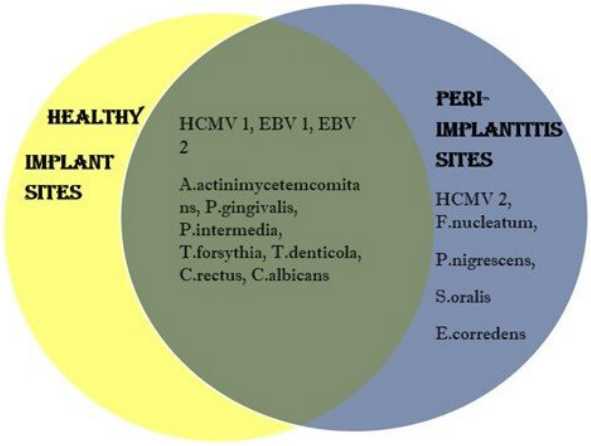

Figure 3. Venn diagram showing microbiome specific to Healthy and Diseased Implant Sites based on PCR Technique.

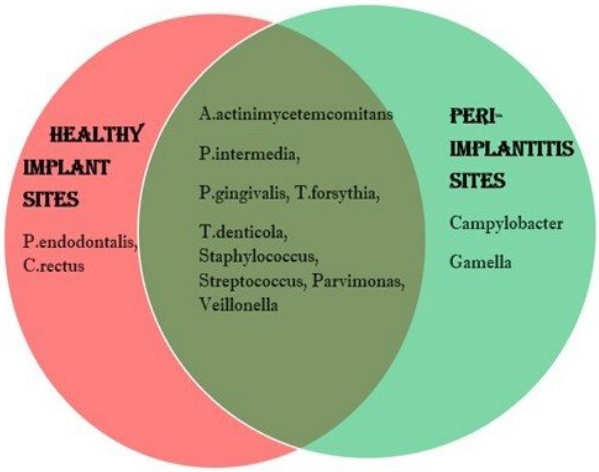

Figure 4. Venn diagram showing microbiome specific to Healthy and Diseased Implant Sites based on Hybridization Technique.

Table 2: Studies assessing microbiome based on PCR technique.

Around healthy peri-implant sites				
S. No	Study	Year	Author	Microbiome
1.	Correlation between different genotypes of human cytomegalovirus and Epstein-Barr virus and peri-implant tissue status.	2011	Jankovic et.al	HCMV 1, EBV 1, EBV 2
2.	Identification of Periodontal Pathogens in Healthy Peri-implant Sites	2011	Casado.et.al	*A.actinomycetemcomitans, P.gingivalis, P.intermedia, T.forsythia, Tdenticola*
3.	Frequency of Periodontal Pathogens in equivalent Peri-implant and Periodontal Clinical Statuses	2013	Cortelli.et.al	*A.actinomycetemcomitans, P.gingivalis, P.intermedia, T.forsythia, Tdenticola, C.rectus*

4.	Real Time PCR Analysis of Fungal Organisms and Bacterial Species at Peri-implantitis Sites	2015	Schwarz.et.al	*C.albicans*
5.	Genetic-Relatedness od Peri-implants and Buccal Candida Albicans Determined by RAPD- PCR	2016	Bertone.et.al	*Candida. albicans*
6.	Prevalence of Epstein-Barr virus DNA and *Porphyromonas gingivalis* in Japanese periimplantitis patients	2017	Kato.et.al	*EBV and P.gingivalis*
7.	The Evaluation of Bacterial flora in Progress of Peri-implant Disease	Sato.et.al	2011	*A.actinomycetemcomitans, P.gingivalis, T.forsythia, T.denticola*
	Around Failing Peri-implant Sites			
1.	Correlation between different genotypes of human cytomegalovirus and Epstein-Barr virus and peri-implant tissue status.	Jankovic et.al	2011	*HCMV1, HCMV 2, EBV 1 and EBV 2*
2.	Identification of Periodontal Pathogens in Healthy Peri-implant Sites	Casado. et.al	2011	*A.actinomycetemcomitans, P.gingivalis, P.intermedia, T.forsythia, T.denticola*
3.	The Evaluation of Bacterial flora in Progress of Peri-implant Disease	Sato.et.al	2011	*A.actinomycetemcomitans, P.gingivalis, T.forsythia, T.denticola*
4.	Frequency of Periodontal Pathogens in Equivalent Peri-implant and Periodontal Clinical Statuses	Cortelli.et.al	2013	*A.actinomycetemcomitans, C.rectus, P.gingivalis, P.intermedia, T.denticola,T.forsythia*
5.	Intra Individual Variation in Core Microbiota in Peri-implantitis and Periodontitis	Maruyama.et.al	2014	*Actinomyces.species, F.nucleatum, P.gingivalis, P.nigrescens, S.oralis, T.forsythia, T.denticola*
6.	Real Time PCR Analysis of Fungal Organisms and Bacterial Species at Peri-implantitis Sites	Schwarz.et.al	2015	*Candida.albicans*

7.	Clinical, Radiographic and Microbiological Evaluation of High-Level Laser Therapy, a New Photodynamic Therapy Protocol in Peri-implantitis Treatment; a Pilot experiance	Caccianiga.et.al	2016	*A.actinomycetemcomitans, C.rectus, Eikenell.corredens, F.nucleatum P.gingivalis, T.forsythia, T.denticola*
8.	Genetic Relatedness of Peri-implant and Buccal *Candida.albicans* Determined by RAPD-PCR	Bertone.et.al	2016	Candida. albicans
9.	Prevalence of Epstein Barr Virus DNA and Porphyromonas. gingivalis in Japanese Peri-implantitis Patients	Kato.et.al	2017	EBV and *P.gingivalis*

Studies assessing different species based on Hybridization technique

Three studies using the hybridization technique to assess different genera were included. In all studies *Actinomyces* spp, Campylobacter spp., Fusobacterium spp., *Porphyromonas* spp., Treponema spp., and *Tannerella* spp. could be identified in healthy and diseased peri-implant sites. While *Parvimonas* spp., *Staphylococcus* spp., *Veillonella* spp, and *Streptococcus* spp. were detected in one study at healthy and diseased peri-implant sites. No conclusive differences between samples from healthy implants or peri-implantitis could be found (Table 3 and Fig 4).

Table 3: Studies assessing microbiome based on Hybridization technique.

Around healthy peri-implant sites				
S. No	Study	Year	Author	Microbiome
1.	Bacterial Analysis of Peri-implantitis and Chronic Periodontitis in Iranian subjects	2012	Ebadian et.al	*A.actinomycetemcomitans, F.nucleatum, P.endodontalis, P.intermedia, P.gingivalis, P.nigrescens, T.forsythia, T.denticola*
2.	Cluster of Bacteria Associated with Peri-implantitis	2013	Persson et.al	*A.actinomycetemcomitans, C.rectus, Staphylococcus spp, Streptococcus spp, Fusobacterium spp. Prevotella spp, Porphyromonas spp, Parvimonas spp, Tannerella*

				spp, Treponema spp, Veillonella spp.
Around failing peri-implant sites				
1.	Clinical and Microbiological Characteristics of peri-implantitis Cases: a retrospective Multicentric Study	Charalampakis.et.al	2011	*Actinomyces spp, Porphyromonas spp, Prevotella spp, Tannerella spp, Treponema spp Campylobacter spp, Fusobacterium spp, Gamella spp, Streptococcus spp, Parvimonas spp,*
2.	Bacterial Analysis of peri-implantitis and Chronic Periodontitis in Iranian Subjects	Ebadian.et.al	2012	*Actinomyces spp, Porphyromonas spp, Prevotella spp, Tannerella spp, Campylobacter spp, Fusobacterium spp*
3.	Cluster of Bacteria Associated with Peri-implantitis	Persson.et.al	2014	*Actinomyces spp, Porphyromonas spp, Tannerella spp, Treponema spp, Prevotella spp, Campylobacter spp, Fusobacterium spp, Streptococcus spp, Parvimonas spp, Veillonella spp, Staphylococcus spp.*

Studies assessing different species based on Pyrosequencing technique

Four studies used this technique for microbial assessment. Following genera were positively detected in healthy and diseased peri-implant sites *Actinomyces* spp., *Campylobacter* spp., *Fusobacterium* spp., *Gemella* spp., *Parvimonas* spp., *Porphyromonas* spp., *Prevotella* spp., *Rothia* spp., *Staphylococcus* spp., *Streptococcus* spp., *Treponema* spp., *Veillonella* spp., and *Tannerella* spp. None of the positively detected Genera showed any specificity, i.e., complete absence or presence in either peri-implantitis or healthy implant samples (Table 4 and Fig 5).

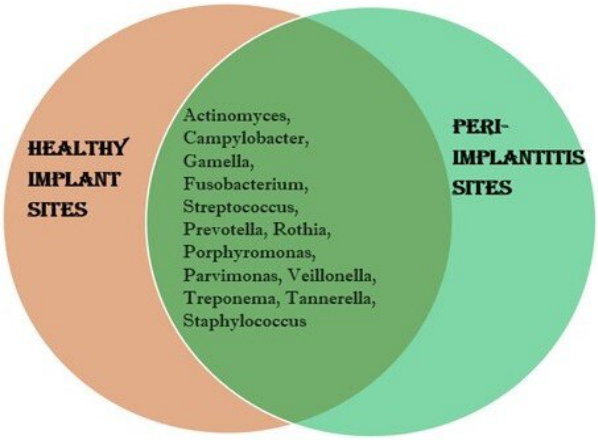

Figure 5. Venn diagram showing microbiome specific to Healthy and Diseased Implant Sites based on Pyrosequencing Technique.

Studies assessing different species based on Illumina-sequencing technique

Three such studies were included for review, of which 2 studies assessed both healthy and diseased peri-implant sites while one study only assessed peri-implantitis sites. Genus *Porphyromonas* spp., *Prevotella* spp., *Tannerella* spp., *Treponema* spp., *Streptococcus* spp., *Fusobacterium* spp., were found in two studies at peri-implantitis sites while *Rothia* spp. was found in only one study at peri-implantitis sites. One study found Streptococcus spp., *Veillonella* spp., and *Rothia* spp. at healthy peri-implant sites. Species *A.actinomycetemcomitans, P.gingivalis, T.forsythia, T.denticola, P.intermedia* were found in one study at healthy peri-implant sites (Table 5 and Fig 6). *Fusobacterium* spp. was completely absent at healthy peri-implant sites while *Veillonella* spp. was completely absent at peri-implantitis sites.

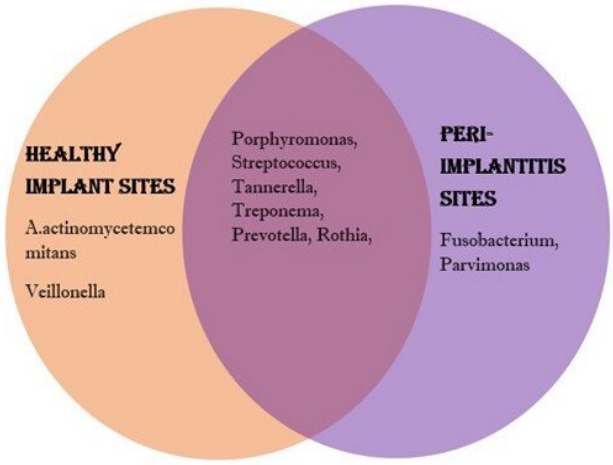

Figure 6. Venn diagram showing microbiome specific to Healthy and Diseased Implant Sites based on Illumina-sequencing Technique.

Table 4: Studies assessing microbiome based on Pyrosequencing technique.

Around healthy peri-implant sites				
S. No	Study	Year	Author	Microbiome
1.	Pyrosequencing reveals unique microbial signatures associated with healthy and failing dental implants	2012	Kumar et.al	*Actinomyces spp, Porphyromonas spp, Prevotella spp, Treponema spp, Campylobacter spp, Gamella spp, Streptococcus spp, Rothia spp Parvimonas spp, Veillonella spp,*

2.	Patient Specific Analysis of Periodontal and Peri-implant Microbiome	2013	Dabdoud et.al	*Actinomyces spp, Porphyromonas spp, Prevotella spp, Treponema spp, Tannerella spp, Campylobacter spp, Fusobacterium spp, Gamella spp, Streptococcus spp, Rothia spp, Staphylococcus, Veillonella spp.*
3	Microbiological Diversity of Peri-implantitis Biofilm by Sanger Sequencing	2014	da Silva et.al	*Actinomyces spp, Porphyromonas spp, Campylobacter spp, Fusobacterium spp, Gamella spp, Parvimonas spp, Rothia spp, Veillonella spp.*
4.	Subgingival Microbiome in Patients with Healthy and Failing Implants	2015	Zheng et.al	*Actinomyces spp, Gamella spp, Veillonella spp*
Around failing peri-implant sites				
1.	Pyrosequencing reveals unique microbial signatures associated with healthy and failing dental implants	2012	Kumar et.al	*Actinomyces spp, Porphyromonas spp, Prevotella spp, Treponema spp, Campylobacter spp, Fusobacterium spp, Gamella spp, Streptococcus spp, Rothia spp Parvimonas spp, Veillonella spp, Staphylococcus spp.*
2.	Patient Specific Analysis of Periodontal and Peri-implant Microbiome	2013	Dabdoud et.al	*Actinomyces spp, Porphyromonas spp, Prevotella spp, Tannerella spp, Treponema spp, Campylobacter spp, Fusobacterium spp, Gamella spp, Rothia spp, Staphylococcus spp, Streptococcus spp, Veillonella spp.*
3	Microbiological Diversity of Peri-implantitis Biofilm by Sanger Sequencing	2014	da Silva et.al	*Actinomyces spp, Porphyromonas spp, Campylobacter spp, Fusobacterium spp, Gamella spp, Parvimonas spp, Rothia spp, Veillonella spp.*

4.	Subgingival Microbiome in Patients with Healthy and Failing Implants	2015	Zheng et.al	*Actinomyces spp, Treponema spp, Fusobacterium spp, Gamella spp, Veillonella spp*

Table 5: Studies assessing microbiome based on Illumina-sequencing technique.

I- Around healthy peri-implant sites				
S. No	Study	Year	Author	Microbiome
1.	Exploring the Microbiome of Healthy and Diseased Per-implant sites Using Illumina Sequencing	2017	Martin et.al	*Streptococcus spp, Rothia spp and Veillonella spp*
2.	Subgingival microbiome Colonization and Cytokine Production During Early Dental Implant Healing	2017	Payne et.al	*A.actinomycetemcomitans, P.gingivalis, T.forsythia, T.denticola, P.intermedia*
II. Around failing peri-implant sites				
1.	Exploring the Microbiome of Healthy and Diseased Peri-implant Sites using Illumina Sequencing	Martin.et.al	2017	*Porphyromonas spp, Tannerella spp, Treponema spp, Fusobacterium spp, Streptococcus spp,*
2.	Microbiome of Peri-implantitis affected and Healthy Dental Sites in Patients with a History of Chronic Periodontitis	Apatzidou.et.al	2017	*Porphyromonas spp, Prevotella spp, Tannerella spp, Treponema spp.*
3.	Subgingival Microbiome Colonization and Cytokine Production During Early Dental Implant Healing	Payne.et.al	2017	*Prevotella spp, Fusobacterium spp, Rothia spp, Streptococcus spp. Parvimonas spp.*

Studies assessing different species based on Meta-transcriptomic technique

Only one such study was included in this review which analyzed the microbiome at peri-implantitis sites. The genera identified from peri-implantitis affected implant sites were *Actinomyces* spp., *Campylobacter* spp., *Parvimonas* spp., *Porphyromonas* spp., *Prevotella* spp., *Rothia* spp., *Streptococcus* spp., *Tannerella* spp., *Treponema* spp., (Table 6). The genera *Gemella* spp. and *Veillonella* spp. was not found in peri-implantitis sites.

Table 6: Studies assessing microbiome based on Meta-transcriptomic technique.

I- Around failing peri-implant sites				
S. No	Study	Year	Author	Microbiome
1.	Distinct interacting Core Taxa in co-occurance networks enable Discrimination of Polymicrobial Oral Diseases with Similar Symptoms	Shiba.et.al	2016	*Actinomyces spp, Campylobacter spp, Porphyromonas spp, Parvimonas spp, Prevotella spp, Rothia spp, Streptococcus spp, Tannerella spp, Treponema spp.*

Comparison of Microbiome around Healthy and Failing Implants

Fourteen such studies were included in this review (Casado et al, 2011, Jankovic et al, 2011, Ebadian et al, 2012, Kumar et al, 2012, Cortelli et al, 2013, Dabdoub et al, 2013, Silva et al, 2014, Persson et al, 2014, Schwarz et al, 2015, Zheng et al, 2015, Bertone et al, 2016, Kato et al, 2017, Martin et al, 2017, Payne et al, 2017) (Table 7).

Based on PCR and Hybridization technique *A.actinomycetemcomitans, P.gingivalis, P.intermedia, T.forsythia, T.denticola and C.rectus*, were present at both healthy and diseased peri-implant sites.

However, PCR detection frequency percentage varied in healthy and peri- implantitis sites for *A. actinomycetemcomitans* (3-8 for healthy; 4-42 for peri-implantitis), *P.gingivalis* (9-2 for healthy; 8 to 93 for per-implantitis), *P.intermedia* (4-22 for healthy; 7-32 for peri-implantitis), T.forsythia (8 for healthy; 9-40 for peri-implantitis), *T.denticola* (7-10 for healthy; 7 to 54 for peri-implantitis) and *C.rectus* (0-44 for healthy; 0 to 70 for peri-implantitis) . *C.albicans*, EBV2 and HCMV1 were found at both healthy and diseased peri-implant sites while HCMV 2 and EBV 1 were present at peri-implantitis sites only with the PCR technique.

Prevalence percentage at peri-implant sites with Hybridization technique varied in healthy and peri-implantitis sites for *A.actinomycetemcomitans* (17%-23.1% for healthy; 23.1%-38% for peri-implantitis), *P.gingivalis* (27.7%-30.8% for healthy; 53.8%-56% for peri-implantitis), *P.intermedia* (21.3%-30.8% for healthy; 30.8%-45.8% for peri-implantitis), *T.denticola* (7.7%-14.9% for healthy; 8.3%-45.2% for peri-implantitis), *T.forsythia* (5.5%-46.1% for healthy; 61.5% for peri-implantitis), *P.endodontalis* (7.7% for healthy; 15.4% for peri-implantitis), *P.nigrescorens* (15.4% for healthy; 23.1% for peri-implantitis), *C.rectus* (00-27.7% for healthy; 15.4%-61.4% for peri-implantitis), and *F.nucleatum* (40.4%-61.5% for healthy; 38.5%-58.4% for peri-implantitis). *A.actinomycetemcomitans, P.gingivalis, C.rectus* were seen at both healthy and diseased peri-implant sites with pyrosequencing method. (Table 7).

Table 7: Studies comparing similarity and difference of microbiome around healthy and failing Implants.

S No	Study	Author	Year	Similarity	Differences	
I. Based on PCR						
1.	Correlation between	Jankovic .et.al	2011	HCMV and EBV were	Healthy Site	Peri-implantitis Sites

	different genotypes of human cytomegalovirus and Epstein-Barr virus and peri-implant tissue status.			found at both healthy implant sites and peri-implantitis site. No significant difference in distribution of HCMV-1 and EBV-2 at healthy and diseased peri-implant sites.	HCMV-2 and EBV-1 were not detected. HCMV-1 and EBV- 2 were recorded in 8% healthy peri-implant sites.	HCMV-2 was detected in 53.3%, HCMV-1 in 13.33%, EBV-1 in 36.66% and EBV-2 in 10% sites.
2.	Identification of Periodontal Pathogens in Healthy Peri-implant Sites	Casado. et.al	2011	Detection frequency of *A.actinomycetemcomitans, P.gingivalis, P.intermedia, T.forsythia, T.denticola* was same around healthy and diseased peri-implant sites.	<u>Detection Frequency at Healthy Site</u> *A.actinomycetemcomitans- 3, P.gingivalis-9, P.intermedia-4, T.forsythia-8, T.denticola-7*	<u>Detection Frequency at Peri-implantitis Sites</u> *A.actinomycetemcomitans- 4, P.gingivalis-8, P.intermedia-7, T.forsythia-9, T.denticola-7*
3.	Frequency of Periodontal Pathogens in equivalent Peri-implant and Periodontal Clinical Statuses	Cortelli. et.al	2013	Same species were found but in different frequency.	<u>Bacterial Frequency % at Healthy Implant Sites-</u> *A.actinomycetemcomitans-* 8 *P. gingivalis- 12* *P.intermedia-* 22 *T. forsythia- 8* *T. denticola-*10 *C. rectus-* 44	<u>Bacterial Frequency % at Peri-implantitis Sites-</u> *A.actinomycetemcomitans-* 42 *P. gingivalis-* 54 *P.intermedia-* 32 *T. forsythia- 40* *T. denticola-* 54 *C. rectus-* 70
4.	Real Time PCR Analysis of Fungal Organisms and Bacterial	Schwaz. et.al	2015	Fungal organisms were found at both healthy and peri-implantitis sites	<u>Healthy Implant Sites-</u> Fungal organisms were present at 40.0% of subjects. Candida dubliniensis	<u>Peri-implantitis Sites-</u> Fungal organisms were present at

	Species at Peri-implantitis Sites			and these were co-colonized with *P.micra* and *T.forsythia*.	Cladosporium. cladosporioides	31.6% of subjects. C. albicans, Candida.boidinii Cladosporium. cladosporioides
5.	Genetic-Relatedness od Peri-implants and Buccal Candida Albicans Determined by RAPD- PCR	Bertone. et.al	2016	Different candida species colonized the peri-implant sulcus irrespective of the implant health.	Healthy Implant sites- Candida species was present at 50% healthy implant sites. *C.albicans* *C.dubliniensis*	Peri-implantitis Sites- Candida species was present at 53% peri-implantitis sites. *C.albicans* *C.dubliniensis*
6.	Prevalence of Epstein-Barr virus DNA and Porphyromonas gingivalis in Japanese periimplantitis patients	Kato.et. al	2017	Both healthy (9 of 15) and diseased (13 of 15) implant sites showed presence of EBV and *P.gingivalis*	Frequency at Healthy-implant sites- EBV- 60% *P.gingivalis-26.7%*	Frequency at Peri-implantitis Sites- EBV- 86.7% *P.gingivalis-93.3%*
II. Based on Pyrosequencing						
1.	Pyrosequencing reveals Unique Microbial signatures Associated with Healthy and Failing Dental Implants	Kumar.et.al	2012		Healthy Implant Sites- *Streptococcus mutans* *Treponema* *Butyrivibrio, Catonella*, Lactococcus, *Leptotrichia, Prevotella, Propionibacter.*	Peri-implantitis sites-Higher levels of *Actinomyces, Butyrivibrio* *Campylobacter, Peptococcus, non-mutans* *Streptococcus* and *S.mutans.*
2.	Patient Specific Analysis of Periodontal and Peri-implant Microbiome	Dabdoub.et.al	2013		Healthy Implant Sites - *Actinomyces.bovis, Actinomyces.gerencseriae, Actinomyces.meyeri,*	Peri-implantitis Sites - *Staph.pettenkoferi, Hylemonella species,*

					V.dispar, Calubacter species, F.nucleatum, H.influenza, Mycoplasma.faucium, Peptostreptococcus.anaerobius, Streptococcus species Veillonella species	*Staph.homonis, Prevotella.baroniae, Streptococcus.agalactiae, Atopobium.rimae, Prevotella.oralis, Megasphaera.elsdenii, Prevotella.loescheii, Aggregatibacter.aphrophilus, Arthrobacter species, Campylobacter.sputorum, Streptococcus.parasanguinis, Clostridium.botulinium, Neisseria.elongata, Veillonella parvula, Actinomyces.meyeri*
3.	Microbiological diversity of peri-implantitis biofilm by Sanger sequencing	da Silva.et. al	2014		Mean Proportion ± Standard deviation at Healthy Implant Sites- *Actinomyces* 18.28 ± 10.78 *Porphyromonas* 0.41 ± 0.87 *Fusobacterium* 3.73 ± 4.88 *Atopobium* 1.90 ± 2.09 *Campylobacter* 3.61 ± 5.92 *Catonella*0.00±0.00 *Desulfobulbus* 0.00 ± 0.00 *Dialister* 0.44 ± 0.93	Mean Proportion ± Standard deviation at Peri-implantitis Sites- *Actinomyces* 3.93 ± 3.78 *Porphyromonas* 4.70± 4.65 *Fusobacterium* 8.53± 5.31 *Atopobium* 0.63± 1.43 *Campylobacter* 4.82 ± 5.88

					Eubacterium 2.96 ± 3.65 *Filifactor* 0.62 ± 1.00 *Gemella* 5.08 ± 3.85 *Mitsuokella* 0.21 ± 0.67 *Parvimonas* 1.04 ± 1.46 *Pseudoramibacter* 0.00 ± 0.00 *Rothia* 0.00 ± 0.00 *Veillonella* 10.07 ± 8.43	*Catonella* 0.61± 1.38 *Desulfobulbus* 1.71 ± 1.32 *Dialister* 6.58±4.18 *Eubacterium* 4.39±5.07 *Filifactor* 2.93± 3.05 *Gemella* 0.44± 0.92 *Mitsuokella* 3.77± 3.98 *Parvimonas* 4.12± 4.68 *Pseudoramibacter* 2.27±3.90 *Rothia* 0.62± 1.39 *Veillonella* 3.85± 4.03
4.	Subgingival microbiome in Patients with Healthy and Ailing Implants	Zheng.et.al	2015	*P.gingivalis and T.forsythia* showed similar relative abundance	Healthy Implant Sites- *Leptotrichia.goodfellowii, Selenomonas, Brevundimonas.nasadae, Ochrobactrum, Delftia.acidovorans, Abiotropia.defective, Actinomyces.gerencseriae, Nisseria.flavescensActinomyces.dentalis, Streptococcs.parasanguinis*	Peri-implantitis Sites- *Leptotrichia hofstadii, Eubacterium infirmum, Kingella denitrificans, Actinomyces cardiffensis, Eubacterium minutum, Treponema lecithinolyticum, and Gemella sanguinis* These showed higher relative abundance
III. Based on Illumina Sequencing						
1.	Exploring the microbiome of	Martin.et.al	2017	*Rothia.dentocoriosa,*	Healthy Implant Sites- *Streptococcus,*	Peri-implantitis Sites-

	healthy and diseased peri-implant sites using Illumina Sequencing			*Streptococcus, C.gracilis, Fusobacterium , V.dispar, H.parainfluenzae*	*Veillonella, Rothia species and Haemophilus* were present at higher relative abundance	*P.gingivalis, T.forsythia, T.denticola Filifactor.alocis, Fretibacterium. fastidiosum, P.micra, and P.endodontalis*
2.	Subgingival Microbiome Colonization and Cytokine Production during Early Dental Implant Healing	Payne.et.al	2017	No significant difference between genus present at healthy and diseased implant sites.	Peri-implantitis Sites- After 4 weeks elevated level of *Oribacterium* and at 12 weeks elevated *Parvimonas* level.	
IV. Based on Hybridization						
1.	Bacterial Analysis of Peri-implantitis and Chronic Periodontitis in Iranian Subjects	Ebadian .et.al	2012	Insignificant Difference between healthy implant sites and peri-implantitis sites for all species.	Prevalence percentage at Healthy Implant Sites- *A.actinomycetemcomitans*- 23.1%, *P.gingivalis*-30.8%, *P.intermedia*- 30.8%, *T.forsythia*- 46.1%, *T.denticola*- 7.7% *C.rectus*- 00 *F.nucleatum*-61.5%, *P.endodontalis*- 7.7% *P.nigrescens*- 15.4%	Prevalence percentage at Peri-implantitis Sites- *A.actinomycetemcomitans*- 23.1%, *P.gingivalis*- 53.8%, *P.intermedia*- 30.8%, *T.forsythia*- 61.5% *T.denticola*- 8.3% *C.rectus*- 15.4% *F.nucleatum*- 38.5%, P.*endodontalis*- 15.4% *P.nigrescens*- 23.1%
2.	Cluster of Bacteria	Persson et.al	2014	*P. gingivalis, T. forsythia, Tdsocranskii, S.aureus,*	Prevalence percentage at Healthy Implant Sites-	Prevalence percentage at Peri-implantitis Sites-

	Associated with Peri-Implantitis			*S.intermedius, S. mitis, and H. influenzae* were present at 14.1% of healthy and 30.2% diseased implant sites.	*A.actinomycetemcomitans*-17 *P.gingivalis*-27.7%, *P.intermedia*-21.3%, *T.forsythia*- 25.5%, *T.denticola*- 14.9% *C.rectus*- 27.7% *F.nucleatum*-40.4%,	*A.actinomycetemcomitans*-38%, *P.gingivalis*-56%, *P.intermedia*-45.8%, *T.forsythia*-61.4%, *T.denticola*-45.2% *C.rectus*- 61.4 *F.nucleatum*-58.4%

DISCUSSION

This systematic review focused on the microbiome of healthy and diseased peri-implant sites and compared the microbiome of healthy implant sites with that of peri-implantitis sites. Considering the findings from all the studies included in this review, specific or unique microbiome could not be identified around healthy or diseased peri-implant sites. Few studies detected the presence of single microbial species but still no consistent difference was noted around these sites. Therefore, the hypothesis that distinct microbial flora is found around peri-implantitis sites and healthy implant sites could not be confirmed.

A direct comparison of the outcomes of all the studies was not possible as different microbial analysis techniques were used in different studies, having their advantages and disadvantages.

The selected studies (n=20) in this review used different microbial analysis techniques. PCR was used in 45% of the studies (n=9); 20% of the studies used pyrotag sequencing; 15% used illumina sequencing (n=3) and hybridization technique (n=3) and only 5% used meta-transcriptomic analysis technique (n=1). Study quality was evaluated based on: study group selection, methods for assessing microbial species, peri-implant surroundings, standardization of outcome assessors, implant site comparison, microbiologic outcome assessment.

Rapid and proper identification of microbes is an important factor and since conventional methods of bacterial identification are based on the microbial culture which is labor-intensive, time-consuming, and often inadequate to differentiate phenotypically similar and anaerobic species (Tong et al, 2010, Szeliga et al, 2011, Duskov et al, 2012, Salplachta et al, 2013). Now, molecular biology methods—such as 16S ribosomal RNA (rRNA) gene sequencing, DNA-DNA Hybridization, polymerase chain reaction (PCR), Pyrosequencing, Illumina sequencing, Meta-transcriptomic analysis, and other related PCR-based methods are very popular. These techniques allow the assessment of a much broader range of microbiota in both health and disease. The microbial culture was used earlier for the identification of different species but in the last two decades these more advanced techniques have been used for bacterial identification.

DNA-DNA Hybridization is one of the first used methods for bacterial identification (Socransky et al, 1994). This technique uses a single membrane for the hybridization of a multitude of species, it was the first developed molecular technique. This review included three studies using this technique (Table 1 and Fig 2). PCR enables enzymatic replication of DNA without using living organisms (Mullis et al,

1986). This was the most common method used in included studies. A total of nine such studies were included (Table 1 and Fig 2). The pyrosequencing method allows rapid and accurate sequencing of the microbial genome (Cummings et al, 2013). This technique has been used for the identification of microbial species, differentiation of bacterial strains, and detecting genetic mutations (Cummings et al, 2013). Four studies using this technique were included (Table 1 and Fig 2).

Illumina sequencing allows rapid profiling of relevant microbial communities. It offers a greater depth of sequencing, reduced costs, and a smaller number of errors. However, it does not make the taxonomic assignment simple (Shiba et al, 2016). Three studies using this method were included (Table 1 and Fig 2). A meta-transcriptomic analysis is a more advanced method for microbial identification. This method not only identifies the microbial sample but also tells the gene expression. Only one such study was included (Table 1 and Fig 2).

Studies assessing microbiome using PCR technique found *A. actinomycetemcomitans, P. gingivalis, T. forsythia, T.denticola, P. intermedia, P. micra, Prevotella nigrescens, F. nucleatum, C. rectus,* and *E. corrodens* around healthy and diseased peri-implant sites (Table 2). In respect to viruses, HCMV 2 was not found at healthy peri-implant sites (Table 2). No major difference was found between the healthy and diseased peri-implant sites (Table 2 and Fig 3)

Considering the findings of studies comparing microbiome of healthy implant sites and peri-implantitis sites, *Actinomyces, P.gingivalis, T.forsythia, Treponema, Fusobacterium, Gemella* were detected at both healthy sites and peri-implantitis sites in many studies (Casado et al, 2011, Ebadian et al, 2012, Cortelli et al, 2013, da Silva et al, 2014, Persson et al, 2014, Zheng et al, 2015). However, in the case of viruses consistently higher levels of EBV and HCMV were found at peri-implantitis sites (Jankovic et al, 2011, Kato et al, 2017). In the case of fungal organisms, no major difference was noted between these sites (Kumar et al, 2012, Caccianiga et al, 2016). Jankovic et al, in their study using PCR assay, found a higher prevalence of HCMV 2 and EBV 1 from peri-implantitis sites. 53.3% and 46.6% of peri-implantitis sites harbored HCMV 2 and EBV, respectively (Jankovic et al, 2011). 76% of the healthy implant sites showed an absence of viral DNA while this percentage was only 26.6% in the case of peri-implantitis sites (Jankovic et al, 2011). Also, Kato. et.al in their study using the PCR method found a higher association of EBV at peri-implantitis sites of Japanese patients. They found coexistence of EBV and *P.gingivalis* in 80% of peri-implantitis sites and only 13.3% of healthy implant sites (Kato et al, 2017). It can be due to inflammatory cytokine release caused by EBV, which results in increased osteoclastic activity leading to more colonization of periodontal pathogens (Kato et al, 2017). Hence, it can be concluded that the presence of viral DNA is related to peri-implantitis. This is in relation to the study by Verdugo et al, that the co-existence of periodontal pathogens and EBV may severe the level of peri-implant disease (Verdugo et al, 2015).

In the case of fungal organisms, two studies assessing candida species and their relation with peri-implant tissue were included in this review (Bertone et al, 2016, Cacciangia et al, 2016). A study by Schwarz et al. found a higher association of candida species at peri-implant sites as compared to periodontal sites (Schwarz et al, 2015). In their study Candida species were frequently associated with both healthy (40%) and peri-implantitis sites (31.6%). Also, Bertone et al. in their study found an association of Candida species irrespective of the implant health (Bertone et al, 2016). 50% of the healthy implant sites and 53% of peri-implantitis sites were colonized by Candida species. 43% of the implants colonized by Candida species had bone resorption while 43% of the implants did not have resorption. Hence, presence of only fungal species is not indicative of peri-implant health or disease (Schwarz et al, 2015, Bertone et al, 2016).

A study by Casado et al, assessed the presence of periodontal pathogens in healthy peri-implant sites. They found that *A.actinomycetemcomitans,*

P.gingivalis, P.intermedia, T.forsythia, T.denticola were present at both healthy and peri-implantitis sites (Casado et al, 2011). They concluded that the presence of only these pathogens at peri-implant sites will not lead to the destruction of peri-implant tissues, rather a combination of the genetic, inflammatory response, and occlusal overload will lead (Casado et al, 2011). Similarly, the study by Cortelli et al also found all the species at both healthy implant sites and peri-implantitis sites. However, the bacterial frequency was higher at peri-implantitis sites when compared to healthy sites except for *P.intermedia* ($p>0.05$) all the species showed a significant difference between the sites ($p<0.05$) (Cortelli et al, 2013).

Genera *Actinomyces* spp, *Campylobacter* spp., *Fusobacterium* spp., *Porphyromonas* spp., *Treponema* spp. and *Tannerella* spp. could be identified in healthy and diseased peri-implant sites. No consistent difference was noted between healthy and failing implant sites (Table 3 and Fig 4). According to Dabdoub et al. all periodontal pathogens are not capable of surviving in the peri-implant sulcus and hence they solely are not responsible for a peri-implant disease. They also said that *Staphylococcus* and *Treponema* are significantly associated with diseased implants but it is not true in every case and most of the genera, which were present at diseased sites, were also present at healthy implant sites (Dabdoub et al, 2013). Similar findings were obtained in a study conducted by Ebadian et al, who found a non-significant difference between bacterial species at peri-implantitis and healthy implant sites (Ebadian et al, 2012). In their study, only 37.5% of the species were higher at peri-implantitis sites.

A significant difference between microbiome at peri-implantitis and healthy implant sites was obtained in a study by Persson et al. (Persson et al, 2014). 19 bacterial species were found in higher count at peri-implantitis sites, of which seven species showed significant differences. Seven species *(T. forsythia, P.gingivalis, T.socranskii, S.aureus, S.anaerobius, S. intermedius, and S.mitis)* comprised 30.2% of peri-implantitis sites while only 14.1% of healthy implant sites. The total bacterial load of these species at peri-implantitis sites was four times than at healthy implant sites, thus bacterial burden as such may play an important role in peri-implantitis (Ebadian et al, 2012).

According to the findings based on pyrosequencing methods, it was noted that both healthy and diseased peri-implant sites harbored genera *Actinomyces* spp., *Porphyromonas* spp., *Prevotella* spp., *Treponema* spp., *Tannerella* spp, *Campylobacter* spp., *Fusobacterium* spp., *Gemella* spp., *Parvimonas* spp., *Rothia* spp., *Staphylococcus* spp., *Streptococcus* spp., *Veillonella* spp., and. None of the genera was completely absent or present in either peri-implantitis or healthy implant or periodontitis samples (Table 4 and Fig 5).

However, a significant difference between the two sites was noted by Kumar et al, 2012. Gram-negative bacteria were found to be significantly associated with peri-implantitis (Kumar et al, 2012). Marked bacterial difference is noted in the biofilm around healthy and failing implants, marked reduction in the beneficial bacteria and increase in putative pathogens is found around failing implants (Silva et al, 2014). Pathogens from the orange complex (*Fusobacterium nucleatum, Prevotella intermedia, Prevotella nigrescens, Parviomonas micra, Eubacterium nodatum,* and various *Campylobacter* species) were predominantly associated with peri-implantitis and *Actinomyces* was found associated with healthy implant sites (Silva et al, 2014). Shibli et al. also found the association of *Actinomyces* species with healthy implant sites (Shilbi et al, 2008).

Illumina-based studies found that genus *Fusobacterium* was completely absent at healthy peri-implant sites while *Veillonella* was completely absent at peri-implantitis sites (Table 5 and Fig 6). However, a higher diversity was noted in diseased peri-implant sites than healthy implant sites. A longitudinal study assessing the microbiome colonization and cytokine production during early healing of dental implants found that the only

difference between healthy and failing implants was after 4 weeks *Oribacterium* was only the elevated species around failing implants and after 12 weeks *Parvimonas* was elevated around failing implants (Payne et al, 2017). *Streptococcus, Prevotella, Neisseria*, and *Fusobacterium* were present at both healthy and diseased implant sites (Payne et al, 2017). However, the study duration was only 12 weeks and the oral environment can change thereafter, therefore the findings are still not very reliable.

Martin et.al in their study using the Illumina sequencing method, studied the core microbiome of healthy and diseased implant sites (Martin et al, 2017). Higher diversity was noted at diseased sites. Higher levels of classic pathogens such as *T.forsythia*, *T.denticola*, and *P.gingivalis* were present at peri-implantitis sites. They suggested that the microbiome of health and disease is quite different at peri-implant sites. The peri-implantitis sites were enriched in pathogens at the expense of depletion of host compatible species and harbors species often associated with periodontal inflammation (Martin et al, 2017).

Only one study using the meta-transcriptomic analysis studied the microbiome at the peri-implantitis sites. Red complex species (*P.gingivalis, T.denticola*, and *T.forsythia*) were detected at peri-implantitis sites and *Veillonella and Gamella* were not identified at peri-implantitis sites (Table 6). These species are commonly associated with diseased periodontal sites. Since this study does not evaluate the microbiome of healthy implant sites a comparison cannot be obtained.
A.actinomycetemcomitans, P.gingivalis, P.intermedia, T.forsythia, and T.denticola were the most common species identified at healthy implant sites in different studies (Table 7). Viral species of HCMV and EBV were also found at healthy implant sites in the studies included (Jankovic et al, 2011, Kato et al, 2017). Studies assessing fungal species found a higher prevalence of these species with healthy implant sites (Bertone et al, 2016, Schwarz et al, 2015). *A.actinomycetemcomitans, P.gingivalis, P.intermedia, T.forsythia, T.denticola* were the most common species found at peri-implantitis sites. It has been found that the detection rate of T. *forsythensis* and *A.actinomycetemcomitans* increases with the increase in the severity of the peri-implant disease or found to be associated with deeper bone defect (Sato et al, 2011). A consistently higher level of EBV is noted around peri-implantitis sites indicating their role in peri-implant disease (Table 7).
Considering these findings, it can be said that the prevalence of peri-implantitis is not entirely dependent on the composition of the peri-implant biofilm but also on associated factors like age, occlusal load, chronic systemic diseases, history of periodontal disease, general body health, nutrition, psychological stress, smoking and implant material, and design. Although, the influence of these factors is difficult to assess their role cannot be denied completely (Singh et al, 2017, Kumar et al, 2019). Genetic, dietary, protocol, lifestyle and environment changes along with microbial species play an important role in peri-implantitis.

CONCLUSION

Considering the findings of all the reviewed studies, it can be concluded that-
a) A.actinomycetemcomitans, P.gingivalis, P.intermedia, T.denticola, T.forsythia and C.rectus, were present at both healthy and diseased implant sites. However, there was an increase in detection frequency (not established statistically) of these bacterial species at peri-implant sites seen with both PCR and hybridization techniques.
b) EBV 1 and HCMV 2 were associated with peri-implantitis sites only while C.*albicans, EBV 2 and HCMV* 1 were found to be present at both healthy and diseased implant sites.
c) Increase in detection frequency of above-mentioned bacteria and presence of signature viruses at peri-implantitis sites offers promising avenues for research in early detection of susceptible sites and relevant pharmacotherapeutic

management, which will improve implant prognosis.

d) A better understanding of the peri-implant environment, genetic, dietary, and lifestyle changes is also needed to clearly demarcate between healthy and diseased peri-implant sites.

Merits

This review includes only recent studies which were published in last ten years. Studies included in this review used only newer molecular biology techniques for microbial analysis, none of the studies used conventional culture-based method hence the data is more accurate and reliable. It will offer scope for future research and developing pharmacotherapy as well as diet, lifestyle, protocol and environment changes to promote microbes improving peri- implant health and remove those causing peri-implantitis.

Limitations

Although the review included recent studies using advanced microbial analysis technique, the number of studies included is less to draw any consistent variation in the microbiome of healthy and diseased peri-implant sites. Further research using more studies and collecting larger data can overcome this limitation. Larger efforts are still needed to understand the role of microbiome as well as to determine the role of other factors in peri-implant health and disease.

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Author's contribution

AP performed the literature search. AP, SVS and DA wrote the draft. SVS and DA critically reviewed the manuscript

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