Curr Oral Health Rep (2014) 1:262–271
DOI 10.1007/s40496-014-0033-8

EPIDEMIOLOGY (M LAINE, SECTION EDITOR)

Subgingival Distribution of Microorganisms

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Published online: 20 September 2014

Abstract Composition of subgingival microbiotas has long been seen as being of primary importance in the etiology of periodontal diseases. Development of advanced molecular methods has improved our knowledge about the role of traditional periodontal pathogens as well as resulted in the recovery of novel pathogen candidates. Detection rates of these microorganisms vary considerably between geographical regions but also between ethnic groups within a country. We have gathered information of various types of microorganisms inhabiting subgingival sites of individuals living in different parts of the world for the present review, the purpose of which is to highlight the potential impact of geography and ethnicity on subgingival findings, especially in chronic periodontitis.

Keywords Bacteria · Biofilm · Dental plaque · Ethnicity · Geographic locations · Gingiva · Microorganism · Molecular methods · Periodontal pathogen · Periodontitis · Population · Smoking · Viruses · Yeasts

Introduction

Each site in the human body harbors a typical microbiota of its own. A recent study looking for the bacterial microbiome in ten selected sites of the digestive tract, including seven intraoral sites, of more than 200 healthy adults, revealed four clusters on the basis of a similar community composition [1•]. Tooth-associated bacterial communities, namely supra- and subgingival plaque, formed one cluster, being clearly distinct from those of other intraoral or extraoral sites. Furthermore, the major difference between these two types of dental biofilms was the lower redox potential subgingivally, leading to increased abundances of strictly anaerobic genera (e.g., *Fusobacterium*, *Prevotella*, and *Treponema*) in subgingival biofilms [1•]. Although *Porphyromonas*, *Tannerella*, and *Treponema* proved to be highly prevalent genera in the examined healthy adults, it is important to keep in mind that, at the species level, these genera include both periodontitis-associated and periodontal health-associated organisms.

Bacterial communities in subgingival sites are highly diverse [2], and this diversity increases in diseased conditions [3•, 4, 5] when the overall composition rather than a single pathogen determines the pathogenicity of these biofilm communities. Indeed, the microbial profiles of subgingival biofilms differ significantly with respect to periodontal health status; bacterial diversity is greatest in diseased sites of periodontitis patients, in comparison with shallow sites or with subgingival findings in subjects without periodontitis [3•].

Health behavior of an individual, such as smoking and oral hygiene, can considerably influence the composition and abundance of subgingival microbiotas. Smoking is known as an important risk factor for periodontitis, which could be, at least partly, explained by the microbial shift. It has been shown that, on one hand, smoking favors the rapid shift towards pathogen-enriched biofilms in periodontal pockets and, on the other hand, causes a decrease in the proportion of health-compatible species [6, 7]. Oral hygiene has an

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impact on subgingival biofilms irrespective of the smoking status [8]. Gingival inflammation, however, did not lead to a distinct subgingival microbiome in periodontal pockets [9•].

The environment (geography) and genetic factors (ethnicity) may have a fundamental impact on microbial findings in the oral cavity [10••, 11••]. In the present review, we give an overview of the distribution of subgingival microorganisms in subjects of different ethnic origin and/or living in different continents and countries.

Dental Plaque Below the Gumline

Four decades ago, it was demonstrated by electron microscopy techniques that the microbial composition of subgingival plaque differs from that of supragingival plaque and that the composition varies in relation to periodontal health status [4]. While both plaque types consisted mainly of coccoid cells with features of Gram-positive organisms in periodontal health, various types of bacterial populations with flagellated bacteria and spirochetes dominated in periodontal disease-associated subgingival plaques. Dental biofilm formation and maturation occur via intergeneric and bacteria–environment interactions [12]. The use of specific oligonucleotide probes and fluorescent in situ hybridization has confirmed the principal findings of the structure of dental plaque where specific bacterial taxa are localized in different layers of the tooth-attached biofilms [13]. In subgingival biofilms, the bottom layer is mainly composed of *Actinomyces*. The intermediate layer consists of *Fusobacterium nucleatum*, *Tannerella forsythia*, and possibly *Tannerella*-like organisms, while the *Cytophaga-Flavobacterium-Bacteroides* cluster and the *Synergistes* group A are located in the top layer. Known periodontal pathogens, *Porphyromonas gingivalis*, *Porphyromonas endodontalis*, *Prevotella intermedia*, and *Parvimonas micra*, form microcolonies, colonizing already formed biofilms, whereas spirochetes dominate outside the biofilm [13]. The gradual maturation and subsequentially changing microbial composition influence the pathogenicity of subgingival biofilms [5].

A wide variety of microorganisms colonizing dental plaque at the gingival margin and below the gumline have been suggested as potentially being harmful to periodontal tissues. These include several bacterial species but also not-yet-cultivated phylotypes [2, 3•, 5, 9•, 14, 15], *Archaea* [16], some viruses as triggering organisms [17], and yeasts as opportunistic pathogens [18]. Among bacteria, Gram-negative species especially are considered major culprits of periodontal destruction due to their biologically active lipopolysaccharide-containing cell wall structure [19], although evidence on the involvement of various Gram-positive species in periodontal pathogenesis is also considerable [3•, 9•, 15, 20–22].

Role of Different Microorganisms as Periodontal Pathogens

Bacteria

Etiologic agents of periodontal diseases include both known cultivable species and several not-yet-cultivable phylotypes. According to Socransky and Haffajee [5], the three red-complex bacterial species, *P. gingivalis*, *T. forsythia*, and *T. denticola,* are the key pathogens, while Gram-negative *P. intermedia*, *Prevotella nigrescens*, *F. nucleatum*, *Campylobacter rectus*, and *Campylobacter showae* and Gram-positive *P. micra*, *Eubacterium nodatum*, and *Streptococcus constellatus* in the orange complex are putative pathogens. The use of open-ended molecular methods has further highlighted the composition of subgingival biofilms in disease. In the landmark study of Paster et al. [2], 347 species/phylotypes were found in subgingival samples from 31 subjects (11 subjects with 'refractory periodontitis', nine with periodontitis, two with HIV-associated periodontitis, four with necrotizing ulcerative gingivitis, and five periodontally healthy subjects). The majority of the findings represented unknown bacterial taxa; for example, *Synergistetes* (formerly *Deferribacteres*) and TM7 organisms were common in subgingival plaque of patients with periodontal disease. *P. endodontalis* and two red-complex species, *P. gingivalis* and *T. forsythia*, were exclusively associated with the disease, whereas *T. denticola* was common both in health and disease. Gram-positive *Atopobium parvulum*, *Atopobium rimae*, *Eubacterium saphenum*, and *Filifactor alocis* were found as potential pathogens [2]. In fact, it has been suggested that Gram-positive bacteria, such as *Filifactor* and *Peptostreptococcus*, could be the major players in periodontal disease [22]. In a recent study, using 454 pyrosequencing of 16S rRNA genes, clear differences were observed between subgingival plaque samples collected from subjects with chronic periodontitis and periodontitis-free subjects, the major periodontitis-associated organisms being *F. alocis*, *P. gingivalis*, and *T. denticola* [3•]. Interestingly, in the latter study, most findings represented cultivable species.

Of the Gram-negative organisms, *A. actinomycetemcomitans* is considered a major periodontal pathogen in aggressive periodontitis, particularly in its localized form [10••], while *P. gingivalis* has been long known as a typical member of subgingival biofilms in patients with chronic periodontitis [5]. It is notable that, in some geographical areas, subjects with aggressive periodontitis are primarily infected by *P. gingivalis* [10••]. In young adults, *P. gingivalis* and *T. forsythia* have been connected with the progression of gingivitis to periodontitis [23, 24]. Therefore, *T. forsythia* could be useful as an indicator bacterium of periodontal destruction in its early phase. In addition to *T. denticola*, other *Treponema* species, such as *T. lecithinolyticum* and *T. socranskii*, can also be harmful to

periodontal tissues, and the presence of subgingival spirochetes has been linked to both chronic and aggressive forms of periodontitis [25, 26•]. Among Gram-positive organisms, *F. alocis* has the strongest evidence as a periodontal pathogen [2, 3•, 9•, 15, 20, 22] but several *Eubacterium* species have also been strongly associated with chronic periodontitis [9•, 21]. Table 1 presents bacterial taxa found in chronic periodontitis, obtained in three recent studies using high-throughput 16S rRNA pyrosequencing [3•, 9•, 15].

There can be considerable differences in the pathogenicity between the clones and genotypes within a species. Of the *A. actinomycetemcomitans* serotypes, serotypes b and c seem to have an increased virulence, which may explain why they are commonly detected in subjects with aggressive periodontitis [10••]. Within serotype b, the JP2, a specific clone with highly enhanced leukotoxicity, exposes its carrier to high risk of aggressive periodontitis [27]. Variation in the virulence of *P. gingivalis* may depend on the type of its fimbriae. Most *P. gingivalis*-positive subjects carry a single genotype, and distinct fimbrial genotypes are found in periodontitis patients in comparison with periodontally healthy subjects; *fimA* type II, in particular, has been associated with periodontitis [28–32]. Overall, the distribution of different clonal types of periodontal pathogens may vary considerably, depending on the geographic location and/or ethnicity.

Table 1 Bacterial taxa considered as periodontal pathogens in chronic periodontitis. Data are adapted from three studies using 454-pyrosequencing of the 16S rRNA gene

Phylum/Class	Bacterial species (phylotype)	Reference(s)
Bacteroidetes	*Capnocytophaga granulosa*	[15]
	Porphyromonas gingivalis	[3•, 9•, 15]
	Porphyromonas endodontalis	[3•, 9•]
	Prevotella denticola	[3•, 15]
	Prevotella intermedia	[3•]
	Prevotella tannerae	[3•]
	Tannerella forsythia	[3•, 9•, 15]
Spirochaetes	*Treponema denticola*	[3•, 9•]
	Treponema lecithinolyticum	[3•, 9•]
	Treponema maltophilum	[3•, 9•]
	Treponema socranskii	[3•, 9•]
	Treponema vincentii	[3•]
Fusobacteria	*Fusobacterium nucleatum*	[15]
Proteobacteria	*Campylobacter gracilis*	[15]
	Campylobacter rectus	[3•]
	Kingella oralis	[15]
Firmicutes/Negativicutes	*Anaeroglobus geminatus*	[3•]
	Dialister invisus	[15]
	Dialister pneumosintes	[15]
	Selenomonas sputigena	[3•, 9•, 15]
Firmicutes/Bacilli	*Streptococcus anginosus*	[15]
Firmicutes/Clostridia	*Eubacterium brachy*	[9•]
	Eubacterium nodatum	[9•]
	Eubacterium saburreum	[15]
	Eubacterium saphenum	[3•, 9•, 15]
	Filifactor alocis	[3•, 9•, 15]
	Mogibacterium timidum	[9•, 15]
	Parvimonas micra	[9•, 15]
	Peptostreptococcus stomatis	[3•, 15]
Synergistetes		[3•, 9•, 15]
TM7		[3•, 9•, 15]

Viruses

To date, little is known about the oral and periodontal viromes. Most information on viruses in periodontal literature exists on two herpes viruses, Epstein-Barr virus (EBV) and cytomegalovirus (CMV) [17]. Significantly altered viral load found in deep periodontal pockets supports the role of viruses in the pathogenesis and progression of periodontal disease [33, 34]. On one hand, the predominance of bacteriophages, including bacteriolytic myoviruses, may have a role in shaping the bacterial microbiota and altering its diversity in subgingival biofilms; the proportions of myoviruses were highest in periodontitis, whereas siphoviruses were significantly more abundant in health [34]. On the other hand, herpes viruses, particularly CMV, possess several potential pathogenicity mechanisms that can lead to periodontal tissue destruction (see a comprehensive review by Contreras et al. [33]). Indeed, elevated genomic copies of EBV and CMV have been detected in subgingival plaque, especially in subjects with untreated or progressive periodontitis [17]. Moreover, higher detection rates of viral co-existence or virus–anaerobe synergy have been found in deep periodontitis lesions in comparison with healthy sites [35, 36]. According to the herpes viral–bacterial interactive model [17], viruses are considered triggering organisms for periodontitis-associated bacteria; however, the role of subgingival virus load in the periodontal pathogenesis remains unsolved. In fact, the solidity of the theory is challenged by studies reporting high detection rates of herpes viruses in periodontally healthy subjects [35–37] or failing to detect any viral contribution to periodontitis lesions [38–40]. Conflicting results in the occurrence of periodontal herpes viruses may be explained, at least in part, by variations in the clinical and viral diagnostic methods used in different studies or by true population-, ethnicity-, or age-related differences.

Yeasts

Although yeasts are aerobic organisms, considerable detection rates (17–48 %) have been reported from diseased periodontal pockets [41–45]. *Candida albicans* is clearly the most

common *Candida* finding in subgingival samples, while other species, such as *C. dubliniensis* and *C. parapsilosis*, are much less common and often recovered together with *C. albicans* [42, 44–46]. Other yeast species are only occasionally isolated from immunocompetent subjects. In HIV-infected periodontitis patients, a high frequency of yeasts has been reported from subgingival samples [18]. In this study, including 54 HIV patients, 55 % had *C. albicans*, 48 % *C. dubliniensis*, and 17 % *C. glabrata* subgingivally, whereas only one healthy subject in the HIV-negative control group had a subgingival culture with light growth of *C. albicans*. The role of *Candida* in the periodontal pathogenesis, if any, has not been elucidated yet.

Impact of Geography and Ethnicity

Marked differences can be observed in the frequencies of periodontal pathogens among different populations, which may reflect the geography and ethnicity as well as the epidemiology of different forms of periodontal disease [5, 10••]. Figure 1 presents polymerase chain reaction (PCR)-based detection rates of two known periodontal pathogens, *A. actinomycetemcomitans* and *P. gingivalis*, giving an overview at a glance on their presence in different regions of the world.

Subgingival Microbial Findings in Different Countries

Major bacterial findings reported in studies from different countries are summarized in Table 2 [31, 35, 47–66]. As can be seen, a variety of microbial identification methods, including culture, DNA-DNA checkerboard hybridization, and several types of PCR, was used in these studies. Besides different identification methods, inclusion criteria, disease definition, clinical status of selected subgingival sites, and subgingival sampling methods vary between studies. In addition, often a relatively small number of subjects, even divided in subgroups, have been examined, which brings about uncertainties when interpreting the data. Therefore, it is plausible that differences in prevalence rates are not caused by geography solely. Some of the studies included subject groups from several countries but otherwise used the same study design and methodology for investigating the collected samples, thus improving the reliability of the comparison. Two culture-based studies were conducted in the Netherlands and Spain [54] and in Spain, Chile, and Colombia [49], including around 40 chronic periodontitis patients in each study group. Despite the similarity of the two Spanish study groups, there were slight differences in detection rates of examined pathogens; for example, for *A. actinomycetemcomitans* 3 % [54] versus 17 % [49] and for *T. forsythia* 65 % versus 36 %, respectively. Except for the low rate of 3 % for *A. actinomycetemcomitans*, the microorganism was found in a fifth of the subjects in Spain, Netherlands, Chile, and Colombia. In general, subgingival *P. gingivalis* was a prevalent finding (65–84 %) in chronic periodontitis patients; however, in the Netherlands the prevalence was only 37 %. Furthermore, *P. intermedia/P. nigrescens* was isolated from 19 % of Chilean patients in contrast to high detection rates (73–97 %) in other populations examined. The most remarkable difference was observed for *P. micra*; it was detected in 97 % of Dutch subjects but only in one patient in the Colombian study group. Another study [55], presenting data on subgingival samples from 259 periodontitis patients in the Netherlands and comparing culture and PCR for detecting periodontal target microorganisms confirmed this high detection rate of *P. micra* previously presented [54]. A PCR-based study compared the prevalence of periodontal pathogens between 88 German and 91 Korean subjects suffering from chronic periodontitis [56]. *A. actinomycetemcomitans* and *T. denticola* were more common in German than in Korean subjects (48 % vs 27 % and 96 % vs 81 %, respectively), whereas high rates of *T. forsythia* and *P. gingivalis* were found in both countries. In a study using checkerboard DNA-DNA hybridization, subgingival proportions of 40 microorganisms were compared between chronic periodontitis patients in the US, Brazil, Chile, and Sweden [67]. Marked differences were detected for 13 target microorganisms; for instance, the adjusted mean proportion of *P. gingivalis* in subgingival plaque was highest in Chile and that of *T. forsythia* in Brazil, whereas the lowest proportions of these species were found in Sweden [67].

The methodology utilized for the detection of herpes viruses is an important matter when comparing the prevalence rates between different studies. Nowadays, nested PCR is a popular technique for subgingival virus detection, and data are available from various study populations. For example, CMV in periodontitis patients versus periodontitis-free subjects has been reported as follows: 35 % versus 12 % in Canada [68], 50 % versus 48 % in Brazil [35], 50 % versus 5 % in Iran [59], and 79 % versus 77 % in China [36], respectively. In contrast, by real-time PCR, extremely low rates of CMV (0–2 %) both for periodontally healthy and diseased subjects have been obtained from studies conducted in the US, UK, and Germany [38–40]. In a demographically homogenous Chinese study population, including 143 patients with chronic periodontitis and 141 periodontitis-free subjects, genotype profiles of CMV differed in relation to the periodontal health status. While CMV gB-I predominated in healthy and gingivitis subjects, CMV gB-II alone or in combination with EBV-1 had a significant association with periodontitis [36]. According to nested PCR-based studies from Brazil, Iran, Japan, and China, EBV-1 (or EBV) is common in subgingival plaque collected from deepened pockets [35–37, 59]. Again, real-time PCR resulted in much lower detection rates of EBV, varying

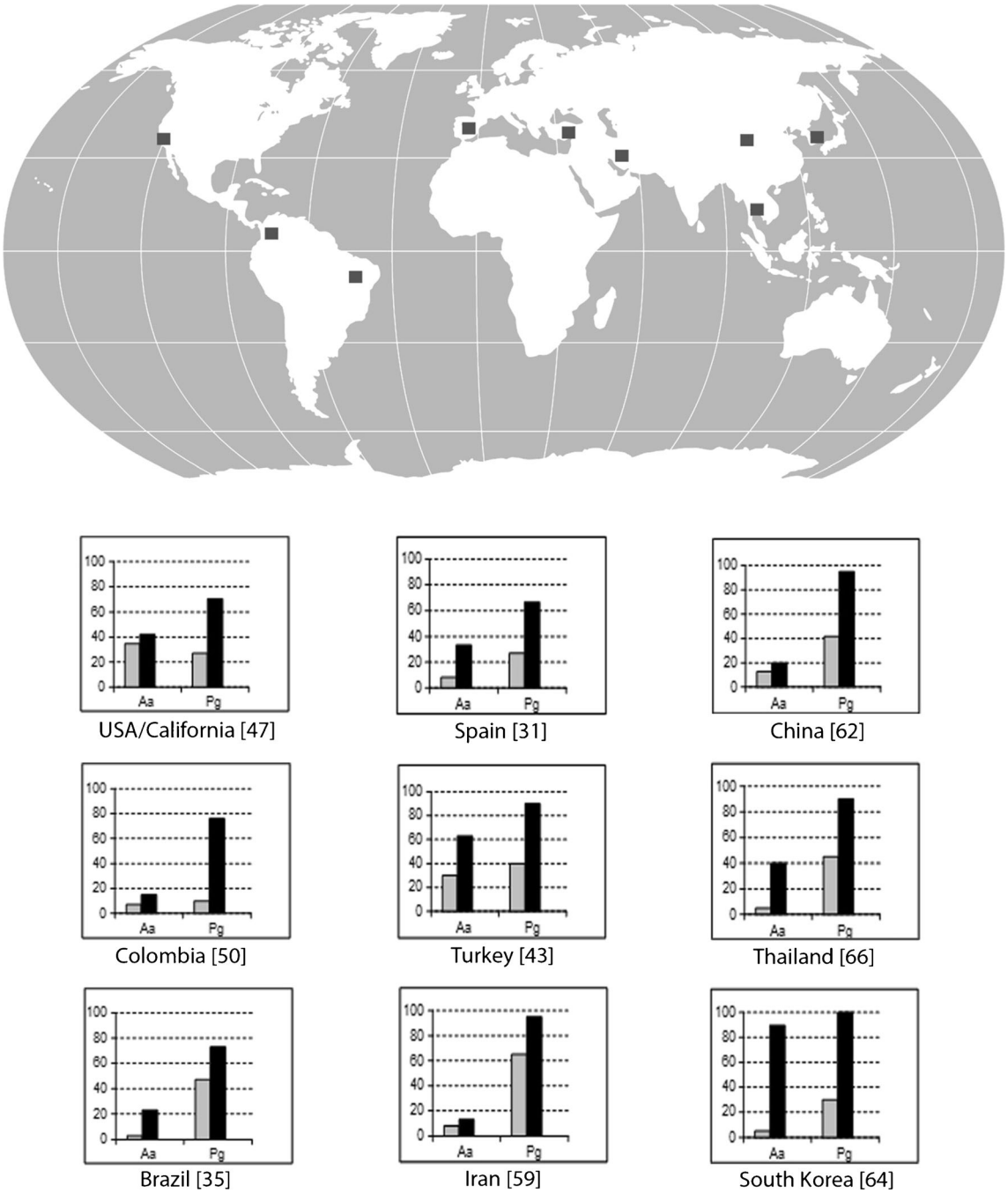

Fig. 1 Polymerase chain reaction (PCR)-based detection rates of *A. actinomycetemcomitans* and *P. gingivalis* in subjects with chronic periodontitis (*black bars*) and periodontitis-free controls (*grey bars*) in different regions of the world. The detailed information about the prevalence rates of the major periodontal pathogens is given in Table 2

between 6 and 28 %, in American, English, and German periodontitis patients [38–40]. Herpes simplex virus type 1 (HSV-1) was among the target microorganisms in the German study using real-time PCR [40] and in the Brazilian study using nested PCR [35], both studies including an aggressive periodontitis group. In the latter study, HSV-1 was found in 37 % of the periodontally healthy subjects and in 87 % of the patients with aggressive periodontitis, while the microorganism was found only in 2 % of both healthy and diseased German subjects. It remains open whether this remarkable difference is explained by different methods or geography.

Subgingival yeasts in periodontitis patients have rather similar detection rates in different geographical locations. In two large-scale studies, 19.6 % of the 535 samples collected in Sweden [41] and 16.8 % of the 500 samples collected in the

Table 2 Geographical distribution of major bacterial recoveries from subgingival plaque in chronic periodontitis subjects

Country [Reference]	Population		Method	Prevalence (%)										
	N	Age range (mean±SD)		*Aa*	*Pg*	*Pi* sensu lato	*Pi*	*Pn*	*Td*	*Tf*	*Syn*	*Fa*	*Pm*	TM7
USA [47]	101	35–83	PCR	42	70		57	85	76	83				
Mexico [48]	44	31–75	Checkerboard	86	98		95	93	95	96				
Colombia [49]	41	25–64	Culture	17	66	73				39			2	
Colombia [50]	68	(42.3±11.3)	PCR	15	77	75				50				
Colombia [51]	325	(45.6±10.6)	PCR	17	68		~27	~12		59				
Brazil [35]	30	(42.7±6.7)	PCR	23	73		87			33				
Chile [49]	37	31–60	Culture	19	84	19				16			30	
Cameroon [52]	21	20–62	Checkerboard	52	86		86	91	67	82			91	
Sudan [53]	25	22–70	DNA probe/ Culture	16/28	96/36	NA/40	76/NA			96/NA				
Norway [53]	18	30–61	Culture	33	87	78								
Netherlands [54]	30	29–63	Culture	23	37	90				73			97	
Netherlands [55]	259	>25	RT PCR/Culture	27/22	46/43	NA/64	83/NA			89/83				
Germany [56]	88	(43.3±8.6)	RNA probe	48	82				96	96				
Switzerland [57]	47	(50.5±ND)	RT PCR	19	81		72		89	96				
Spain [49]	36	26–67	Culture	17	78	97				36			61	
Spain [54]	31	26–62	Culture	3	65	74				65			58	
Spain [31]	33	(43.4±ND)	PCR	33	67				49	70				
Romania [58]	36	30–68	Culture	42	76	55								
Turkey [43]	14	37–59	PCR/Culture	50/~43	93/~71	NA/~86				NA/~86			NA/~29	
Iran [59]	40	(40.9±12.2)	Multiplex PCR	13	95					75				
Yemen [60]	20	30–50	RT PCR	68	98				100	100			100	
Yemen [61]	40	35–47	RT PCR		71				91	100	100	100	100	95
China [62]	60	(35.3±9.1)	RT PCR	20	95		98							
China [63]	306	(52.2±9.6)	RT PCR	~11	~81		~67		~50	~47				
Korea [64]	29	29–49	PCR	90	100		90			97			97	
Korea [56]	90	(46.6±10.9)	RNA probe	27	81				81	89				
Japan [65]	20	(43.6±11.1)	RT PCR	25	75					85				
Thailand [66]	20	(46.5±10.1)	PCR	35	95				80	95				

Aa = *A. actinomycetemcomitans*; *Fa* = *F. alocis; Pg* = *P. gingivalis*; *Pi* = *P. intermedia*; *Pm* = *P. micra*; *Pn* = *P. nigrescens*; *Syn* = *Synergistetes*; *Td* = *T. denticola*; *Tf* = *T. forsythia*; NA = no data available; RT PCR = Real-time PCR

US [45] were reported to be positive for yeasts. In an Argentinian study population, *C. albicans* was found in 24 %, *C. parapsilosis* in 5.6 %, and *C. dubliniensis* in 4.4 % of the 180 periodontitis patients examined [46]. Smaller studies conducted in Brazil [42], Turkey [43], and Ireland [44] have presented detection rates of 30 %, 23 %, and 48 %, respectively, for yeasts in subgingival plaque from diseased periodontal pockets.

Distribution of Some Virulent Clones/Types

A comprehensive overview on the colonization of potential microorganisms related to aggressive periodontitis in geographically and ethnically diverse populations was recently provided by Könönen and Müller [10••]. Traditionally, *A. actinomycetemcomitans* has been seen as the major pathogen and its leukotoxin production as the main virulence factor contributing to this form of periodontal disease. In general, of its seven serotypes, *A. actinomycetemcomitans* serotype b is common in periodontally diseased Caucasians in Europe, while in American and Asian subjects, serotype c dominates. However, serotype c and serotype a, in particular, are also common in *A. actinomycetemcomitans*-positive subjects without the disease (see Table 1 in the reference [10••]). In contrast, the presence of the specific JP2 clone results in a highly increased risk for aggressive periodontitis. This clone is nearly exclusively found in Moroccan adolescents and in some young populations of West African origin in Brazil and the US [10••]. Also, in chronic periodontitis patients colonized by *A. actinomycetemcomitans*, serotype distribution varies

between countries. In Brazil, serotype c dominates and serotype a is relatively prevalent, contrasting with the findings in Finnish and Taiwanese patients where serotype b dominates, while on the Eastern coast of the US, serotypes a, b, and c are distributed equally [10••, 69].

Prevalence of fimbrial genotypes of *P. gingivalis* has been studied in various regions of the world. Although *fimA* type II seems to predominate in periodontitis patients irrespective of the geographical location, there are remarkable differences in the detection rates of other genotypes [28–32]. In Japan and China, the distribution of *fimA* genotypes proves to be similar; besides *fimA* type II, *fimA* type IV was common in subgingival plaque collected from subjects with periodontitis, whereas *fimA* type I was highly prevalent and also *fimA* type V was rather common in periodontally healthy subjects [28, 32]. In Brazil, the distribution differs as regards to *fimA* type IV, which was found in half of the periodontitis-free subjects but only occasionally in periodontitis patients [30]. In the latter study, including a multi-ethnic study population, the detection rates of *P. gingivalis* did not differ between Caucasians (88 %) and African-Brazilians (94 %) with periodontitis; however, no separate data on the prevalence of *fimA* genotypes in these ethnic groups were given.

Ethnic Differences

The influence of ethnicity is most reliably studied in countries with a multi-ethnic population. In the US, Craig et al. [70] examined a series of clinical, environmental, demographic, and microbiologic factors in an urban study population of 185 subjects, consisting of three minority groups living in the greater New York metropolitan area. Subgingival plaque samples were collected from 47 African-American, 40 Asian-American, and 27 Hispanic subjects and examined by the DNA-DNA checkerboard method. Although *Actinomyces oris* (formerly *Actinomyces naeslundii* genospecies 2) was the most common species in all groups, the mean proportions of some bacterial species differed significantly between the three ethnic groups; for example, *P. micra* (formerly *Peptostreptococcus micros*) was elevated in the African-American group, whereas *A. actinomycetemcomitans* and *Selenomonas noxia* were elevated in the Asian-American group. However, elevated levels of periodontal pathogens were found especially in the unskilled group with worsened periodontal status, higher proportions of males and smokers, and being older than subjects in the skilled and professional groups [70]. In other words, the ethnicity itself was not the explanatory factor for differences seen in subgingival bacterial profiles.

In a study conducted in Los Angeles, California, oral samples (subgingival plaque, saliva) were collected from four ethnic groups, including 52 Caucasians, 50 Hispanics, 49 African-Americans, and 48 Asian-Americans, for PCR-based microbiologic analyses, including six periodontal bacteria [47]. The ethnicity, indeed, had an impact on the occurrence of target bacteria; Asian-Americans and Hispanics had a significantly increased risk for harboring *A. actinomycetemcomitans* and *P. gingivalis* subgingivally.

A recent US study was specifically targeted to examine the impact of ethnicity on the oral microbiome [11••, 71]. Altogether 192 periodontally and dentally healthy subjects, divided into Caucasian, African-American, Latino, and Chinese groups (48 subjects in each group), were carefully questioned for their demographics, oral hygiene (the frequency of brushing, flossing, smoking history, and visits to the dentist) and diet (standard American, Hispanic/Latino, Asian, and one not fitting to any of the three diets). When subgingival plaque samples from 25 subjects from each ethnic group were examined using an advanced sequencing method, discriminative microbial fingerprints with a 62 % accuracy, 58 % sensitivity, and 86 % specificity were found. Although Caucasians and African-Americans shared a common food, nutritional and lifestyle heritage, a significant microbial divergence between these two groups was observed [11••, 71], indicating that ethnicity itself is the determining factor for distinct bacterial profiles.

Concluding Remarks

To summarize the findings of the reviewed studies, it can be stated that subgingival detection rates of periodontal pathogens vary considerably in different regions of the word. The variation in methodologies leads in part to differences in microbiologic findings; however, geography and ethnicity have an impact on the composition of the human oral microbiota, including subgingival biofilms. Therefore, in studies with multi-ethnic study populations, it would be advisable to give the data separately for each ethnic group.

Acknowledgments We thank Klaus Könönen for generating the figure for this article.

Compliance with Ethics Guidelines

Conflict of Interest Eija Könönen and Mervi Gürsoy declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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