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Review Article

Ectopic colonization by oral bacteria as an emerging theme in health and disease

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Abstract

The number of research papers published on the involvement of the oral microbiota in systemic diseases has grown exponentially over the last 4 years clearly demonstrating the growing interest in this field. Indeed, accumulating evidence highlights the central role of ectopic colonization by oral bacteria in numerous noncommunicable diseases including inflammatory bowel diseases (IBDs), undernutrition, preterm birth, neurological diseases, liver diseases, lung diseases, heart diseases, or colonic cancer. There is thus much interest in understanding the molecular mechanisms that lead to the colonization and maintenance of ectopic oral bacteria. The aim of this review is to summarize and conceptualize the current knowledge about ectopic colonization by oral bacteria, highlight wherever possible the underlying molecular mechanisms and describe its implication in health and disease. The focus lies on the newly discovered molecular mechanisms, showcasing shared pathophysiological mechanisms across different body sites and syndromes and highlighting open questions in the field regarding the pathway from oral microbiota dysbiosis to noncommunicable diseases.

Keywords: oral microbiota; ectopic colonization; dysbiosis; noncommunicable diseases; molecular mechanisms

Introduction

Microbial communities (microbiomes) usually play important roles in the ecosystems or hosts where they live. In the case of animal microbiomes, they contribute to the overall maintenance of healthy physiological functions, development, and nutrition and provide protection against infectious microorganisms. These microbe–microbe and microbe–host interactions include competition for resources and niches, production of antibiotic substances, metabolic inhibition as well as modulation of host immunity, influencing in turn also the composition of the microbial community (Ursell et al. 2012, Belkaid and Harrison 2017, Vonaesch et al. 2018). When these stable (healthy) communities are perturbed, they enter a dysbiotic state, which is defined as a microbial community displaying an imbalanced composition associated with a disease status. A common hallmark of dysbiosis are taxonomic changes in the overall microbial community, e.g. due to ectopic colonization by exogenous or endogenous microorganisms (Al-Rashidi 2022).

After the fecal microbiota, the oral microbiota is the second most important microbial community inhabiting the human body, both regarding absolute numbers as well as in terms of diversity (Escapa et al. 2018). The oral microbiota plays a role in oral health but also in that of many systemic sites. For instance, the oral microbiota contributes to the establishment and maintenance of a healthy immune barrier (Hooper et al. 2012), provides protection against pathogens that invade the oral space (Wade 2013), and contributes to cardiovascular health through the production of vasodilatory compounds such as nitric oxide (Cyr et al. 2020).

Oral commensals are considered good colonizers in general since they can colonize almost any part of the buccal cavity (Caselli et al. 2020). Similarly, under certain conditions, specific species of oral origin, or even more likely, oral communities can colonize ectopically in distant sites and trigger dysbiosis in the colonized environment. Ectopic colonization by oral bacteria in the intestinal tract has been known since a long time. However, in the last years, there has been accumulating evidence highlighting the central role of ectopic colonization by oral bacteria in distant places including the intestine, placenta, nasal tissue, lungs and upper airways, and aorta, contributing to many noncommunicable diseases including inflammatory bowel diseases (IBDs) (Atarashi et al. 2017, Dinakaran et al. 2019, Hu et al. 2021, Molinero et al. 2022, Rojas-Tapias et al. 2022, Rashidi et al. 2023), undernutrition (Vonaesch et al. 2018, 2022, Chen et al. 2020, Collard et al. 2022), preterm birth (Van der Haar et al. 2018, Yin et al. 2021), neurological diseases (Nicholson and Landry 2022), liver diseases (Joossens 2021), lung diseases (Joossens 2021), heart diseases (Hodel et al. 2023), or colonic cancer (Flemer et al. 2018, Rashidi et al. 2023). There is thus an increased interest in understanding the molecular mechanisms that lead to the colonization and maintenance of oral bacteria at ectopic sites. Several mechanisms have been proposed for ectopic colonization to occur, such as swallowing the oral bacteria with saliva or mechanical injuries in the oral cavity that allow microbes to directly enter the bloodstream and spread to distant sites. Nevertheless, the specific circumstances that can lead to permanent colonization and expansion of these bacteria in secondary places are only poorly understood to date. Furthermore, there is an ongoing debate whether the oral strains found at ectopic sites are constantly seeding in from the oral cavity or if they have adapted to the new niche, i.e. within the lower gastrointestinal (GI) tract (Schmidt et al. 2019,

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Gough et al. 2020). Also, regarding community dynamics, it remains unclear whether the ectopic colonization by oral bacteria is leading to an expansion of oral bacteria at distant sites or if a decrease in the resident microbiota is leading to a relative increase in these oral bacteria, while the absolute counts remain constant (Liao et al. 2022). Finally, to date microbiome research has often leaned towards the oral and intestinal microbiomes, because of their easier accessibility, leading to the identification of numerous strains in these extensively studied environments. This inclination fosters a misconception that some specific bacterial species are predominantly oral inhabitants, overshadowing the versatile nature of certain bacterial species that can potentially primarily inhabit less investigated body sites.

Despite large efforts directed on understanding the role and function of the human microbiome during the past decades, the causal association between ectopic colonization by oral bacteria and pathophysiology remains poorly understood. Recent literature starts to shed light on the molecular mechanisms that link ectopic colonization by oral bacteria with pathophysiological disturbances such as inflammation (Atarashi et al. 2017, Vonaesch et al. 2022, Bergsten et al. 2022, Conde-Pérez et al. 2023, Miao et al. 2023), immune system subversion (Bergsten et al. 2022, Conde-Pérez et al. 2023) or decreased lipid absorption (Vonaesch et al. 2022). The aim of this review is to provide an overview, summarize and conceptualize the current knowledge about ectopic colonization by oral bacteria and describe its implication in health and disease. Understanding the molecular mechanisms leading to the observed pathophysiology will be crucial to find successful interventions to diagnose, treat, and prevent disease linked to this ectopic colonization. Further, we aim to showcase shared pathophysiological mechanisms across different body sites and syndromes linked to ectopic colonization by oral bacteria, to then highlight open questions in the field regarding the pathway from oral microbiota dysbiosis to noncommunicable diseases.

Oral microbiota

After the fecal microbiota, across all human-associated habitats, the oral microbiota is the second most abundant both in terms of total biomass (absolute number of microorganisms) and in terms of variety of bacterial species (Wade 2013, De Vos et al. 2022). The oral cavity is easily accessible and samples for microbiome analysis can be taken in a noninvasive way, compared to other body sites [i.e. lower (GI) tract compartments]. For that reason, the composition of the oral microbiota has been widely studied across different geographic areas and life stages and information on its taxonomy and ecology have been summarized and made freely accessible on the Human Oral Microbiome Database website (Dewhirst et al. 2010) and the Oral Microbiome Bank of China database (Xian et al. 2018). Nevertheless, it is undeniable that so far, oral microbiome studies have neglected populations from nonindustrialized countries: to date, there are only a few studies on African, Asian, or Indigenous cohorts (Yang et al. 2019, Nath et al. 2021, Yeo et al. 2022, Araújo et al. 2023). Recent research has clearly shown that the oral bacterial community structures of healthy individuals is distinct from individuals suffering from diseases such as periodontitis, dental caries, oral cancer, IBDs, colonic cancer, and preterm birth (reviewed by Peng et al. 2022). Here, we provide a brief overview of the composition of the oral microbiota in healthy subjects as well as the main changes occurring during states of disease.

Healthy oral microbiota

The oral microbiota encompasses a variety of bacteria, viruses, archaea, fungi, and protozoans distributed in almost any part of the oral tissue, including tongue, teeth, saliva, mucosa, and gingiva. These microhabitats differ in their exact taxonomic composition yet share a large set of species (Verma et al. 2018, Caselli et al. 2020). In the whole microbiome field, the definition of a "healthy" microbiome remains a controversial topic. This is not different for the oral microbiome, where attempts to define a "normal" microbiota have been based on studying the oral microbiota in seemingly healthy subjects. Despite the high level of interindividual variation, *Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Fusobacteria*, *Actinobacteria*, and *Spirochaetes* are typically representing around 96% of the total sequences derived from oral bacteria (Bik et al. 2010, Dewhirst et al. 2010, Verma et al. 2018, Caselli et al. 2020) and *Streptococci*, *Neisseria*, *Prevotella*, *Veillonella*, and *Rothia* are the most typical genera (Bik et al. 2010). On the species level, there is high prevalence of different species of *Streptococci*, including *S. mitis*, *S. oralis*, *S. salivarius*, and *S. sanguinis* (Dewhirst et al. 2010, Caselli et al. 2020). In addition, although representing a smaller part of the total biomass of the oral microbiome, several fungi are typically present in the oral cavity of healthy subjects. These include members of the genera *Candida*, *Cladosporium*, *Aspergillus*, *Fusarium*, *Saccharomycetales*, *Aureobasidium*, and *Cryptococcus* (Ghannoum et al. 2010, Caselli et al. 2020). Further, the protozoa *Entamoeba gingivalis* and *Trichomonas tenax*, as well as bacteriophages from the *Caudovirales* are frequently recovered (Caselli et al. 2020). Together, this microbial community plays important roles in overall human health, influencing immune responses and providing protection against pathogens invading the oral space. As an example, *Streptococcus* species, the earliest colonizers of the upper GI tract after birth, and one the most frequently detected bacterial taxa in the buccal cavity, produce hydrogen peroxide and bacteriocins, which possess antimicrobial properties capable of inhibiting the growth of multiple pathobionts (Baty et al. 2022).

The microorganisms found in the oral cavity rarely live as planktonic cells, but they frequently form associations and live in microbial communities that can then easily evolve in a biofilm when they attach to a tissue (Welch et al. 2016, Simon-Soro et al. 2022). Only 3% of the microorganism in saliva are found as single-free-living cells, thus not associated with other bacterial or oral epithelial cells (Simon-Soro et al. 2022). Certain taxa are more prone to form aggregates, as it is the case of *Fusobacterium*, *Rhodanobacter*, and *Porphyromonas*, while others such as *Streptococcus*, *Prevotella*, *Veillonella*, and *Neisseria*, can be found in both forms, as single-free-living-cells and forming aggregates (Simon-Soro et al. 2022). Interestingly, recent work from Simon-Soro and collaborators showed that polymicrobial aggregates formed by *Streptococcus*, *Haemophilus*, *Veillonella*, *Gemella*, *Neisseria*, *Fusobacterium*, *Porphyromonas*, and *Prevotella* colonize and grow faster than single-free-living-cells of these species in *in vitro* biofilm models (Simon-Soro et al. 2022). These findings provide a better knowledge on oral bacterial communities than previous models of sequential single-free-living-cells of early colonizer species attachment and biofilm development in an orderly ecological succession of different species (Welch et al. 2016, Bowen et al. 2018).

Oral microbiota dysbiosis

Oral microbial communities, as any microbiome, can also experience perturbations leading to an altered composition (dysbiosis) and, eventually, to disease. Changes in microbial composition

usually consist of changes in abundance, expressed as relative abundance in most of the oral microbiome studies published so far. Indeed, it has been described that strains associated with disease can still be found in healthy individuals, but in lower relative abundance compared to diseased individuals (Bik et al. 2010, Caselli et al. 2020). For example, *S. mutans*, a carcinogenic bacterium that promotes tumor progression in oral cancer, is detected in low relative abundance in the oral microbiota of healthy subjects but is found in higher abundance in the teeth biofilm of precarcinogenic and carcinogenic subjects (Caselli et al. 2020, Tsai et al. 2022). In addition, specific co-occurrences between bacteria correlate with disease. *Fusobacterium nucleatum* and *Porphyromonas gingivalis* often coaggregate in the oral cavity of patients with oral cancer, where they interact to promote each other's growth and lead to enhanced inflammation and faster tumor progression (Diaz et al. 2002, Gallimidi et al. 2015). Similarly, high relative abundance of *Veillonella*, *Porphyromonas*, *Fusobacterium*, and *Candida* correlates with severe dental caries. It is believed that the presence of species from these genera facilitates adherence of pathogenic bacteria within the oral cavity, which ultimately leads to disease (Darrene and Cecile 2016, Verma et al. 2018). The same bacterial communities are also associated with systemic disease including IBDs and cardiovascular disease (reviewed by Thomas et al. 2021, Peng et al. 2022). To date, the specific molecular mechanisms behind these associations remain unclear.

The most immediate and well-known consequences of oral microbiota dysbiosis are dental caries and periodontitis. Changes in the oral microbiome of patients with these oral diseases typically indicate a decrease in alpha diversity. Alpha diversity is commonly assessed by measuring the number of different bacterial taxa and species present in the oral cavity. Furthermore, they are associated with the presence of pathobionts, which are nonharmful microorganisms under normal circumstances, but which potentially cause disease in a susceptible individual (Belstrøm et al. 2015). Moreover, an unbalanced oral microbial community can affect human health also beyond the oral cavity. Widely known examples of this systemic effects are the association of poor oral health and metabolic diseases such as obesity and diabetes, IBDs, Alzheimer or rheumatoid arthritis (reviewed by Thomas et al. 2021, Peng et al. 2022). This effect is usually due to the generation of a sustained inflammatory microenvironment at the site of the disease (i.e. periodontitis) that contributes to the secretion of proinflammatory cytokines into the bloodstream. This then creates inflammatory environments in distal body sites and contributes to disease (Lira-Junior and Boström 2018, Kitamoto et al. 2020). However, the effect can also be due to translocation of oral bacteria in the bloodstream and ectopic colonization at distant sites. Studies describing the latter case will be reviewed in the next section of this paper. Although fungal members of the human oral microbiota have been less studied compared to bacteria, recent evidence suggest that oral fungi (i.e. *Candida*, *Cladosporium*, *Fusarium*, and *Malassezia*) likewise impact several systemic diseases, including IBDs, Crohn's disease (CD), chronic respiratory diseases, and hepatitis B (Ghannoum et al. 2010, Dupuy et al. 2014; and reviewed by Baker et al. 2017). Despite a clear correlation between these fungal taxa and disease, little is known about the underlying molecular mechanisms (Cui et al. 2013; and reviewed by Baker et al. 2017). Recent work indicates the presence of cross-kingdom interactions, exemplified through the fungus *Candida albicans*, which interacts with members of the bacterial genus *Streptococcus* increasing the severity of oral diseases. *C. albicans* interaction with *S. oralis* results in increased levels of μ-calpain, a molecule that degrades epithelial junctions and thus enhances tissue invasion by these microorganisms (Diaz et al. 2012, Xu et al. 2016; and reviewed by Baker et al. 2017). In contrast, in the presence of macrophages, interaction between *C. albicans* and the bacterium *F. nucleatum* results in reduced growth but increased survival of both the bacterium and the fungus. This then leads to dampening of the host immune response (Allison et al. 2016; and reviewed by Baker et al. 2017).

There is, thus a clear link between oral dysbiosis, local ill-health as well as a variety of systemic diseases. This phenomenon will be discussed in detail in the following sections of this review.

Ectopic colonization by oral bacteria

Under certain conditions microorganisms of oral origin ectopically colonize distant body sites. This phenomenon has been associated with negative health outcomes, mainly noncommunicable diseases (previously reviewed in Lu et al. 2019, Peng et al. 2022). In this chapter, we aim to give a short overview of the latest research on these diseases (Fig. 1). We will discuss the specific molecular mechanisms associated with this ectopic overgrowth in the next sections of this review.

Ectopic colonization and intestinal diseases

In the past, there have been several studies that have shown an association between ectopic colonization by oral bacteria in the intestinal tract and different intestinal diseases. The most studied phenomenons include IBDs (Atarashi et al. 2017, Dinakaran et al. 2019, Hu et al. 2021), colorectal cancer (CRC; Castellarin et al. 2012, Flemer et al. 2017, 2018, Osman et al. 2021, Conde-Pérez et al. 2023) and childhood undernutrition (Vonaesch et al. 2018, 2022, Chen et al. 2020, Collard et al. 2022, Donowitz et al. 2022). In a hallmark study, Atarashi et al. (2017) analyzed the fecal microbiota of patients with CD, ulcerative colitis (UC), primary sclerosing cholangitis (a chronic liver inflammatory disease), gastroesophageal reflux disease, and alcoholism using amplicon sequencing and compared them with fecal samples of healthy individuals. They found that several genera typically belonging to members of the oral microbiota, including *Rothia*, *Streptococcus*, *Neisseria*, *Prevotella*, and *Gemella*, were significantly more abundant in the samples from diseased patients (Atarashi et al. 2017). Interestingly, the same authors also found that some species that make up only a minor portion of the oral microbiota can proliferate and get establish in the intestinal tract. This was proven by administering saliva samples from healthy individuals and patients suffering from CD and UC into germ-free (GF) mice. *Klebsiella pneumoniae*, which was present in low abundance in saliva samples of patients suffering of CD, grew to high absolute abundance and was recovered alive in the fecal samples collected from the mice that where transplanted with saliva from the CD patients. Similarly, *Klebsiella aeromobilis*, present in low absolute abundance in the saliva from UC donors was recovered on agar plates from the fecal samples of the transplanted mice, indicating successful ectopic colonization (Atarashi et al. 2017). A further study using amplicon sequencing showed increased relative abundance of species of the oral genera *Streptococcus*, *Staphylococcus*, *Peptostreptococcus*, *Prevotella*, *Veillonella*, and *Fusobacterium* in the feces of individuals with CD and UC compared to healthy controls (Dinakaran et al. 2019). In a more recent study using shotgun metagenomic sequencing, Hu et al. (2021) found ectopic intestinal colonization by oral bacteria in an Asian cohort of patients suffering of CD (Hu et al. 2021). In this study, *S. salivarius* was one of the most prevalent species both in the oral and intestinal microbiome. Of note, the oral and fecal strains were clustering

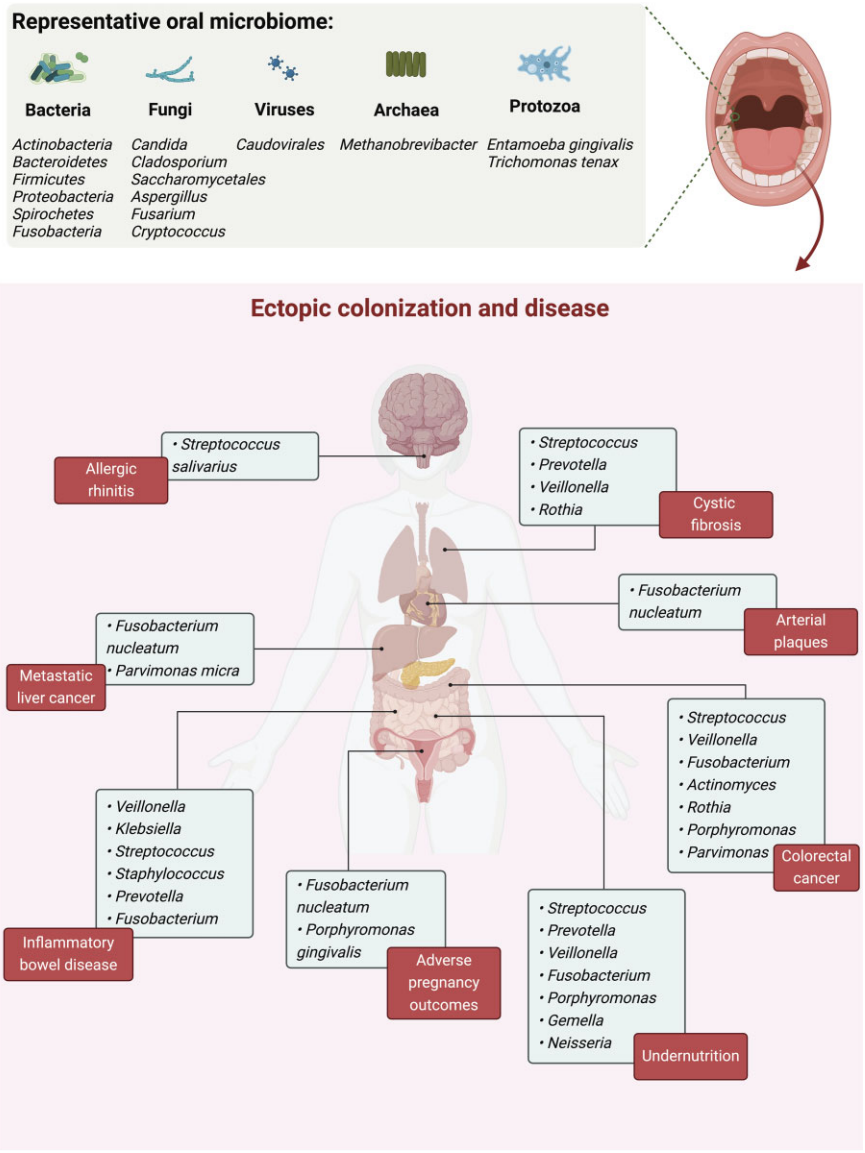

Figure 1. Ectopic colonization of oral microbiota in different body sites and associated diseases.

together in CD patients based on multiple sequence alignment of specific marker genes of *S. salivarius*. This phenotype was especially pronounced in patients suffering of active disease, yet not observed in healthy patients (Hu et al. 2021).

Another intestinal disease with a well-established link with ectopic colonization by oral bacteria is CRC. In the last decade, several studies have reported a higher relative abundance of oral bacteria in intestinal samples of cancer patients, including in whole feces, mucosa samples as well as colonic tumor tissue compared to healthy controls (Castellarin et al. 2012, Flemer et al. 2017, 2018). These studies reported members of the genera *Fusobacterium*, *Actinomyces*, *Rothia*, *Veillonella*, and *Streptococcus*, as potentially colonizers of oral origin by metagenomic sequencing and qPCR confirmation (Castellarin et al. 2012) and 16S rRNA sequencing (Flemer et al. 2017, 2018). Of note, Castellarin et al. (2012) were also able to recover *F. nucleatum* isolates from colonic tumor sections. Another study reveals that at the species level, the periodontal pathogens *F. nucleatum*, *P. gingivalis*, and *Parvimonas micra* are overrepresented in CRC tumor tissues, assessed by 16S rRNA amplicon sequencing (Osman et al. 2021). In recent years, numerous studies have found an increased presence of *F. nucleatum* in both colorectal tissues and stools of individuals with CRC or precancerous conditions (Flanagan et al. 2014, Mima et al. 2016, Osman et al. 2021; and reviewed by Alon-Maimon et al. 2022). The presence of *F. nucleatum* in the intestine appears to be linked to periodontal sites, which may provide a plausible explanation for the observed association between periodontal disease and CRC (Flemer et al. 2018, Xuan et al. 2021). Moreover, *F. nucleatum* and *F. necrophorum* have also been isolated and detected by qPCR in liver metastatic tissues (Bullman et al. 2017). Similarly, *P. micra* is typically found as a commensal with low absolute abundance within the subgingival cavity, respiratory system, and GI tract. However, under certain conditions, it can exhibit opportunistic pathogenic behavior, particularly in the context of periodontal disease (Conde-Pérez et al. 2023). Recent reports based on 16S rRNA gene amplicon sequencing show that this bacterium is present in higher relative abundance in fecal samples from CRC patients and can be isolated from colonic carcinoma tissue (Osman et al. 2021, Zhao et al. 2022, Bergsten et al. 2022). Moreover, *P. micra* was also detected by 16S rRNA

gene amplicon sequencing in liver tissue in a patient with liver metastasis, although in this case the authors were unable to isolate the bacterium (Conde-Pérez et al. 2023). These observations suggest a causal link between invasion of distant places by *P. micra* and CRC. However, the causal relationship as well as the molecular mechanism associated with this phenomenon remain to be elucidated. Regarding the existence of a tumor microbiome or the infiltration of bacteria within tumors, we acknowledge it remains a focal point of discussion within the microbiome research community. In fact, recent research has raised doubts on the presence of bacteria in tumor tissues (Braitwieser et al. 2019, Manni and Zdobnov 2020). These studies have identified human DNA sequences within multiple microbial assemblies, some of which were subsequently annotated as protein-coding sequences, resulting in the creation of erroneous bacterial proteins that have spread throughout public databases. Thus, the controversy surrounding this issue stems from challenges in distinguishing between bacterial and host DNA, particularly in instances where genomic reads mistakenly match bacteria instead of host DNA. This highlights the potential limitations of metagenomics-based microbiome quantification in tumor tissues, which should be confirmed by other approaches (i.e. qPCR or 16S rRNA amplicon sequencing), and reinforces the need of increasing efforts toward isolating live bacteria to corroborate ectopic colonization. Despite this controversy, most of the studies mentioned in this review pointed out ectopic colonization by oral bacteria in CRC through a combination of metagenomic analysis confirmed by qPCR (Castellarin et al. 2012), 16S rRNA amplicon sequencing (Flemer et al. 2017, 2018, Osman et al. 2021), and direct isolation of the ectopic colonizer strain (Bullman et al. 2017, Conde-Pérez et al. 2023). Thus, we consider they have provided solid evidence for ectopic colonization by oral bacteria in tumor tissue.

Stunting is a syndrome characterized by a low height-for-age, resulting from chronic undernutrition. Two studies of the small intestinal content of stunted children using 16S rRNA gene amplicon sequencing revealed an overgrowth by oral bacteria in two different countries in Africa (Afribiota project) (Vonaesch et al. 2018, 2022, Collard et al. 2022) as well as in Bangladesh (Chen et al. 2020, Donowitz et al. 2022). In all three countries, an increased absolute abundance of members of the genera *Prevotella*, *Streptococcus*, *Prophyromonas*, *Neisseria*, *Fusobacterium*, *Veillonella*, and *Gemella* was observed. Furthermore, in the Afribiota project, there was confirmation of small intestinal bacterial overgrowth by culture methods, showing that these bacteria are viable (Vonaesch et al. 2018, 2022, Collard et al. 2022), while the study team in Bangladesh could show that absolute abundance of these strains is correlated with stunting severity (Chen et al. 2020). Finally, an association between this oral bacterial overgrowth and small intestinal inflammation has been described in the two African sites (Vonaesch et al. 2022). Together, these studies suggest that ectopic colonization by oral bacteria is strongly associated with inflammation in the small intestine.

Ectopic colonization and lung diseases

The oral cavity is the major gateway not only to the intestinal tract but also to the lungs. Several studies have reported the presence of oral species in the respiratory system, especially in the context of pulmonary diseases. Studies from cystic fibrosis (CF) patients showed that oral bacterial species are overrepresented in respiratory secretions and distal lung areas of diseased compared to healthy subjects (Tunney et al. 2008, Brown et al. 2014). A more recent study assessed for associations between early life ectopic colonization of the respiratory system and disease (Muhlebach et al. 2018). The authors characterized the microbiome of the lower airways of CF toddlers and found that the microbiome is dominated by oral bacteria as exemplified by a higher relative abundance of *Rothia*, *Prevotella*, *Veillonella*, and *Streptococcus* (Muhlebach et al. 2018). In children aged 3–5 years, there seems to be a transition to a pathogen dominated phenotype characterized by the presence of *Staphylococcus*, *Haemophilus*, *Pseudomonas*, and *Moraxella*, which increases inflammation and worsens the disease overall (Muhlebach et al. 2018). Based on these results, the hypothesis arises that early ectopic colonization by oral bacteria of lower airways can facilitate respiratory infections later in life. There is clearly more research needed to elucidate the exact links between ectopic colonization by oral bacteria, colonization resistance toward lung pathogens and lung disease.

Ectopic colonization and adverse pregnancy outcomes

Probably less expected, but well documented, is the ectopic colonization of the maternal placenta by oral bacteria, which is associated with negative pregnancy outcomes, including preterm birth and stillbirth (reviewed by Van der Haar et al. 2018). One of the main bacteria put in relation with stillbirth is the oral pathobiont *F. nucleatum*. This species has been isolated from intra-amniotic samples and has been detected by 16S rRNA gene amplicon sequencing in placental and fetal tissues from women with preterm birth (Chaim and Mazor 1992, Cahill et al. 2005, Doyle et al. 2014; and reviewed by Van der Haar et al. 2018). Similarly, in a stillbirth case, the same strain of *F. nucleatum* has been detected in the maternal subgingival plaque, the placenta, and the stillborn respiratory and GI tract tissues (Han et al. 2010). Further, *P. gingivalis*, another oral pathobiont, has been found in uterus-related samples in adverse pregnancy outcomes including in amniotic fluid and placenta of mothers experiencing preterm birth and in placenta of women with preeclampsia (Barak et al. 2007, León et al. 2007, Kotz et al. 2009).

The placenta is not the only body site ectopically colonized by oral bacteria in the context of preterm birth. Recent studies point to a positive correlation between preterm birth and ectopic colonization of the intestinal tract by members of the oral genera *Porphyromonas*, *Streptococcus*, and *Fusobacterium* (Yin et al. 2021). Indeed, the relative abundance of common oral bacteria was significantly higher in fecal samples from mothers delivering preterm compared to mothers that delivered on term (Yin et al. 2021). Therefore, ectopic colonization by oral bacteria seems to influence pregnancy outcomes. The molecular mechanisms underlaying this phenomenon remain however largely unknown.

Ectopic colonization and autoimmune diseases

There is less literature investigating the role of ectopic colonization by oral bacteria in autoimmune diseases. Nevertheless, a study on systemic lupus erythematosus, a chronic inflammatory autoimmune condition, reports higher relative abundances of oral strains from the species *Actinomyces massiliensis*, *Shutleworthia satelles*, and *Atopobium rimae* in fecal samples from patients compared to healthy controls (Chen et al. 2021).

Recent research has also associated periodontal disease caused by the oral pathogen *Porphyromonas gingivalis* with an increased risk of rheumatoid arthritis (RA) (Scher et al. 2012, Mikuls et al. 2014; and reviewed by Maeda and Takeda 2019). Periodontitis is associated with higher levels of anticyclic citrullinated peptide (anti-CCP) antibodies in RA patients and is closely tied to RA

disease activity. *P. gingivalis* expresses gingipain, an enzyme, i.e. correlated with anti-CCP antibody production in RA patients. However, whether ectopic colonization by *P. gingivalis* is causally linked with RA remains unclear. Currently the most accepted hypothesis is that *P. gingivalis* causing chronic periodontitis increases sustained systemic inflammation that exacerbates autoimmunity in RA.

Allergic rhinitis (AR) is a chronic inflammatory autoimmune disease that causes sneezing, itching, and nasal obstruction. A recent study has found that the nasal microbiome of patients suffering from AR shows a distinct composition compared to healthy individuals. The AR nasal microbiome shows increased relative and absolute abundance of the oral bacterium *S. salivarius*, although its oral origin has not been confirmed (Miao et al. 2023). In the same study, the authors investigate whether *S. salivarius* is implicated in the pathophysiology of AR. Their findings are discussed in chapter 5 of this review.

Ectopic colonization and neurological diseases

Although a positive correlation between oral dysbiosis and neurogenerative diseases including Alzheimer and Parkinson's disease has been established by many authors (reviewed by Nicholson and Landry 2022), there is little evidence of ectopic colonization linked to neurological diseases. We found a single review that postulates that periodontal pathogens can translocate from the oral cavity to the bloodstream and travel to the brain where they might eventually colonize (Olsen 2008). Experimental evidence of such a phenomenon has, however, not been reported so far.

Ectopic colonization and cardiovascular diseases

As in the case of neurological diseases, there is still little evidence to support a role for ectopic colonization by oral bacteria in cardiovascular diseases. Very recently, Hodel et al. (2023) pointed out a positive correlation between the detection of antibodies against the oral pathobiont *F. nucleatum* and a higher risk of suffering from coronary heart disease. A previous study demonstrated that *F. nucleatum* can migrate from the oral cavity to arterial plaques (Figuero et al. 2011). This is an important finding since *F. nucleatum's* ability to ectopically colonize has been previously associated with inflammation and disease. This suggests that ectopic colonization by *F. nucleatum* might indeed directly contribute to inflammation and cardiovascular disease, yet further research is needed to elucidate a possible association and investigate the underlying mechanisms.

In conclusion, the phenomenon of ectopic colonization by oral bacteria and associated (inflammatory) disease has emerged as an important area of research with far-reaching implications for human health. This chapter provided a comprehensive overview of the diverse diseases and conditions associated with ectopic colonization by oral bacteria. Although much remains to be explored, a growing body of research underscores the need for a deeper understanding of the mechanisms underlying ectopic colonization and its implications for human health.

Ectopically colonizing strains: insights into their origin and adaptation

Several controversial questions remain open in the field of ectopic colonization by oral bacteria. As an example, the transmission dynamics of ectopically colonizing oral species and the relationship between strains in distant body sites and oral strains remain unclear. It is uncertain if ectopically colonizing oral species are consistently transmitted from the oral cavity. Alternatively, strains in distant body sites may be closely related to oral strains of the same species, having adapted and diverged as longer-term residents in new niches. Further, if there is indeed such a "constant seeding," we ignore if this seeding is a rare event or a more frequently encountered process. In this chapter, we will summarize the main findings regarding these questions and highlight knowledge gaps regarding the colonization process.

For years, the exact origin and nature of ectopically colonizing oral strains has been an open question in the field. Indeed, most of the studies assessing for ectopic colonization by oral bacteria are based on 16S rRNA gene amplicon sequencing data (Muhlebach et al. 2018, Dinakaran et al. 2019, Chen et al. 2020, 2021, Yin et al. 2021, Hodel et al. 2023), thus not allowing for strain-level inference and comparisons. In recent years, several studies have started to tackle this question by analyzing in parallel the microbiome in the saliva as well as from ectopically colonized sites. To infer strains and perform strain-level comparisons, the authors applied either shotgun metagenomic sequencing, genomic comparisons of isolated strains, or arbitrarily primed PCR (AP-PCR). These studies have confirmed that ectopically colonizing strains are indeed closely related to oral strains in patients with CRC and preterm birth (Han et al. 2010, Atarashi et al. 2017, Komiya et al. 2019, Schmidt et al. 2019, Chen et al. 2021, Hu et al. 2021).

Recently, Schmidt et al. (2019) hypothesized that the transmission of oral strains could be more common than previously thought, since the ectopic colonization of oral microbiota has been neglected in healthy subjects. Thus, using shotgun metagenomic sequencing and reconstruction of metagenome-assembled genomes they compared 310 bacterial species originating from saliva and feces from 470 subjects, both healthy and diseased, including patients with RA, type-1 diabetes, and CRC from Fiji, China, Luxembourg, France, and Germany. They showed that 125 of these species were equally prevalent in the oral cavity and the intestine. Moreover, the authors performed single nucleotide variant profiling allowing for strain-level analysis within each species and were able to delineate two categories of species transmitted along the oral-fecal axis: frequent oral-fecal transmitters, whose transmission along the GI tract was found in most of the individuals independently of their health status, include strains of the genera *Streptococcus*, *Veillonella*, *Actinomyces*, and *Haemophilus*; and occasionally transmitted species, such as members of the genus *Prevotella* (Schmidt et al. 2019). Furthermore, they showed that the high absolute abundance of bacteria from oral origin in the fecal samples exceeds the order of magnitude that can be explained by passive transmission alone (i.e. through saliva ingestion) (Schmidt et al. 2019). They, thus speculate that there must be active colonization by oral strains in the lower intestinal tract.

The studies described focus on sequencing-based comparisons, as do most of the other studies published to date (Table 1). The first few studies to compare isolated strains focused on the comparison of strains of the oral species *F. nucleatum* in between the oral cavities and ectopically colonized sites from the same patients. A first study reported the same clone of *F. nucleatum* in the subgingival tissue and placenta of a woman after stillbirth (Han et al. 2010). Later, a study on cancer compared strains of *F. nucleatum* in the oral cavity and cancerous colonic tissue in patients with CRC (Komiya et al. 2019). The authors isolated *F. nucleatum* strains from saliva and CRC of 14 patients and characterized the strains by AP-PCR. They confirmed that strains of oral and CRC origin were identical in 42.9% of the patients. Another study, isolated *Fusobacterium* spp. from CRC tissues and liver metasta-

Table 1. Oral microbiota strains ectopically colonizing associated with disease across body sites.

Taxa/species	Disease	Technique	Body site colonized	Confirmed oral origin*	Mechanism	Publication
K. pneumoniae (strain Kp-2H7)	CD	16S[a] and isolation	Colon and cecum (mouse model)	Yes	Antibiotic resistance and inflammation	Atarashi et al. (2017)
K. aeromobilis (Ka-11E12)	UC	16S and isolation	Colon and cecum (mouse model)	Yes	Antibiotic resistance and inflammation	Atarashi et al. (2017)
Streptococcus spp. (not defined beyond genus level)	Intestinal bowel disease (mixed cohort: UC and CD)	16S	Colon	No	N/A[b]	Dinakaran et al. (2019)
	CRC	16S	Colonic mucosae and colorectal polyps	No	N/A	Castellarin et al. (2012), Flemer et al. (2017, 2018)
	Undernutrition	16S and isolation	Feces, gastric, and duodenum	No	N/A	Vonaesch et al. (2018, 2022)
	CF	16S	Bronchoalveolar lavage	No	N/A	Munchlebach et al. (2018)
	Preterm birth	16S	Feces	No	N/A	Yin et al. (2021)
S. salivarius	CD	Shotgun metagenomics	Feces	Yes	N/A	Hu et al. (2021)
	Undernutrition	16S and isolation	Feces, gastric, and duodenum	No	Inflammation and lipid malabsorption	Gough et al. (2020), Vonaesch et al. (2022)
	AR	16S and isolation	Nasal swabs	No	Inflammation	Miao et al. (2023)
Staphylococcus spp. (not defined beyond genus level)	Intestinal bowel disease (mixed cohort: UC and CD)	16S	Colon	No	N/A	Dinakaran et al. (2019)
Prevotella spp. (not defined beyond genus level)	Intestinal bowel disease (mixed cohort: UC and CD)	16S	Colon	No	N/A	Dinakaran et al. (2019)
	Undernutrition	16S and isolation	Feces, gastric, and duodenum	No	N/A	Vonaesch et al. (2018, 2022)
	CF	16S	Bronchoalveolar lavage	No	N/A	Munchlebach et al. (2018)
Veillonella spp. (not defined beyond genus level)	Intestinal bowel disease (mixed cohort: UC and CD)	16S	Colon	No	N/A	Dinakaran et al. (2019)
	CRC	16S	Colonic mucosae and colorectal polyps	No	N/A	Castellarin et al. (2012), Flemer et al. (2017, 2018)
	Undernutrition	16S and isolation	Feces, gastric, and duodenum	No	N/A	Vonaesch et al. (2018, 2022)
	CF	16S	Bronchoalveolar lavage	No	N/A	Munchlebach et al. (2018)

Table 1. Continued

Taxa/species	Disease	Technique	Body site colonized	Confirmed oral origin*	Mechanism	Publication
V. parvula	Intestinal bowel disease	16S and isolation (mouse model)	Colon and feces (mouse model)	No	Nitrite reduction cluster genes (*nar*) for nitrite respiration	Rojas-Tapias et al. (2022)
Fusobacterium spp. (not defined beyond genus level)	Intestinal bowel disease (mixed cohort: UC and CD)	16S	Colon	No	N/A	Dinakaran et al. (2019)
	CRC	16S	Colonic cancer tissue, colonic mucosae, and colorectal polyps	No	N/A	Castellarin et al. (2012), Flemer et al. (2017, 2018), Osman et al. (2021)
	Undernutrition	16S and isolation	Feces, gastric, and duodenum	No	N/A	Vonaesch et al. (2018, 2022)
	Preterm birth	16S	Feces	No	N/A	Yin et al. (2021)
F. nucleatum	CRC	16S and isolation	Cancerous colonic tissue	Yes	Fap2 adhesin	Flanagan et al. (2014), Mima et al. (2016), Osman et al. (2021), Komiya et al. (2019), Abed et al. (2016)
	Preterm birth	16S	Placenta	Yes	Fap2 adhesin	Chaim and Mazor (1992), Cahill et al. (2005), Doyle et al. (2014), Abed et al. (2016)
	Stillbirth	16S and isolation	Placenta and infant tissue	Yes	N/A	Han et al. (2010)
	Cardiovascular disease	qPCR	Arterial plaques	No	N/A	Figurero et al. (2011)
Actinomyces spp. (not defined beyond genus level)	CRC	16S	Colonic mucosae and colorectal polyps	No	N/A	Castellarin et al. (2012), Flemer et al. (2017, 2018)
A. massiliensis	*Lupus erythematosus*	Shotgun metagenomics[c]	Feces	Yes	Microbial peptides inducing inflammation	Chen et al. (2021)
Rothia spp. (not defined beyond genus level)	CRC	16S	Colonic mucosae and colorectal polyps	No	N/A	Castellarin et al. (2012), Flemer et al. (2017, 2018)
	CF	16S	Bronchoalveolar lavage	No	N/A	Munchlebach et al. (2018)
Porphyromonas spp. (not defined beyond genus level)	Undernutrition	16S	Feces, gastric, and duodenum	No	N/A	Vonaesch et al. (2018, 2022)
	Preterm birth	16S	Feces	No	N/A	Yin et al. (2021)
P. gingivalis	CRC	16S	Cancerous colonic tissue	No	N/A	Osman et al. (2021)
	Preterm birth	16S	Uterus, amniotic fluid, and placenta	No	N/A	Leon et al. (2007), Barak et al. (2007), Kotz et al. (2009)

Table 1. Continued

Taxa/species	Disease	Technique	Body site colonized	Confirmed oral origin*	Mechanism	Publication
P. micra	CRC	16S	Cancerous colonic tissue	No	N/A	Osman et al. (2021)
		16S and isolation (not from liver metastasis)	Cancerous colonic tissue and liver (metastasis)	Yes	Th17 cells infiltration, upregulation of proinflammatory pathways and inflammatory reprograming	Conde-Pérez et al. (2023), Xhao et al. (2022), Bergsten et al. (2023)
Gemmella spp. (not defined beyond genus level)	Undernutrition	16S and isolation	Feces, gastric, and duodenum	No	N/A	Vonaesch et al. (2018, 2022)
Neisseria spp. (not defined beyond genus level)	Undernutrition	16S and isolation	Feces, gastric, and duodenum	No	N/A	Vonaesch et al. (2018, 2022)
S. satelles	*Lupus erythematosus*	Shotgun metagenomics	Feces	Yes	Microbial peptides inducing inflammation	Chen et al. (2021)
A. rimae	*Lupus erythematosus*	Shotgun metagenomics	Feces	Yes	Microbial peptides inducing inflammation	Chen et al. (2021)

*Confirmed oral origin of the ectopic colonizing strains by whole genomic comparison or strain profiling of the isolates or sequences.
[a]16S: 16S rRNA gene amplicon sequencing.
[b]N/A: not assessed in this study.
[c]Shotgun metagenomics: Shotgun metagenomic sequencing.

sis from two patients (*F. nucleatum* in patient 1 and *F. necrophorum* in patient 2). By applying whole genome sequencing to the isolates, they concluded that the isolates corresponded to the same strain as they shared 99.9% nucleotide similarity and speculate that *Fusobacterium* species not only ectopically colonize the colon but can also reach distant places such as the liver, probably migrating together with metastatic cancer cells (Bullman et al. 2017). In line with this observation, very recently, a study has demonstrated that *P. micra* can also translocate from the oral cavity to CRC tissues, as the same strains were isolated from the subgingival space and CRC tissues from the same patient (Conde-Pérez et al. 2023). Applying again whole genome sequencing to the isolates, the authors revealed that the strains share 99.2% identity within each patient, a score much higher than that obtained for interindividual strain similarity (97%) (Conde-Pérez et al. 2023). Moreover, *P. micra* strains isolated from different body sites from the same patient cluster together in a pangenome clustering of *P. micra* genomes sourced from this study and from NCBI (Conde-Pérez et al. 2023). Finally, Chen et al. (2021) discovered similar findings regarding the oral species *A. massiliensis*, *S. satelles*, and *A. rimae* in fecal samples from individuals with systemic lupus erythematosus compared to healthy controls, with a high similarity between all strains based on single nucleotide polymorphisms (Chen et al. 2021). To the best of our knowledge these are the only studies that go beyond sequencing-based comparisons and report the isolation and identification of identical strains/clones in the oral cavity and diseased tissue from the same subject. While these studies are indeed pioneering, they were restricted to a low sample size with a single placenta sample in the study on still-birth (Han et al. 2010), 14 patients in the study of *F. nucleatum* in CRC (Komiya et al. 2019), and five patients with positive isolation of *P. micra* in CRC (Conde-Pérez et al. 2023). Furthermore, they did not include healthy controls. There is thus clearly more work needed to understand the process of oral ectopic colonization in different tissues at distant body sites. Approaches using bacterial isolation will be especially valuable as it will allow for clear strain comparison and experimental follow-up studies, thus helping to validate possible causal relationships between ectopic colonization and disease.

There is an ongoing debate whether the oral strains found at ectopic sites are constantly seeding in from the oral cavity (Muhlebach et al. 2018, Schmidt et al. 2019) or if they have adapted to the new niche, and are thus long-term colonizers in the new body site (Gough et al. 2020). To shed light on this question, Schmidt et al. (2019) analyzed the stability of the oral and fecal strains of oral bacteria in a group of 46 individuals for which longitudinal sequencing data was available. Their findings demonstrated that these populations remain stable over time in terms of fecal strains turnover by oral bacteria, leading the authors to conclude that oral transmission is a frequent and ongoing process, with oral strain populations consistently re-establishing themselves in the gut (Schmidt et al. 2019). In contrast, the strain-level analysis of gut-resident *Streptococci* in a children's cohort from Zimbabwe conducted by Gough et al. (2020) showed that *S. salivarius* strains present in fecal samples are different from *S. salivarius* reference genomes isolated from the oral cavity, although the exact genomes used as reference were not specified. This observation could indicate that oral *S. salivarius* strains ectopically colonizing the gut have evolved to adapt to the new environment. Interestingly, Conde-Pérez et al. (2023) made a similar observation for one of the CRC patients in their cohort where the paired *P. micra* isolates from gingival and tumor samples exhibited less similarity between each other compared to isolates from the same body site from other patients. This observation suggests that the isolates may either belong to different strains with independent origin or share a common ancestor in the oral cavity and adapted to the new niche once transmitted to the intestinal tract. Such adaptations might lead to changed interactions within the overall bacterial community and thus be important to consider when developing treatment options for syndromes linked to ectopic oral bacterial overgrowth. Another possibility is that the intestine-resident strains are not originating in the oral cavity but that they are natural members of the intestinal microbiota. Of note, Gough et al. (2020) did not use salivary and fecal samples from the same individuals in their comparison but rather used oral samples from subjects from another country. It is, thus not possible to clearly infer if strains adapted to the lower GI tract or if they just diverge from the previously published oral data due to interindividual and geographic differences in strain occurrence. Likewise, as the publication by Schmidt et al. is based on a pooled dataset (Schmidt et al. 2019), there might be artifacts induced by technical issues, i.e. linked to differences in sample extraction or sequencing runs. Therefore, the debate on the origin and evolution of ectopically colonizing oral strains remains an open question. Given the few studies published so far, there is a clear need for larger, rigorously controlled and ideally longitudinally conducted studies. These studies should include culture as well as shotgun metagenomic analysis of samples from both, the oral cavity, and the ectopically colonized sites. Such studies will allow to better understand the origin, frequency, and disease relevance of ectopic colonization by oral bacteria, the strains capable of translocating to distant sites and an eventual long-term colonization that they might engage in. Further studies are especially also warranted for extraintestinal sites, which have been largely neglected in previous research. In addition, it should be noticed that the oral and colonized sites environments exhibit variances in pH levels, oxygen concentration, nutrient availability, host immune responses, and indigenous microbial populations, which create competitive pressures. These factors collectively have the potential to significantly influence the process of ectopic colonization and shape the ability of colonizing bacteria as well as specific bacterial taxa or groups to adapt to their new niche. Therefore, future studies should take these factors into account.

Pathophysiological and molecular mechanisms associated with ectopic colonization by oral bacteria

Although we have made great progress on elucidating correlations between a variety of diseases and ectopic colonization by oral bacterial species (Fig. 1 and Table 1), our understanding of the causal mechanisms leading to the observed pathophysiology remains limited. The last years have seen an increase in studies aimed at elucidating the mechanisms leading to colonization and persistence of oral strains at distant body sites as well as the bacteria–host interactions they engage in, ultimately leading to disease. The focus has been especially on mechanisms underlying the role of ectopically colonizing oral bacteria in inflammatory diseases (i.e. IBDs, CRC, and inflammatory autoimmune diseases) (Abed et al. 2016, Atarashi et al. 2017, Chen et al. 2021, Bergsten et al. 2022, Rojas-Tapias et al. 2022, Zhao et al. 2022). Recently, pioneering studies have shown an additional implication of oral bacteria in pathophysiological changes that are not strictly associated with inflammation of the intestinal tract, including childhood undernutrition (Chen et al. 2020, Von-

aesch et al. 2022) and adverse pregnancy outcomes (Han et al. 2004, 2005, Ikegami et al. 2009, Fardini et al. 2011, Coppenhagen-Glazer et al. 2015). This chapter aims to summarize the existing knowledge of these bacteria-host interactions and highlight gaps in our understanding of the molecular mechanisms underlying the ectopic colonization by oral bacteria and the role they play in pathophysiology.

Molecular mechanisms favoring ectopic colonization by oral bacteria

To elucidate the ability and mechanisms leading to ectopic colonization of oral strains in the intestinal tract Atarashi et al. (2017) orally transplanted saliva samples from CD and UC patients to GF mice. They then used 16S rRNA gene amplicon sequencing combined with bacterial isolations to characterize the salivary bacteria able to colonize the intestinal tract of these mice (Atarashi et al. 2017). Their results confirmed that *K. pneumoniae* isolated from patients with CD (strain Kp-2H7) can colonize the intestinal tract, leading to especially high absolute abundances in the colon and cecum. They further observed that this ectopic colonization leads to significant accumulation of interferon-gamma-positive $CD4^+$ T cells (Th1 cells) in the intestinal lamina propria of the colonized colonic tissue. This proinflammatory signal was specific to the colon and not found in non-colonized sites such as the small intestine and oral tissues (Atarashi et al. 2017). The authors then performed a second colonization experiment in specific pathogen free (SPF) mice pre-treated with the antibiotics ampicillin and tylvalosin to break colonization resistance. Also here, they were able to colonize the mice, favored by the resistances of *K. pneumoniae* to these antibiotics (Atarashi et al. 2017). Similar results were found for *K. aeromobilis* isolated from saliva of UC patients in the same study (Atarashi et al. 2017).

Veillonella strains are versatile commensals inhabiting various regions of the human body, including the oral cavity, lungs (Dickson et al. 2017), and vagina (Piot et al. 1982). *Veillonella parvula* is a primary inhabitant and early colonizer of the oral cavity (Giacomini et al. 2023). Regarding potential ectopic colonization from the oral cavity to distant sites, this bacterium has been shown to colonize the intestinal tract in a mouse model of colitis. To do so, it relies on a metabolic shift from fermentation toward anaerobic nitrite respiration, thus allowing it to colonize. This might potentially also give it an advantage over the resident microbiota, although it has not been confirmed in this study (Rojas-Tapias et al. 2022). Nitrite is an abundant metabolite in inflammatory environments, such as IBDs (Hu et al. 2020). *V. parvula* uses the *nar* genes (cluster of genes implicated in nitrate reduction) for nitrite respiration and in consequence, a *V. parvula* mutant lacking *narG* showed lower colonization ability in the lower GI tract in a mouse model overexposed to this oral bacterium. Moreover, the authors also observe a higher relative abundance of *V. parvula* in fecal and colonic tissue of mice with colitis compared to wild-type mice and observe a reduced colonization by *V. parvula* in the colon of mice unable to produce nitric oxid (iNOS knock out mice) (Rojas-Tapias et al. 2022).

In a study in humans, Rashidi et al. (2023) analyzed the oral and fecal microbiota composition in a diverse group of subjects including healthy volunteers treated with an antibiotic, acute leukemia patients and stem cell transplant recipients, which both receive antibiotics for their medical follow-up. They used 16S rRNA gene amplicon sequencing and exact amplicon sequence variants to compare oral and fecal microbiota members. The authors observed an opposite phenomenon to the role of colonization resistance, as no ectopic colonization was observed in antibiotic-treated healthy patients and, even with significant antibiotic exposure, only one oral species colonized the gut in each of the latter two groups, *Actinomyces odontolyticus* in acute leukemia patients and *Streptococcus* spp. in stem cell transplant recipients. The authors, thus concluded that colonization resistance impairment may not be the primary factor allowing ectopic colonization by oral bacteria and that additional mechanisms are needed for successful ectopic colonization at distant body sites (Rashidi et al. 2023). While this is an interesting study and hypothesis, it is important to keep in mind that the study was retrospective and purely based on sequencing data. The conclusions thus need to be corroborated by additional *in vitro* and *in vivo* studies. In line with this observation, recent studies in animal models support the existence of specific additional colonization factors beyond simple colonization resistance. For *F. nucleatum*, one of the most studied oral pathobionts associated with CRC, it has been shown that the lectin-type adhesin Fap2 is an essential factor mediating bacterial adhesion to tumorigenic tissue, and thus a crucial colonization factor for this bacterium in CRC (Abed et al. 2016). If *F. nucleatum* is intravenously injected in an adenocarcinoma mouse model (mimicking the transient bacteremia during periodontal disease), it has been observed that *F. nucleatum* comes to the cancerous sites and uses Fap2 to bind to a host polysaccharide (Gal-GalNAc) overexpressed in CRC cells. As a result of this Fap2-Gal-GalNAc adhesion, the absolute abundance of *F. nucleatum* is higher in tumor tissues compared to adjacent healthy tissues (Abed et al. 2016). Interestingly, in the same study, there was no predisposition of ectopic colonization by *P. gingivalis* in the lower GI tract of CRC patients, suggesting that the molecular mechanisms used by oral bacteria to ectopically colonize distant body sites vary in between different species. Nevertheless, the same mechanism can be used by the same bacteria to colonize diverse body sites. Indeed, Fap2 has been shown to be likewise essential for *F. nucleatum* to colonize the placenta, whose cells also express Gal-GalNAc in high levels (Coppenhagen-Glazer et al. 2015). Further, there is evidence suggesting that this mechanism could be extended for *F. nucleatum* colonization of other adenocarcinomas (prostate, ovary, colon, uterus, pancreas, breast, and esophagus), since co-occurrence of high levels of Gal-GalNAc and *F. nucleatum* DNA has been found in these tissues (reviewed by Alon-Maimon et al. 2022). In fact, in murine breast cancer models, the intravascular inoculation of *F. nucleatum* expressing *fap2* resulted in the specific colonization of mammary tumors, while tumor colonization was compromised when *fap2* was inactivated (Parhi et al. 2020).

Regarding community dynamics, it remains unclear whether the ectopic colonization by oral bacteria is leading to an expansion of oral bacteria at distant sites or if a decrease in the resident microbiota is leading to a relative increase in these oral bacteria, while the absolute count remains constant (Liao et al. 2022). Liao et al. (2022) have recently provided evidence that tips the balance in favor of the second option. Indeed, displacement of autochthonous strains has been observed in several diseases, including IBDs (Metwaly et al. 2020) or childhood undernutrition (Vonaesch et al. 2018, 2022) and it is thus not clear if the disappearance of these strains and/or the overrepresentation of oral strains are the main disease-inducing entities. Chen et al. (2020) reported that an increase in absolute abundance of oral species in stunted children correlates with stunting severity. Clearly, more studies reporting absolute abundance data are needed to shed light onto community dynamics during colonization of distant sites by oral bacteria.

Mechanisms leading to inflammation at sites ectopically colonized by oral bacteria

Dysregulated immune responses and persistent inflammation are recognized as key factors in the pathogenesis of IBDs, CRC, and undernutrition (Belkaid and Hand 2014, Chen et al. 2020). It has been postulated that this inflammation is linked to ectopically colonizing oral bacteria (reviewed by Peng et al. 2022). In the study of Atarashi et al. (2017), ectopically colonizing *K. pneumoniae* strains strongly induce Th1 cells in the colon of colitis-mouse model compared to wild-type mice, leading to an upregulation of proinflammatory genes such as *cxcl9*, *tnfa*, and *ifng*, and an increase in the production of IL-18 as well as to visible cell damage in histology section. Of note, all these changes that are not observed in wild-type animals after colonization with orally derived *K. pneumoniae*. Later, Federici et al. (2022) analyzed the composition of fecal microbiome of four separate IBDs cohorts (France, USA, Israel, and Germany) comprising a total of 537 participants by shotgun metagenomic sequencing. They detected a specific group of strains of *K. pneumoniae* with a distinct antibiotic resistance and mobilome profile (Kp2 strains). Additionally, the presence of these strains in fecal samples from IBDs patients strongly correlated with the exacerbation and severity of the disease (Federici et al. 2022). The introduction of these *K. pneumoniae* strains into mice with a predisposition to colitis resulted in an increased intestinal inflammation, exemplified through induction of colonic Th1 cells, increased expression of *ifng* and *il17* and elevated fecal lipocalin levels. While the results of this study sustain the findings by Atarashi et al. (2022), it must be noted that Federici et al. (2022) did not confirm the oral origin of the inflammation-inducing *K. pneumoniae* strains in their study. Furthermore, Rojas-Tapias et al. (2022) showed a correlation between a higher relative abundance of *Veillonella* species, in particular *V. parvula* and *V. dispar*, in the feces and increased levels of fecal calprotectin in patients with UC. This suggests that the intestinal inflammation encountered in the context of IBDs facilitates ectopic colonization by *Veillonella* spp., as well as *Klebsiella* spp. (Atarashi et al. 2017). It thus seems that inflammation encountered in the context of IBDs does not only create a more permissive environment for the colonization by orally derived bacteria but that it also exacerbates intestinal inflammation thus contributing to the perpetuation of chronic inflammation. To date, it is unclear which molecular mechanisms underly this immune activation clearly demonstrating that more work is needed to understand this phenomenon in detail.

Yet another oral bacterium, *P. micra,* has been also widely associated with CRC (Zhao et al. 2022, Conde-Pérez et al. 2023). Recent mechanistic studies performed *in vivo* have demonstrated that ectopic colonization by this oral pathobiont in the colon promotes colonocytes proliferation and metastasis and leads to increased Th17 cell infiltration and upregulation of the proinflammatory cytokines Il-17, Il-22, and Il-23 (Zhao et al. 2022). Another study in mice shows that Th17 cells infiltration and upregulation of proinflammatory cytokines is likewise associated with *P. gingivalis* after its translocation from subgingival tissue to the intestinal tract (Nagao et al. 2022). In the intestine, *P. gingivalis* is internalized in Peyer's patches, facilitating Th17 cell differentiation and infiltration to peripheral immune tissues. Moreover, Th17 cells can then migrate to and accumulate in the oral cavity, inducing inflammatory cytokines (i.e. CCL20) and worsening periodontitis. Importantly, the development of periodontitis through these *P. gingivalis*-responsive Th17 cells seems to be directly influenced by the intestinal microbiome, as alterations with antibiotics lead to an ameliorated phenotype (Nagao et al. 2022). Taken together, these studies suggest that ectopic colonization by *P. micra* and *P. gingivalis* accelerates cancer progression and periodontitis, respectively, and creates a proinflammatory microenvironment that worsens disease. At the same time, this inflammation also potentially facilitates ectopic colonization by other oral bacteria, such as *F. nucleatum*, profiting from the proinflammatory environment. A recent study conducted by Bergsten et al. (2022) confirmed that *P. micra* leads to upregulation of inflammatory pathways in colonic mucosa samples from CRC patients. In addition, the authors elucidate the involvement of *P. micra* in the epigenetic reprogramming of human primary intestinal epithelial cells, altering the methylation profile of promoters for oncogenes and tumor-suppressor genes, thus showing a direct and causal role of this bacterium in the development of cancer (Bergsten et al. 2022).

A few recent studies have pointed towards an association between autoimmune diseases and ectopic colonization by oral bacteria and started to shed light on the underlying molecular mechanisms: A first study in a Chinese cohort of 49 patients with RA and 25 healthy patients observed that absolute abundance of *F. nucleatum* in the feces correlated with RA severity. This association was especially strong for known biomarkers as RA such as serum levels of the proinflammatory cytokines IL-6 and TNFα, C-reactive protein as well as changes to the erythrocytes sedimentation state (a blood test to indirectly assess for systemic inflammation) (Hong et al. 2023). On an experimental level, oral administration of *F. nucleatum* to a mouse model of collagen-induced arthritis resulted in increased arthritis severity, characterized by higher levels of proinflammatory cytokines in the serum, higher bone erosion and infiltration of macrophages and neutrophils in the joints. Moreover, this phenotype was reversible upon antibiotic depletion of *F. nucleatum* from the intestinal tract of these mice (Hong et al. 2023). At molecular level, the induction in inflammation seems to be due to *F. nucleatum*-secreted outer membrane vesicles containing the adhesin protein FadA, an essential factor for *F. nucleatum* dissemination to distant sites. FadA interacts with E-cadherin, and thus enables bacterial adhesion and invasion of E-cadherin-positive cells in carcinogenic tissues (Rubinstein et al. 2013). In addition, FadA is involved in inflammation induction as it directly interacts with the host protein Rab5a, a transcriptional regulator, to stimulate transcription of proinflammatory genes, and ultimately induce inflammation (Hong et al. 2023). Of note, FadA and Rab5a are both detected in joint fluids and synovium tissues of RA patients. For ethical reasons, it was, however, not possible to compare the levels of these two proteins in the joint fluids of RA patients and healthy controls. Also in China, another study demonstrated an increase in relative abundance of the oral species *A. massiliensis*, *S. satelles*, and *A. rimae* in fecal samples of individuals suffering from systemic *lupus erythematosus* (117 patients) compared to healthy controls (115 subjects), pinpointing a role of secreted microbial peptides in inducing inflammation (Chen et al. 2021). Given the difficulties to isolate these bacterial species, the authors could not experimentally test their findings. They thus used an indirect approach where they synthesized autoantigens based on the genomic sequences (MAGs) obtained from the microbiome of the patients' samples. Coculturing the microbial peptides with peripheral blood mononuclear cells they confirmed the ability of these peptides to trigger secretion of IL-17A and IFN (Chen et al. 2021). Another study assessed for a role of the oral commensal bacterium *S. salivarius* in AR. In this study, the authors isolated *S. salivarius* from nasal swabs of patients suffering from AR (Miao et al. 2023). Further *in vitro* and *in vivo* studies demonstrated that the isolated *S. salivarius* adhere to the nasal epithelium more efficiently under AR con-

ditions compared to healthy epithelial cells. This adhesion takes place through Mucin5AC, a mucin protein overexpressed in epithelial cells during AR (Evans et al. 2015). Once adhered to the nasal epithelium, the *S. salivarius* isolates increased the expression of genes encoding inflammatory cytokines (*il1β*, *il6*, *tnfa*, *il25*, and *il5*), thus contributing to the development and worsening the severity of AR (Miao et al. 2023). Taken together, these studies provide a promising foundation for exploring the mechanisms behind the association of ectopic colonization by oral bacteria and non-intestinal inflammatory diseases. This research is, however, at the beginning and there are clearly further research efforts needed to truly understand the molecular mechanisms underlying this phenomenon.

SIBO of oral origin (SIOBO), is a different entity compared to SIBO (reviewed in Yersin and Vonaesch 2024) and has been well-documented in the context of childhood undernutrition (Vonaesch et al. 2018, 2022, Chen et al. 2020, Collard et al. 2022, Donowitz et al. 2022). Several recent studies have shown that there is a clear link between SIOBO in stunted children and induction of different proinflammatory cytokines including IL-6, MCP1, IL-1β, and IL-12 (Chen et al. 2020, Vonaesch et al. 2022). Chen et al. (2020) administered a bacterial community consisting of 14 oropharyngeal strains isolated from the duodenum of stunted children into undernourished mice and compared the inflammatory profile and small intestinal histology to mice colonized with cecal content from a SPF mouse (control condition). They observed that the 14-member consortium led to an inflammatory infiltration of lymphocytes in the small intestinal lamina propria (Chen et al. 2020). This infiltration was associated with disruption of the small intestinal epithelial barrier suggesting a causal relationship between SIOBO and the pathophysiology observed in stunted children. The authors, however, did not manage to explain the molecular mechanism leading to the observed inflammatory changes. In a different study, Vonaesch et al. (2022) assessed for the effect of several oral strains isolated from the duodenum of stunted children on polarized epithelial cells. They observed a clear increase in permeability induced by several of the oral strains. Focusing on inflammation, they assessed then for the proinflammatory potential of a *S. salivarius* strain isolated from duodenal samples and assessed for inflammation *in vitro* using coculture with polarized murine small intestinal cells and *in vivo* by orally administering *S. salivarius* to antibiotic pretreated SPF mice. As the authors failed to observe inflammation in these systems, they concluded that either a full bacterial community, rather than a single strain must be responsible for the inflammation observed *in vivo* or that the bacterial load needs to be higher (Vonaesch et al. 2022).

Implication of ectopic oral bacteria in nutrient absorption in childhood undernutrition

Beside intestinal inflammation, there is recent evidence of the role of oral bacteria in nutrient malabsorption (Vonaesch et al. 2022). In this study, Vonaesch et al. (2022) cocultured polarized murine small intestinal cells with a collection of bacterial species isolated from the duodenum of undernourished African children. The bacteria were chosen to represent a broad taxonomic selection, including *Streptococcus parasanguinis*, *S. salivarius*, *S. oralis*, *Moraxella catarrhalis*, *Neisseria cinerea*, and *Haemophilus influenzae*. They could show that coculture led to a decrease in lipid absorption by all oral strains and that this decrease in lipid absorption seems to be mediated by a low-molecular weight product. Last, they could also confirm the decreased lipid absorption in the jejunum and liver in a mouse model of overexposure to *S. salivarius* (Vonaesch et al. 2022). Of note, similar changes in lipid absorption are also observed in human small intestinal biopsies of undernourished children from Pakistan, although a correlation with microbiota composition and ectopic colonization by oral bacteria was not assessed in this case (Haberman et al. 2021). While this study shows an exciting link between lipid absorption and ectopic oral bacterial colonization, the exact effector and the molecular mechanism remain unknown (Vonaesch et al. 2022). More work is thus needed to elucidate the exact molecular mechanism underlying this phenomenon and to unravel its specific role in the pathophysiology of childhood undernutrition.

Implication of ectopic oral bacteria in adverse pregnancy outcomes

Another disease associated with ectopic colonization by oral bacteria and only involving minimal inflammation is adverse pregnancy outcome. Indeed, adverse pregnancy outcomes is one of the first pathologies associated with ectopically colonizing oral strains. Han et al. (2004) were pioneers in delving into the causal relationship between *F. nucleatum*, one of the oral pathobionts responsible of periodontitis, and preterm birth. Using intravenous infection as a model of transient bacteremia caused by periodontitis, they administered *F. nucleatum* strains isolated from both oral cavities and amniotic fluids of preterm births in pregnant mice. In these mice, all tested *F. nucleatum* strains were initially detected in the blood vessels of the placenta and later invaded the endothelial cells lining these vessels. Subsequently, the bacteria crossed the endothelium, multiplied in nearby tissues, and eventually reached the amniotic fluid. Importantly, the bacterial infection remained confined to the uterus-related tissues, where it correlated with neutrophilic inflammatory response. Moreover, the infection led to an increase in premature delivery, stillbirths, and occasionally nonsustained live births compared to noninfected mice (Han et al. 2004). Of note, vaginal isolates of a different *Fusobacterium* species, *F. gonidiaformans*, were unable of adhering and invading endothelial and epithelial cells (Han et al. 2004). This observation is explained through later research reporting the existence of Fap2 and FadA adhesins in *F. nucleatum* strains (Han et al. 2005, Ikegami et al. 2009, Fardini et al. 2011, Coppenhagen-Glazer et al. 2015). Similarly, translocation of *P. gingivalis* to the placenta of pregnant mice also leads to an increase in proinflammatory cytokines IFNγ, and IL-2, but in this case the outcome results in fetal growth restriction instead of preterm or stillbirth (Lin et al. 2003). Of note, *P. gingivalis* studies in pregnant mice used subcutaneous injection as infection route, which does not replicate the natural infection routes. Later *in vivo* studies administering saliva and subgingival samples intravenously to pregnant mice have provided further evidence of other oral microbiota species that can translocate to the placenta (Fardini et al. 2010). Beside *F. nucleatum*, these include *S. mitis*, *S. salivarius*, *S. parasanguinis*, *Neisseria flavescens*, *V. parvula*, and *V. atypica*. Furthermore, some of the translocated species such as *Aggregibacter segnis* and *Peptostreptococcus stomatis* seem to be enriched in their relative abundance in placental samples compared to the original saliva samples used for colonization, indicating an expansion of oral bacteria at the colonized sites (Fardini et al. 2010). In conclusion, there is thus a clear association between ectopic oral colonization and adverse pregnancy outcomes. Nevertheless, except for *F. nucleatum*, the mechanisms of translocation, colonization, and microbe–host interactions leading to adverse pregnancy outcomes remain unclear. Furthermore, it is also not clear if single bacteria, or rather polymicrobial infections are

leading to negative birth outcomes. A study on *F. nucleatum* suggested that *F. nucleatum* binds to endothelial cells though the adhesin FadA, increasing endothelial permeability and thus allowing infiltration of other bacteria, such as *Escherichia coli* (Fardini et al. 2011). Weather this is true for other oral commensal species, if it contributes to ectopic colonization of oral bacteria at distant sites and if this contributes to the disease outcome needs further research.

In conclusion, the existing literature underscores a clear association between ectopic colonization by oral bacteria and a worsened disease outcome, yet also showcases the intricate nature of the colonization process, which exhibits significant variation between bacterial species and specific diseases. It is thus essential to assess for disease-specific colonization to gain deeper insights into the unique mechanisms associated with this complex phenomenon. While the initial research, especially regarding mechanisms, has mostly concentrated on intestinal inflammatory diseases, there is increased evidence that this ectopic colonization also contributes to certain noninflammatory conditions. More research is needed on the exact molecular mechanisms underlying these phenomena as these endeavors hold the promise of uncovering new insights and therapeutic avenues for a diverse range of health conditions.

Concluding remarks

The complexity and close relationships of microbiomes with their human host, health, and disease has fascinated the scientific community since several decades. In this review, we have summarized the most recent literature on the molecular mechanism leading to ectopic colonization by members of the oral microbiome, the way they interact with their human host and the profound impact this ectopic colonization has on human health and disease.

Despite the significant research progress in this field in the last years, we are only beginning to scratch the surface in understanding the causal links between ectopic colonization by oral bacteria and pathophysiology. Thus, many knowledge gaps remain. First and foremost, while there was substantial progress on understanding the role of ectopically colonizing bacteria on intestinal inflammatory diseases, we know much less about its role in other pathophysiological disturbances such as nutrient absorption, cell proliferation, or other mechanisms, which are yet to be determined. Second, while we know already quite well how certain oral species such as *F. nucleatum*, *P. gingivalis*, or *Veillonella* species colonize the host at distant body sites, much less is known about other members of the oral microbiota. Indeed, the recent literature clearly shows that there are common themes in the way that different oral bacteria colonize at distant sites and predispose/contribute to disease. However, there is also clear evidence that the exact molecular mechanisms are specific to each bacterial species or even strain. We thus, need further studies assessing also for the ectopic colonization of other oral bacteria, as well as fungal or viral species, which have been neglected in research studies so far. Moreover, the existing literature tends to focus on oral and intestinal microbiome research, which has been historically favored for their accessibility, potentially introducing bias in the microbial strains characterized to date. This focus has led to the identification of numerous bacteria as predominantly oral species. In turn, it has overshadowed the potential versatile nature of some bacterial species that may also predominate in less investigated body sites. Recognizing this, we emphasize the need for a more comprehensive exploration of less-investigated environments to avoid perpetuating misconceptions about strain-specific habitat preferences, highlighting the potential versatile nature of certain bacterial strains that can extend beyond their presumed habitat in the oral environment. Finally, the study of polymicrobial communities will be of major importance, as it has been previously observed that ectopic colonization often involves multiple bacterial species acting in concert (Castellarin et al. 2012, Flemer et al. 2017, 2018, Muhlebach et al. 2018, Vonaesch et al. 2018, 2022, Dinakaran et al. 2019, Collard et al. 2022). Understanding the causal links and outcomes of such complex interactions is crucial for unravelling the full spectrum of consequences and mechanisms associated with ectopic colonization, providing a more comprehensive insight into the complex world of microbial communities within the human body.

To be able to prevent and treat such diseases in the future, it will be important to delineate possible host- or microbe-associated features that facilitate or prevent colonization at ectopic body sites, as this will be prime targets to develop targeted intervention strategies. In this context, there is a major open question in the field, namely, if these ectopically colonizing strains are indeed constantly seeding from the oral cavity or if they were translocated once and then adapted and multiplied at the distant body site. To answer this question, we will need experimental approaches in model systems such as mice. Although it is crucial to experimentally investigate and validate ectopic colonization in mouse models, it has often been demonstrated that there are notable disparities in the microbiome composition between various body sites in humans compared to mice, especially in bacterial colonization patterns throughout the GI tract, microbial compositions shifts, and the host effect on host–microbe interactions (Nguyen et al. 2015, Hugenholtz and de Vos 2018). Therefore, mouse experiments are not sufficient and should be combined with longitudinal human studies across diverse geographic and demographic populations. Another open discussion regarding ectopic colonization by oral bacteria is whether the translocated strains are dead or alive. To properly address this question, we highlight the importance to increase efforts on bacterial isolation rather than focusing solely on genomic studies. Moreover, increasing the number of isolates for oral ectopic colonizers will also facilitate performing mechanistic studies, and thus contributing to a better understanding of the causality and pathophysiology of ectopic colonization by oral bacteria. This information will be essential to finding the best preventive methods to restrict ectopic colonization by such bacteria in the future.

In conclusion, our exploration of ectopic colonization by oral bacteria has unveiled a complex and intricate landscape with far-reaching implications for human health. It is evident that these microorganisms have the potential to influence a wide array of health conditions, and a deeper understanding of the underlying mechanisms is essential. We hope this review serves as a valuable resource for researchers, healthcare professionals, and anyone interested in the evolving landscape of oral microbiota and its impact on human health. By focusing on reviewing the newly discovered molecular mechanisms and the shared pathophysiological factors across different body sites and syndromes, we have strived to offer valuable insights into this emerging field as they hold the key to diagnosing, treating, and preventing interventions linked to ectopic colonization by oral bacteria, ultimately enhancing the well-being of individuals around the world.

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