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Transmission of Diverse Oral Bacteria to Murine Placenta: Evidence for the Oral Microbiome as a Potential Source of Intrauterine Infection▽†

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Microbial infection of the intrauterine environment is a major cause of preterm birth. The current paradigm indicates that intrauterine infections predominantly originate from the vaginal tract, with the organisms ascending into the sterile uterus. With the improvements in technology, an increasing number of bacterial species have been identified in intrauterine infections that do not belong to the vaginal microflora. We have demonstrated previously that intrauterine infections can originate from the oral cavity following hematogenous transmission. In this study, we begin to systemically examine what proportion of the oral microbiome can translocate to the placenta. Pooled saliva and pooled subgingival plaque samples were injected into pregnant mice through tail veins to mimic bacteremia, which occurs frequently during periodontal infections. The microbial species colonizing the murine placenta were detected using 16S rRNA gene-based PCR and clone analysis. A diverse group of bacterial species were identified, many of which have been associated with adverse pregnancy outcomes in humans although their sources of infection were not determined. Interestingly, the majority of these species were oral commensal organisms. This may be due to a dose effect but may also indicate a unique role of commensal species in intrauterine infection. In addition, a number of species were selectively "enriched" during the translocation, with a higher prevalence in the placenta than in the pooled saliva or subgingival plaque samples. These observations indicate that the placental translocation was species specific. This study provides the first insight into the diversity of oral bacteria associated with intrauterine infection.

Preterm birth (PTB) is a significant public health concern, affecting 12.7% of births in the United States. This rate reflects a 36% increase in the past 25 years. Among these, the very preterm births, i.e., those occurring before 32 weeks of gestation, are the most costly and medically relevant group, accounting for approximately 2% of all deliveries. Intrauterine infection has been recognized as a major cause of PTB. The infection rate is inversely related to the gestational age and is highly prevalent among the very preterm deliveries (24). Intrauterine infection has also been implicated in other adverse pregnancy outcomes, such as late miscarriage and stillbirth (25). A wide variety of bacterial species have been identified in the infections, with *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Bacteroides* spp., *Gardnerella vaginalis*, and *Fusobacterium nucleatum* (24, 31) as the most prevalent. It has been suggested that the majority of these bacterial species are of relatively low virulence. However, once inside the uterus, they stimulate the synthesis and release of proinflammatory cytokines, neutrophil infiltration and activation, and prostaglandin and metalloprotease synthesis and release, leading to cervical ripening, membrane weakening and rupture, uterine contractions, and PTB (24).

The current paradigm indicates that the majority of intrauterine infections originate in the lower genital tract, with the infectious agents ascending into the otherwise sterile womb. The bacteria can then infect the fetal membranes (causing chorioamnionitis), umbilical cord (funisitis), placenta (placental infection), amniotic fluid (amniotic fluid infection), and the fetus (sepsis) (24). However, an increasing number of studies report intrauterine infections caused by bacterial species not found in the urogenital tract, such as *F. nucleatum* and *Bergeyella*, *Eikenella*, and *Capnocytophaga* spp., all of which are commensal species in the oral cavity (28, 30, 31). Thus, a hematogenous transmission has been proposed as an alternative route of infection. The oral cavity houses more than 700 microbial taxa (1, 43) and hence is a potential reservoir for infection. During periodontal infection, oral bacterial titers increase dramatically, accompanied by gingival inflammation and bleeding. These conditions lead to increased bacteremia, thus enhancing the opportunities for hematogenous transmission.

We have previously demonstrated that hematogenous infection by oral *F. nucleatum* resulted in specific colonization in the mouse placenta, causing localized infection and inflammation, leading to preterm and term stillbirth. The pattern of infection mimicked those observed in ascending infections in humans, i.e., with the bacteria colonizing the decidua, followed by spread to fetal membranes, amniotic fluid, and fetus (29). This was the first experimental evidence that oral bacteria can cause adverse pregnancy outcomes following hematogenous transmission.

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Transmission of oral bacteria into the pregnant uterus has been observed in humans in two cases reported by our group. In one case, using 16S rRNA gene-based culture-independent technology, we identified uncultivated oral *Bergeyella* from the amniotic fluid complicated by PTB at 24 weeks. No *Bergeyella* cells were detected in the mother's vaginal tract, but interestingly, the same clonal type was identified in her subgingival plaque based on the 16S-23S rRNA gene sequences (28). In a second case, oral *F. nucleatum* was identified as the cause of a term stillbirth and was isolated from the lung and stomach of the stillborn infant. Using similar technology, the same clonal type was identified in the mother's subgingival plaque, but not in her supragingival plaque, or vaginal or rectal microflora (27). Such accumulating evidence underscores the importance of oral bacteria in intrauterine infection and adverse pregnancy outcome.

One important question that arises from these studies concerns what proportion of the complex oral microbiome is capable of oral-uterus transmission. With the majority of the bacteria in the oral cavity uncultivated, it is very likely that the involvement of oral bacteria in intrauterine infection has been significantly underestimated, because clinical microbiology laboratories still employ routine culturing methods for bacterial detection (30). As a result, clinical evidence involving oral bacteria in PTB is incomplete, and specifically, sufficient knowledge of the diversity of oral bacterial involved in PTB is lacking. In the current proof-of-concept study, we utilize the pregnant murine model established in our laboratory to identify species of the oral microbiome that can translocate to the placenta. We report that a diverse group of oral bacteria are capable of hematogenous transmission to colonize the murine placenta. Furthermore, most of these species have been reported to be associated with adverse pregnancy outcomes in humans.

MATERIALS AND METHODS

Oral sample preparation. Our study was approved by the Case Western Reserve University Internal Review Board. Saliva samples were collected from five healthy volunteers, with each individual spitting nonstimulated saliva into a sterile tube, and pooled. Subgingival plaque was collected from deep periodontal pockets (pocket depth of >7 mm) of eight periodontitis patients, using sterile curettes, and pooled. The pooled subgingival plaque samples were centrifuged at 14,000 × *g* for 5 min, and the pellets were resuspended in 1 to 3 ml prereduced sterile phosphate-buffered saline (PBS). All samples were kept in a portable anaerobic jar containing ice and were injected into mice within an hour following collection.

Animal handling, infection, and placenta collection. The animal protocol was approved by the Case Western Reserve University Institutional Animal Care and Use Committee. Ten-week-old outbred CF-1 mice (Charles River Laboratories, Wilmington, MA) were mated at a female/male ratio of 2:1. Positive mating was indicated by the presence of a white vaginal plug, and that day was termed day 1 of gestation. The normal length of gestation of CF-1 mice is 20 to 21 days. Injections were performed on days 15 to 17 of gestation. A total of 0.1 ml of undiluted saliva or 0.1 ml of the subgingival plaque suspension was injected into the tail vein of each pregnant mouse. At 24 h postinjection, placentas were harvested. For each mouse, placentas were pooled and stored at −80°C until use.

16S rRNA gene-based PCR and construction of the clone libraries. The genomic DNA from pooled saliva, pooled subgingival plaque, and mouse placentas was extracted as previously described (28). The 16S rRNA gene was amplified from each pooled sample by PCR, using the universal primers A17F and 1512R as previously described (34). The PCR amplicons were cloned into the pCR8/GW/TOPO vector by using the TA cloning kit (Invitrogen, Carlsbad, CA) according to the supplier's instructions. Recombinant plasmids were extracted using the Wizard Plus SV Minipreps DNA purification system (Promega, Madison, WI). Plasmids harboring inserts with the expected size (~1,500 bp) were selected, and the DNA sequences of the inserts were determined by the Genomics Core Facility of Case Western Reserve University (Cleveland, OH) using primers GW1 (GTTGCAACAAATTGATGAGCAATGC) and GW2 (G TTGCAACAAATTGATGAGCAATTA) (Invitrogen, Carlsbad, CA). Sequences were assembled using the VectorNTI program (Invitrogen, Carlsbad, CA).

Analysis of 16S rRNA gene. The 16S rRNA gene sequence analysis was performed by using the Human Oral Microbiome Database (HOMD; http://www.HOMD.org). Sequences displaying an identity percentage of at least 97% were retained for further analysis. Sequences with an identity percentage of <97% were reexamined by blasting each half part of the 16S rRNA gene sequence to identify chimeras. Sequences matching only with a genus but not a species or a defined taxon were discarded.

TABLE 1. Clones analyzed for each 16S rRNA gene library

16S rRNA clone library	No. of clones sequenced	No. of clones retained for analysis
Pooled saliva	64	41
Pooled subgingival plaque	105	81
Placentas infected with saliva	59	56
Placentas infected with subgingival plaque	106	104

RESULTS

Infection of mice, and construction and analysis of 16S rRNA gene clone libraries. The pooled saliva was injected into seven pregnant mice, and the pooled subgingival plaque was injected into 10 pregnant mice, all at gestation of 15 to 17 days. At 24 h postinfection, placentas were collected from each mouse. This time point was chosen because we have shown previously that bacterial colonization in the mouse was cleared by 24 h, except in the placenta, where it continued to proliferate (29). Thus, any bacteria detected at 24 h after injection was likely due to specific colonization, rather than hematogenous diffusion into the placenta.

A 16S rRNA gene clone library was constructed for each mouse. For each of these libraries, 8 to 21 independent clones were sequenced. When combined, a total of 59 clones were sequenced from the seven saliva-infected murine libraries, and a total of 106 clones sequenced from the 10 subgingival plaque-infected libraries (Table 1). Separate libraries were constructed for the pooled saliva and pooled subgingival plaque samples. A total of 64 and 105 clones were sequenced, respectively, from these two libraries (Table 1).

The near full-length 16S rRNA gene sequences obtained from each sequenced clone were analyzed by BLAST against the HOMD. The bacterial identification was accepted if the sequence identity was ≥97%. For the clones with sequence identity of <97% ($n = 52$), the full-length 16S rRNA gene sequences were divided into halves, and each half was reanalyzed by BLAST against the HOMD to identify chimeras formed during PCR. The majority of these low-identity clones appeared to be chimeras ($n = 49$). The remaining three clones shared limited identities (94.6 to 96%) with *Leptotrichia*, *Neisseria*, and *Veillonella*, respectively. Only the sequences with ≥97% identity were retained for the analysis described below. The number of retained clones from each library is shown in Table 1.

TABLE 2. Transmission of salivary bacteria into murine placenta

Bacterium identified in saliva[a]	No. of mice with placental infection of salivary species[b]
Capnocytophaga gingivalis oral taxon 337	
***Eikenella corrodens* oral taxon 577**	
Gemella sanguinis oral taxon 757	
Granulicatella adiacens oral taxon 534	2
Leptotrichia sp. oral taxon 417	
Megasphera micronuciformis oral taxon 122	
Neisseria spp.	
N. flava oral taxon 609	
N. flavescens oral taxon 610	7
***N. subflava* oral taxon 476**	1
***Peptostreptococcus stomatis* oral taxon 112**	1
Porphyromonas spp.	
***P. endodontalis* oral taxon 273**	
Porphyromonas sp. oral taxon 284	
Prevotella sp. oral taxon 308	
Streptococcus spp.	
S. australis oral taxon 073	
S. infantis oral taxon 638	
***S. mitis* oral taxon 398**	2
***S. mitis* oral taxon 677**	3
S. oralis oral taxon 707	
S. parasanguis II oral taxon 411	1
***S. pneumoniae* oral taxon 734**	1
S. salivarius oral taxon 755	1
***Streptococcus* sp. oral taxon 058**	
Veillonella spp.	
V. atypica oral taxon 524	2
***V. dispar* oral taxon 160**	
***V. parvula* oral taxon 161**	4

[a] Species identified in the salivary clone library with sequence identities of ≥97% with those available in the HOMD, with the exception of *P. stomatis* oral taxon 112, which was present in the pooled saliva sample but not detected in the clone library. Taxa overlapping with those identified in subgingival plaque are in boldface type.

[b] A total of seven mice were injected with the pooled saliva sample.

Transmission of salivary bacteria into mouse placenta. From the salivary 16S rRNA gene clone library, a total of 25 taxa belonging to 11 different genera and four different phyla, *Firmicutes*, *Fusobacteria*, *Bacteroidetes*, and *Proteobacteria*, were identified (Table 2). The most prevalent was *Streptococcus* spp. (37%), followed by *Neisseria* spp. (29%), *Veillonella* spp. (10%), *Prevotella* spp. (5%), *Porphyromonas* sp. (5%), and *Capnocytophaga gingivalis*, *Eikenella corrodens*, *Gemella sanguinis*, *Granulicatella adiacens*, *Leptotrichia* sp., and *Megasphera micronuciformis*, with each at 2% (Fig. 1).

A different bacterial spectrum was detected in the mouse placenta (see Table S1 in the supplemental material for details). Except for one mouse, which was colonized only by *Neisseria flavescens*, all animals were infected by two or more different species in their placentas. A total of five different genera were detected (Table 2). Mice were preferentially infected by *Neisseria* spp. (in all seven mice tested), *Streptococcus* spp. (in five mice), *Veillonella* spp. (in four mice), *G. adiacens* (in two mice), and *Peptostreptococcus stomatis* (in one mouse). When each species was evaluated based on all 56 clones analyzed from placenta (Fig. 1), the order of prevalence remained unchanged, with 63% *Neisseria* spp., 18% *Streptococcus* spp., 11% *Veillonella* spp., 5% *G. adiacens*, and 4% *P. stomatis*. With the exception of *P. stomatis*, the species found in the placenta were also detected in the saliva. Using *Peptostreptococcus*-specific primers, the 16S rRNA gene of *P. stomatis* was amplified from the pooled saliva and verified by DNA sequencing (data not shown). Thus, *P. stomatis* is present in the pooled saliva but may exist in low quantities and hence was not detected by our random screening of the salivary clone library. These results suggest that *P. stomatis* was preferentially "enriched" in the mouse placenta. In addition, *Neisseria*, *G. adiacens*, and *Veillonella* were also enriched in the placenta, indicating the specificity of colonization (Fig. 1).

Transmission of subgingival bacteria into mouse placenta. The bacterial composition in the pooled subgingival plaque sample was determined in a manner similar to that for the saliva. A total of 54 taxa belonging to more than 20 different genera/species were identified (Table 3). The seven most prevalent genera/species are listed below, in decreasing order: *F. nucleatum* (17%), *Leptotrichia* spp. (12%), *Prevotella* spp. (11%), *Porphyromonas* spp. (9%), *Streptococcus* spp. (7%), *Selenomonas* spp. (7%), and *Neisseria* spp. (5%). Ten of the 54 taxa were also identified in saliva (Table 3). All together, a total of 69 different taxa were identified in the saliva and subgingival plaque.

The plaque bacteria translocated to the placenta were identified (see Table S2 in the supplemental material for details). Except for two mice which were infected only with *Neisseria subflava*, all mice had mixed infections with two or more different species. A total of 16 different genera/species were detected in the murine placenta (Table 3). Mice were preferentially infected by *Neisseria* spp. (in eight mice); *Streptococcus* spp. (in seven mice); *Aggregatibacter segnis*, *Leptotrichia* spp., and *Selenomonas* spp. (each in three mice); and *Fusobacterium nucleatum* and *Eikenella corrodens* (each in two mice). The remaining species, including *Campylobacter showae*, *Capnocytophaga* sp., *Erysipelothrix tonsillarum*, *Gemella morbillorum*, *Microbacterium* sp., *Parvimonas* sp., TM7 phylum sp., and *Veillonella* spp., were each identified in one mouse (Table 3). When each species was evaluated based on all 104 clones analyzed from the placenta, the order of prevalence remained the same, with 24% *Neisseria* spp., 14% *Aggregatibacter segnis*, 12% *Streptococcus* spp., and 12% *Gemella morbillorum*. The remaining species, *C. showae*, *Capnocytophaga* sp., *E. corrodens*, *E. tonsillarum*, *Escherichia coli*, *F. nucleatum*, *Leptotrichia* spp., *Microbacterium* sp., *Parvimonas* sp., *Selenomonas* spp., TM7 phylum sp., and *Veillonella* spp., showed prevalence of <10% (Fig. 2). With the exception of *E. tonsillarum* and *E. coli*, all species found in placenta were also detected in the dental plaque. Similar to *P. stomatis* discussed above, these two species may exist in low quantities in the subgingival plaque, undetected by random screening of the plaque clone library. Similarly, a few species were preferentially enriched in the placentas, including *A. segnis*, *E. corrodens*, *E. tonsillarum*, *E. coli*, *G. morbillorum*, *Neisseria* spp., *Streptococcus* spp., and *Veillonella* spp. (Fig. 2).

DISCUSSION

Utilizing the 16S rRNA gene-based PCR and clone analysis techniques, we provide initial insight into the diversity of oral bacteria that can translocate to the murine placenta following hematogenous infection. This approach mimics transient bacteremia, which can occur during periodontal infections and dental procedures.

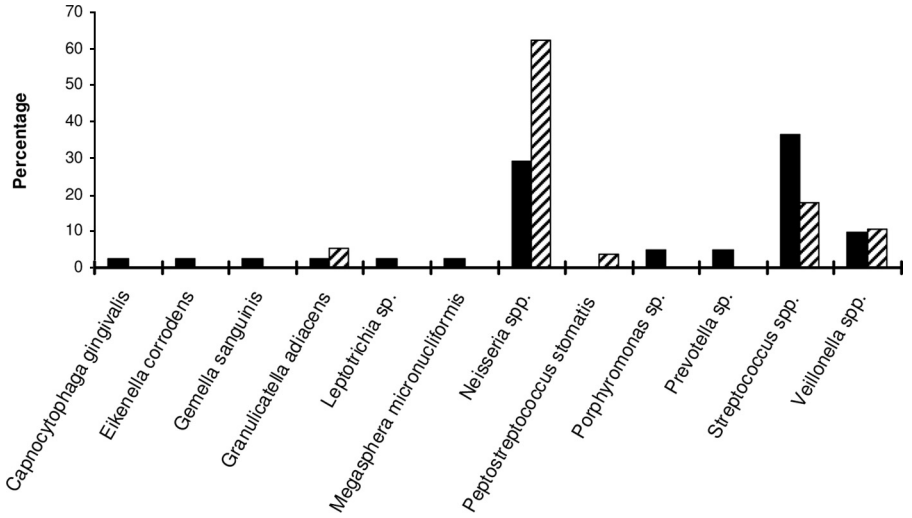

FIG. 1. Transmission of salivary bacteria to murine placentas. The relative abundance of each bacterial genus/species in pooled saliva samples (solid bars) and placentas from all seven infected mice (striped bars) were expressed as the percentage of the species out of the total number of clones analyzed for the pooled saliva samples ($n = 41$) and the murine placentas ($n = 56$), respectively.

Bacteremia caused by bacteria from both saliva and periodontal plaque has been documented, which was why both saliva and subgingival plaque samples were tested in our study (44, 46). These two samples represent two distinct yet closely related floras. The concordance between the two floras has been investigated in several studies, and the correlation was found to vary from species to species (12, 15). Our study, likewise, confirms these findings where the saliva and dental plaque microbiota share similarities and have differences. Out of 69 different taxa identified from both samples, 10 were shared by both. As a result, the species identified in the saliva-infected placenta only partially overlap with those from the plaque-infected placenta. Even for the species that do overlap, their prevalences differ. For example, *Veillonella* was one of the most prevalent bacteria in the saliva while it was not in the dental plaque. Consequently, *Veillonella* was detected in five out of seven mice infected with pooled saliva but only in 1 of 10 mice infected with pooled subgingival plaque. By testing both samples, our study provides a more complete spectrum of oral species capable of transmission to placenta.

Previous studies have shown that when pregnant mice were injected with PBS, no bacteria were recovered from the placenta (29). Thus, all bacteria detected in the placenta originated from the starting material of pooled saliva or pooled subgingival plaque. This was further confirmed by sequencing of pooled saliva and pooled subgingival plaque sample libraries, which identified the same species isolated from the mouse placentas. Studies have shown that each individual harbors approximately 266 taxa in the oral microflora (55). Therefore, only a portion of the oral microbiome was identified, yet it appeared to be sufficient to confirm the origin of the placental infection in our study.

The bacterial prevalence in the placenta and that in the starting materials were compared. A few species that existed in high prevalence in the starting samples, such as *Neisseria* and *Veillonella*, continued to be detected in the placentas with a high prevalence, suggesting a potential dose-dependent effect. On the contrary, bacteria such as *Leptotrichia* colonized the placenta with decreased prevalence compared with the starting samples, suggesting a dose-independent effect. We also note the possibility of selective enrichment of several species, such as *A. segnis* and *P. stomatis*, where they may have existed in relatively low quantities in the starting pooled samples, but were identified in the placentas with increased prevalence. These observations suggest that bacteria utilize specific translocation mechanisms rather than random "diffusion" to colonize the placenta.

The number of clones analyzed from each mouse library was determined by the quality of the library and the bacterial taxa identified. For the majority of the mice, one or few predominant species were identified after sequencing just a few clones (see Tables S1 and S2 in the supplemental material). Analysis of more clones would not significantly alter the prevalence of these species. The prevalence may differ for the species that were identified only once or twice in the library. Yet, it would not affect our conclusion. In this proof-of-concept study, the answer we seek is "yes" or "no." Even if the bacterium is identified only in the placental libraries once, it will suggest that this particular organism is capable of translocating to the placenta. Due to the intrinsic differences between humans and mice, the relative prevalence found in the mouse placenta may not be applicable to humans.

The majority of the species detected in the murine placenta have been associated with adverse pregnancy outcomes (Table 4), validating the relevance of our study. Among them, *F. nucleatum* is the best-recognized oral species. We have shown previously that *F. nucleatum* translocates hematogenously into mouse placenta when pure cultures were used (29). The current study demonstrates that such translocation also occurs in mixed species, better mimicking real-life situations. Furthermore, only *F. nucleatum* was detected in the placenta although additional fusobacteria, such as *Fusobacterium periodontium*, were present in the pooled plaque sample. This is again indicative of a species-specific translocation. This observation is

TABLE 3. Detection of subgingival plaque bacteria transmitted into murine placenta

Bacterium identified in subgingival plaque[a]	No. of mice with placental infection of plaque species[b]
Aggregatibacter segnis oral taxon 762	3
Bacteroidetes sp. oral taxon 511	
Campylobacter showae oral taxon 763	1
Capnocytophaga spp.	
C. granulosa oral taxon 325	
Capnocytophaga sp. oral taxon 326	1
***Eikenella corrodens* oral taxon 577**	1
Erysipelothrix tonsillarum oral taxon 484	1
Escherichia coli oral taxon 574	1
Fusobacterium nucleatum subspecies	
F. nucleatum subsp. *animalis* oral taxon 420	
F. nucleatum subsp. *nucleatum* oral taxon 698	1
F. nucleatum subsp. *polymorphum* oral taxon 202	1
F. nucleatum subsp. *vincentii* oral taxon 200	2
Fusobacterium periodontium oral taxon 201	
Gemella morbillorum oral taxon 046	1
Leptotrichia spp.	
L. buccalis oral taxon 563	
L. goodfellowii oral taxon 845	1
L. holfstadii oral taxon 224	2
L. wadei oral taxon 222	1
Leptotrichia sp. oral taxon 212	
Leptotrichia sp. oral taxon 221	1
Leptotrichia sp. oral taxon 225	
Microbacterium sp. oral taxon 186	1
Neisseria spp.	
N. elongata oral taxon 598	3
N. sicca oral taxon 764	
***N. subflava* oral taxon 476**	5
Parvimonas spp.	
Parvimonas sp. oral taxon 110	1
Parvimonas sp. oral taxon 393	1
***Peptostreptococcus stomatis* oral taxon 112**	
Porphyromonas spp.	
P. catoniae oral taxon 283	
***P. endodontalis* oral taxon 273**	
P. gingivalis oral taxon 619	
Porphyromonas sp. oral taxon 279	
Prevotella spp.	
P. intermedia oral taxon 643	
Prevotella sp. oral taxon 472	
Selenomonas spp.	
S. noxia oral taxon 130	2
Selenomonas sp. oral taxon 126	1
Selenomonas sp. oral taxon 133	
Streptococcus spp.	
S. cristatus oral taxon 578	
***S. mitis* oral taxon 398**	1
***S. mitis* oral taxon 677**	4
***S. pneumoniae* oral taxon 734**	1
S. sanguinis oral taxon 758	2
***Streptococcus* sp. oral taxon 058**	1
Streptococcus sp. oral taxon 064	
Streptococcus sp. oral taxon 071	1
Tannerella spp.	
Tannerella forsythia oral taxon 613	
Tannerella sp. oral taxon 286	
TM7 phylum	
Oral taxon 346	1
Oral taxon 347	
Oral taxon 349	
Treponema spp.	
T. denticola oral taxon 584	
T. medium oral taxon 667	
Veillonella spp.	
***V. dispar* oral taxon 160**	1
***V. parvula* oral taxon 161**	1

[a] Species identified in the subgingival plaque clone library with sequence identities of ≥97% with those available in the Human Oral Microbiome Database, with the exception of *E. tonsillarum* oral taxon 484 and *E. coli* oral taxon 574, which were not detected in the clone library. Taxa overlapping with those identified in saliva are in boldface type.

[b] A total of 10 mice were injected with the pooled subgingival plaque sample.

consistent with those made in humans that only *F. nucleatum*, not other fusobacteria, is associated with intrauterine infections.

Some of the clones translocated to the murine placenta and those identified in intrauterine infection in humans were nonidentical but rather were closely related species of the same genus. They include *Neisseria*, *Leptotrichia*, *Aggregatibacter*, *Campylobacter*, *Capnocytophaga*, and *Peptostreptococcus* (Table 4). The discrepancy observed here may be due to the differences between humans and mice. It may also be due to the discrepancy in microbial nomenclature. It has been reported by our group and others that the nomenclature may differ based on the technology used (30). The fact that the species identified in our study, including *Leptotrichia goodfellowii*, *N. flavescens*, *N. subflava*, *A. segnis*, *Campylobacter showae*, and *Capnocytophaga granulosa*, have been associated with extraoral infections involving hematogenous transmission, such as endocarditis, meningitidis, and extraoral abscesses (2, 16, 19, 36, 50, 53), indicates their ability to translocate to different body parts and their virulence potential.

Some species identified in the placenta exist in multiple maternal microfloras, including *Campylobacter*, *E. coli*, *Leptotrichia*, *Neisseria*, *Peptostreptococcus*, and *Streptococcus* (Table 4). For example, *E. coli* is widely known as an enteric pathogen, but it has been also associated with stillbirth (25, 41). It has been considered that intra-amniotic infection with *E. coli* results from the lower genital tract through an ascending route (25). However, *E. coli* can be isolated from the oral cavity even if it is not one of the most prevalent bacteria (43). Our results indicate that the maternal-fetal infection by *E. coli* and the above-mentioned species can also originate from the oral cavity as a result of bacteremia.

Some bacteria detected in the murine placenta have not been found in intrauterine infections in humans, including *Erysipelothrix*, *Granulicatella*, *Microbacterium*, *Parvimonas* (formerly known as *Micromonas*), *Selenomonas*, and the TM7 phylum. Again, this could be due to the difference between humans and mice. Interestingly, this group includes uncultivated and relatively new species, such as TM7. Thus, it is also possible that they are implicated in human intrauterine infections but have not been identified because the clinical laboratories still use routine culturing to detect microbial infections.

One very interesting observation is that many species that translocate to the murine placenta and are associated with human intrauterine infections are commensal organisms (Tables 2 to 4). For example, we identified *S. mitis*, a ubiquitous oral species, in the mouse placenta. This organism, associated with various infections such as endocarditis and meningitis (14, 35), was recently identified in amniotic fluid from three cases of PTB (17). Similarly, *Veillonella* is also a well-recognized oral commensal species. Several reports described its association with human extraoral infections, including bacteremia, endocarditis, osteomyelitis, and meningitides, confirming its status as an opportunistic pathogen (7, 10, 11, 13, 51, 54). It has also been isolated from amniotic fluid accompanying PTB (47). Therefore, our results indicate the importance of commensal oral species in intrauterine infection.

In contrast, several well-recognized periodontal pathogens, such as *Porphyromonas*, *Tannerella*, and *Treponema*, i.e., the "red-complex" organisms (52), although present in the plaque

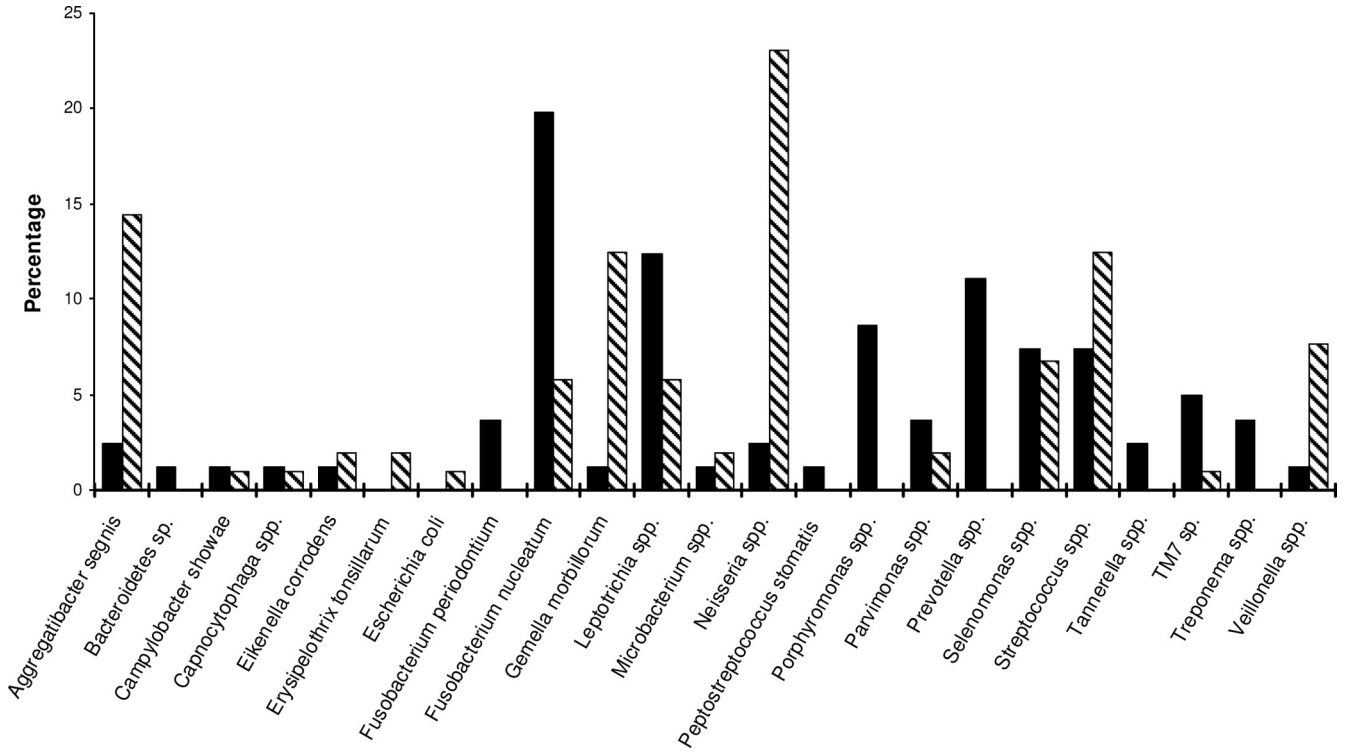

FIG. 2. Transmission of bacteria from pooled subgingival plaque to murine placentas. The relative abundance of each bacterial genus/species in pooled subgingival plaque samples (solid bars) and placentas from all 10 infected mice (slashed bars) were expressed as the percentage of the species out of the total number of clones analyzed for the pooled subgingival plaque samples ($n = 81$) and the murine placentas ($n = 104$), respectively.

sample, were not detected in the placenta. This could be due to several reasons. First, these species existed in low quantities in the plaque, placing them in a disadvantaged position in this "numbers game." Second, the clones in the tested samples may not be the most "transmissive" isolates. Previous studies have shown that placental translocation of *P. gingivalis* is strain dependent (6).

How do these different bacteria colonize the mouse placenta? We have shown previously that *F. nucleatum* colonizes the murine placenta by invading and crossing the endothelial lining (29). This process requires the FadA adhesin of *F. nucleatum* (32). Similarly, we speculate that the placental species identified in our study may possess invasive properties. Our study does not address the question as what happens after these different bacteria colonize in the mouse placenta. We have, however, demonstrated previously that after *F. nucleatum* colonized the placenta, it proliferated quickly to reach a titer of 10^7 CFU per gram of tissue in 72 h and spread to the fetus and amniotic fluid, causing fetal death (29). We have shown that fetal death was the result of localized TLR4-mediated inflammation following the bacterial infection. When TLR4 activation was blocked by its antagonist, *F. nucleatum* still colonized the placenta to the same extent without causing fetal death (37). It will be interesting to see if similar or different events occur with different species in the placenta.

TABLE 4. Bacteria identified in human intrauterine infections

Bacterium	Association with adverse pregnancy outcome in humans	Reference(s)
Aggregatibacter	Preeclampsia	5
Campylobacter	Prenatal and neonatal sepsis; PLBW	20, 21, 26, 39, 49
Capnocytophaga	Chorioamnionitis, PTB	18, 31
Eikenella	PTB	22, 30
E. coli	PTB, chorioamniotis, stillbirth	8, 24, 25
F. nucleatum	PTB	27, 30, 31
Gemella	Amniotic fluid infection	45
Leptotrichia	PTB, abortion, fetal death	9, 17, 30, 48
Neisseria	PTB	17
Peptostreptococcus	PTB	17, 30
Streptococcus	PTB, urinary tract infection, neonatal sepsis	23, 30
Veillonella	PTB	47

In summary, we identified a diverse group of oral bacteria that can translocate to the mouse placenta as a result of bacteremia. These findings are consistent with the observation made in humans that various microbial species have been detected in intrauterine infection. With the exception of the two cases reported from our laboratory, the origins of most intrauterine infections were not determined. Results from the current study suggest that the oral cavity may be a previously overlooked source of intrauterine infection. Furthermore, for the first time, our study indicates the potential significance of commensal oral species in intrauterine infection. This is con-

sistent with the previous report that many intrauterine species are of low virulence. Most of the species reported in this study have been detected in transient bacteremia in humans (4). Previous studies have identified periodontal disease as a potential risk factor for PTB. However, intervention studies employing various periodontal therapies produced inconsistent results among pregnant women (33, 38, 40, 42). One of the most prevalent forms of periodontal disease during gestation is pregnancy-associated gingivitis, affecting at least three-quarters of the expectant mothers (3). Gingivitis is characterized by inflamed gingiva and increased bacterial titers, including those of commensal species. These conditions may lead to frequent bacteremia, thus increasing the opportunity of hematogenous transmission. In the two cases of intrauterine infection with oral *Bergeyella* or *F. nucleatum*, both women showed no signs of periodontitis (a more advanced form of periodontal disease characterized by bone and attachment loss) during postpartum examinations but were suspected to have pregnancy-associated gingivitis (27, 28). Based on our findings, we postulate that periodontal therapies targeted at consistently reducing the total bacterial load in the mother's oral cavity may be effective in improving birth outcomes.

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