**Intestinal enrichment of oral-typical bacteria in feces reflects depletion of gut commensals**

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**SUMMARY**

The association of gastrointestinal diseases with relative enrichment of oral bacteria in human feces can be explained by two alternate hypotheses: it may indicate an increased population density of ectopic oral bacteria in the gut (the *expansion* hypothesis) or a depleted gut commensal bacterial load (the *marker* hypothesis). Through mouse experiments and human microbiome data analysis, we found that the quantitative abundance of oral-typical bacteria in the intestine remains stable as opposed to the marked reduction in the gut bacterial load following antibiotic treatment, thus supporting the *marker* hypothesis. Our mathematical model indicates that the stable oral bacterial population is maintained by a balance between direct antibiotic inhibition and indirect ecological release due to depleted gut commensals. Recognizing that the relative enrichment of oral bacteria does not reflect an ectopic bloom, but results from a depleted gut bacterial population, critically informs the interpretation of microbiome compositional data and interventions to restore healthy gut microbiomes.

**INTRODUCTION**

Healthy people harbor distinct microbiome communities at different body sites (Costello et al., 2009; The Human Microbiome Project Consortium, 2012). The translocation of oral-associated bacteria to the lower gastrointestinal tract and their intestinal enrichment have been linked to a number of digestive system disorders such as inflammatory bowel disease (IBD) (Read et al., 2021) and colorectal cancer (CRC) (Komiya et al., 2019). Compared to the hematogenous route (oral-blood axis) (Abed et al., 2020), the enteral route (oral-gut axis) is a natural and more studied pathway of oral bacterial dissemination (Jin et al., 2022; Kitamoto et al., 2020). For an average person, ~1011 oral-resident bacterial cells are transported to stomach via saliva, food and liquid daily (Segata et al., 2012) but they rarely colonize the healthy gut. Gastric acids, alkaline biles and antimicrobial peptides kill many of those, and survivors face competition from gut-resident bacteria due to colonization resistance (Jin *et al.*, 2022). This is supported by DNA-based surveys that detect very low abundances of oral-resident bacterial DNAs in the feces of health individuals (typically <2%) (Rashidi et al., 2021; Schmidt et al., 2019).

Broad spectrum antibiotics such as ampicillin can weaken the oral-gut barrier by depleting gut anaerobes and promote colonization of translocated oral bacteria (Atarashi et al., 2017). Other than antibiotics, diets, aging, and gut inflammation may also disrupt gut colonization resistance (Kitamoto *et al.*, 2020). All these factors jointly contribute to the clinical associations between ectopic colonization of oral bacteria and a variety of digestive diseases (e.g., Crohn’s disease (Gevers et al., 2014), ulcerative colitis (Schirmer et al., 2018), CRC (Kostic et al., 2013), liver cirrhosis (Qin et al., 2014)). However, nearly all those association studies were based on relative microbiome profiling and thus inconclusive regarding whether the relative enrichment of oral-typical bacteria reflects their active expansion and bloom in the gut. The technical limitation has hampered our ability to associate quantitative microbiome features with disease and led to artificial interpretations from relative microbiome analyses (Vandeputte et al., 2017).

By definition, the relative enrichment of oral bacteria following perturbations can be explained by two alternate hypotheses (Fig. 1). The *expansion* hypothesis proposes that the active expansion of translocated oral bacteria in the gut outweighs the depletion of gut-resident bacteria in determining the relative enrichment. By contrast, the marker hypothesis states the opposite, i.e., the relative enrichment is an artifact caused by loss of gut commensals. The two hypotheses have distinct implications for human health and pathologies: While the expansion hypothesis indicates that the ingested oral bacteria can directly drive or exacerbate digestive diseases, the marker hypothesis indicates indirect effects of microbiome on disease onset and progression due to the loss of gut bacteria. In the following, we distinguished the two hypotheses by quantifying the load of oral-typical bacteria in the gut microbiome of mice and humans.

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**Fig. 1| Expansion versus marker hypotheses to explain the relative enrichment of oral bacteria in human microbiome.** The density of oral-typical bacteria colonizing the human intestine is synergistically determined by a basal transmission influx from the oral cavity, biomass loss due to natural death and fecal excretion, and ecological interactions between bacteria of oral and gut origins. Perturbations such as antibiotics may substantially increase the relative abundance of oral-typical bacteria in the human gut (fecal) microbiome by two alternate mechanisms. The expansion hypothesis proposes that the relative enrichment measures its absolute abundance (i.e., active expansion of the oral populations), while the marker hypothesis views it as an artifact caused by depletion of gut bacterial commensals.

**RESULTS**

**Paired oral and fecal samples from antibiotic-treated mice support marker hypothesis**

To determine whether relative enrichment of oral-derived bacteria reflects their active population expansion, we treated three mice with antibiotic cocktail (ampicillin-vancomycin-neomycin) at day 0 for a week to disrupt colonization resistance by gut commensals (Fig. 2A). We profiled the microbiome compositions at the ASV (amplicon sequence variant) level of oral cavity (oral\_d0) and fecal (fecal\_d0) samples on day 0 prior to treatment as well as fecal samples (fecal\_d8) on day 8 (Fig. 2B). We found that the post-treatment fecal microbiomes shared more identical ASVs (Hamming distance) with the pre-treatment oral microbiomes than the pre-treatment fecal microbiomes and were also compositionally similar (Bray-Curtis distance) in 2 out of the 3 mice (Fig. 2C). Notably, 10%-20% ASVs (the majority from 6 members; Fig. S1) present in the post-treatment feces were detected in the pre-treatment oral microbiomes but not the pre-treatment fecal microbiomes (Fig. 2D), providing strong evidence of oral-gut transmission of bacteria that contain those ASVs. By identifying ASVs typically colonizing the oral cavity of the three mice (see STAR Methods), we found that the antibiotic treatment increased the total fractions of the oral-typical ASVs from nearly 0% to 30% (Fig. 2E), while their quantitative loads reduced by half a log unit (Fig. 2F). The contrast between relative and absolute abundances of oral bacterial populations supports marker hypothesis.

**Additional evidence of marker hypothesis from the literature**

The literature lacks microbiome datasets with both oral and gut samples sequenced in antibiotic-treated mice (Greenberg et al., 2022). To classify oral-typical ASVs from gut microbiome profiles alone, we compiled a reference collection of full-length 16S rRNA genes that represent bacteria typically inhabiting mouse oral cavity (Fig. S2A, see STAR Methods). The resulted collection has 149 sequences (Table S1) from diverse genera with most sequences belonging to *Staphylococcus* and *Streptococcus* (Fig. S2B). Given a new ASV, we can classify whether it is oral-typical or -atypical by exactly matching the ASV to all sequences in the reference collection. We applied this approach to a public dataset of paired oral (swab) and gut (distal intestine) samples from 11 healthy pregnant mice (Theis et al., 2020). On average, 4.5% ASVs in the gut microbiome were found to be oral-typical. In 10 out of the 11 mice, >90% of the total fractions of the oral-typical ASVs in the gut were contributed by those present in the oral cavity of the same mice (Fig. S2C).

Using the approach, we reanalyzed a public study (Reese et al., 2018) that treated mice with an antibiotic cocktail (Ampicillin-Vancomycin-Neomycin-Metronidazole) for 5 days (Fig. 2G). The inferred oral populations, mainly consisting of three ASVs from *Lactobacillus*, *Proteus* and *Escherichia-Shigella*, had substantially higher relative abundances after two days’ exposure (Fig. 2H). However, we did not observe active expansions of the translocated oral populations compared to the control group by tracking the longitudinal changes of the mean oral bacterial loads (Fig. 2I, left). By contrast, the antibiotic cocktail reduced the averaged gut bacterial density by more than 2 orders of magnitudes (Fig. 2I, right). The differential responses of the oral vs. gut populations clearly support the *marker* hypothesis: depletion of gut commensals drove the relative enrichment of oral-typical bacteria in feces. As a result, the oral bacterial fractions are negatively correlated (Pearson’s r = -0.52, P = 3.5e-17) with the total bacterial loads on the log scale (Fig. 2J). The negative association is mostly linear, but several samples with low bacterial loads have notable deviations from the linear fit (Fig. S3). We speculated that the dominant ASVs in these outliers were orally derived but misclassified. Should this be true, the abrupt falls of the oral bacterial fractions (Fig. 2H) and loads (Fig. 2I, left) after day 2 can be explained by the underestimated bacterial translocation.

The *marker* hypothesis was further supported by another public dataset from bone-marrow-transplanted mice that received mono-antibiotic prophylaxis (Staffas et al., 2018). Ampicillin but not streptomycin, aztreonam, or vancomycin substantially reduced gut bacterial loads (Fig. S4A). The oral bacterial loads were stable throughout the experiments for all four antibiotics including ampicillin (Fig. S4B). Similarly, oral bacterial fractions and total bacterial loads exhibited a log-log linear relationship (Pearson’s r = -0.75, P = 5.5e-11) (Fig. S4C).

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**Fig. 2| Oral-gut bacterial translocation in antibiotic-treated mice.** (**A-F**)New mouse experiment in this study. (A) Experimental design. Mice (n=3, labeled as M1, M2, M3) were treated with an antibiotic cocktail of ampicillin, vancomycin, and neomycin for a week. The pre-treatment fecal (fecal\_d0) and oral (oral\_d0) samples were collected on day 0 and the post-treatment fecal samples (fecal\_d8) were collected on day 8. (**B**) Microbiome compositions of all fecal and oral samples**.** (**C**) Microbiome similarity between post-treatment fecal samples and pre-treatment fecal or oral samples. (**D**) Sources of ASVs in the post-treatment fecal samples. (**E,F**) Relative (E) and absolute (F) abundances of oral-typical populations in the feces. (**G-J**) Reanalysis of a public study (Reese *et al.*, 2018). (**G**) Experimental design. Mice were treated with either water (the control group; n=11) or an antibiotic cocktail of ampicillin, vancomycin, neomycin, and metronidazole (AVNM) (the antibiotic group; n=10) for five days. (**H**) Compositional dynamics of oral bacterial ASVs during and after antibiotic cocktail treatment. (**I**) Total loads of ASVs classified as of oral (left) and gut (right) origin averaged across mice in the control and antibiotic groups. Lines and dots: mean; shading: 95% confidence interval (CI). (**J**) Linear relationship between total bacterial loads and oral bacterial fractions in the log-log space. Line: linear regression; shading: 95% CI. Unit of bacterial load: 16S copies per gram of feces.

**Detection of oral-typical bacterial DNAs in human feces without paired oral samples**

The existing human gut microbiome studies with quantitative profiles mostly lack sequencing of paired oral samples. To reuse the quantitative data, we aimed to build a collection of bacterial 16S rRNA gene sequences that typically colonize the human oral cavity. We leveraged the Human Microbiome Project (HMP) that sequenced paired oral (multiple subsites)-gut (stool) microbiomes from 237 healthy volunteers (The Human Microbiome Project Consortium, 2012). Distinct bacterial communities were found inhabiting the two body sites (Fig. 3A), which provides the pragmatic basis for distinguishing oral-typical from oral-atypical ASVs. We followed a previous study (Thomas et al., 2019) for filtering oral-typical ASVs: their mean relative abundance and prevalence must be greater than 0.01% and 5% respectively among all oral cavity samples, and no greater than the same cutoffs among all fecal samples (Fig. 2B,C). The filtering step left 178 ASVs sequenced at the 16S V4-V5 region (Table S2) and they belong to a variety of genera such as *Prevotella* and *Streptococcus* (Fig. 2D). Using the 178 reference sequences, we found that 219 out of 280 HMP stool samples have undetectable oral-typical ASVs. Among the remaining 61 samples, the oral-typical ASVs in 55 samples were all present in at least one paired oral cavity sample of the same persons (Fig. 2E). The average fractions of oral-typical ASVs in the HMP stool samples is as low as 0.05%, consistent with previous findings (Rashidi *et al.*, 2021; Schmidt *et al.*, 2019).

The 178 reference sequences filtered from the healthy people allow us to estimate the abundance of oral-typical bacteria in not only other non-HMP healthy individuals, but also people with diseases. To show the generalizability, we reanalyzed a public study that sequenced paired oral-gut microbiome samples from patients with inflammatory bowel disease (IBD) and their own non-IBD healthy controls. The averaged total fractions of oral-typical ASVs in the gut of the healthy controls is higher than the averaged fraction among the HMP volunteers but remains low at 1.1%. By contrast, the mean oral bacterial fractions in the feces among the patients with Crohn’s disease (CD) and ulcerative colitis (UC) increased ~4 folds up to 4.2% and 4.3% respectively. This follows our expectation, as the relative enrichment of orally derived bacteria in the gut has been associated with both CD and UC (Read *et al.*, 2021). Among 91 out of 101 samples, at least 80% of the estimated total fractions of oral-typical ASVs can be attributed to those present in the paired oral samples (Fig. 2F). Since the most abundant ASVs in feces can be found in the oral cavity when they were classified as of oral origin, our classification is conservative and potentially has low false positive rates in predicting oral bacterial translocation. Finally, we demonstrated that the estimated oral bacterial fractions in the feces are robust against variations of the cutoff thresholds used to filter HMP (Fig. S5).

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**Figure 3. Bacterial DNAs typically found in the human oral cavity but not the intestine.** (**A**) An overview of the 4,354 microbiome samples from The Human Microbiome Project (HMP). T-distributed stochastic neighbor embedding (tSNE) (Van der Maaten and Hinton, 2008) collapses all samples (dots) into distinct clusters based on their compositions. (**B, C**) The mean relative abundance (B) and prevalence (C) of ASVs (dots) among all HMP oral cavity samples (x axis) and the stool samples (y axis). 178 ASVs were identified as oral-typical and highlighted in orange. (**D**) Genus-level distribution of the 178 oral-typical ASVs. (**E, F**) The total fractions of oral-typical ASVs in human feces (circles) and their proportions contributed by ASVs in the paired oral samples. (E) Healthy individuals in HMP (n=280). (F) Patients with inflammatory bowel disease and their own healthy controls (n=101) (F). HC: healthy control; CD: Crohn’s disease; UC: ulcerative colitis. Data sources: panel A-E (The Human Microbiome Project Consortium, 2012); panel F (Imai et al., 2021).

***Streptococcus*-domination states of allo-HCT recipients indicate oral-gut translocation**

To validate the marker hypothesis in humans, we leveraged a pre-compiled large-scale microbiome dataset with quantitative profiles (16S amplicon sequencing and qPCR) for patients receiving allogeneic hematopoietic cell transplantation (allo-HCT) in Memorial Sloan Kettering Cancer Center MSKCC. Allo-HCT is the only curative therapy for a number of hematologic disorders such as leukemias, lymphomas, and myeloma (Shono and van den Brink, 2018). Along with other related procedures (conditioning regime, antibiotic exposure), allo-HCT disrupted the gut microbiome compositions of the MSKCC allo-HCT recipients, where *Enterococcus* and *Streptococcus* are the two most abundant genera that frequently dominate (>30% relative abundance of a single ASV; (Taur et al., 2012)) the gut microbiome (Fig. 4A, top row) (Peled et al., 2020). Among the 10,433 samples from 1,276 patients, 901 were dominated by at least one oral-typical ASV and these ASVs mostly belong to the genera *Streptococcus*, *Actinomyces*, and *Abiotrophia* (Fig. 4A, middle row). The total bacterial loads of a subset of 3,126 samples showed that the fecal microbiome samples highly enriched with oral-typical ASVs have lower bacterial biomass (Fig. 4A, bottom row). We confirmed that the total fractions of oral-typical ASVs detected in the feces are not correlated with sequencing depths (Fig. S6).

One limitation of this cohort is the lack of paired oral microbiome samples. To find supporting evidence of bacterial translocation, we used co-occurrence analysis (see STAR Methods) to identify oral-typical ASVs that were present in the feces more than expected by chance. The co-occurring oral-typical ASVs, if existed, would indicate collective bacterial transmission from the oral cavity to the gut. Not surprisingly, we found 71 such oral-typical-ASV groups of size from 2 to 5 and 55 groups contain *Streptococcus* ASVs (Table S3). To identify the species of the *Streptococcus* ASVs, we extracted shotgun metagenomes from 19 samples which have at least 10% (measured by 16S amplicon sequencing) ASV\_8 (the most abundant *Streptococcus* ASV inferred as of oral origin) from the same MSKCC allo-HCT cohort. We were able to recover 22 high-quality metagenome-assembled genomes, among which 15 were annotated as *S. thermophilus*—a facultative anaerobic lactic acid bacterium. To assess the viability of the *Streptococcus* genomes, we computed the ratio of metagenomic reads near the replication origin to the replication terminus (i.e., peak-to-trough (PTR) ratio) to estimate the simultaneous replication events averaged across the population ([Gao and Li, 2018](https://elifesciences.org/articles/45931#bib3); [Korem et al., 2015](https://elifesciences.org/articles/45931" \l "bib4)). We found that the averaged PTR ratio is 1.50 0.16 (Table S4), suggesting a possible mixture of 50% 2-fork and 50% single-fork cells. The PTR-based growth estimates indicated that these *Streptococcus* genomes generally had slow to intermediate growth at the time of sampling.

**Validation of maker hypothesis in the gut microbiome of allo-HCT recipients**

The allo-HCT recipients require antibiotics to prophylactically minimize the risk of developing infections before immune system reconstitution and/or to treat infections when they develop (Shono and van den Brink, 2018). The allo-HCT procedure in MSKCC usually started antibiotic prophylaxis within a week prior to transplantation. The timing of antibacterial antibiotic administration (Fig. 4B, top) corresponded well to the declined gut bacterial relative (Fig. 4B, middle) and absolute (Fig. 4B, bottom) abundances. Since the mean oral bacterial loads fluctuated over time around a stable level, the increased relative abundance of oral-typical ASVs was mostly driven by the declined gut bacterial density, consistent with the marker hypothesis. As a result, the oral bacterial proportions had a negative correlation with the total bacterial loads (Pearson’s r = -0.27, P = 2.2e-54) (Fig. 4C).

We further examined the bloodstream infections (BSIs) caused by the most common bacterial genera colonizing the oral cavity (*Streptococcus*, *Actinomyces*, *Veillonella*, *Haemophilus*, *Abiotrophia*) in the same cohort of patients. In contrast to *Enterococcus* (Taur *et al.*, 2012) and *Candida spp.* (Rolling et al., 2021; Zhai et al., 2020) whose intestinal dominations were associated with the risk of BSI, we only found 5 infection cases (all *Streptococcus spp.*) in 5 patients between 10 days prior to and 40 days after the transplantation, and none were preceded by intestinal *Streptococcus* domination (Table S5). The few BSI incidences further suggest against active expansion of oral bacteria in the intestine.

The complex antibiotic use patterns in allo-HCT recipients makes it a challenge to uncover the antibiotics associated with intestinal dominations by oral-bacterial ASVs. We conducted a time-varying survival analysis to assess which antibiotic exposures in the past improve the prediction of future occurrence of oral-typical ASV domination above null expectation. We identified that piperacillin/tazobactam (P=5.7e-7) and orally administered vancomycin (P=0.049) significantly increased the risk of oral bacterial domination while quinolones (P=6.1e-4) has a negative impact. Intravenous vancomycin showed no association, likely because it fails to reach the GI tract.

Due to the negative association in Fig. 4C, the strong positive association between piperacillin/tazobactam and the enrichment of oral bacteria simply reflects the anaerobe-killing capacity of this drug. Piperacillin/tazobactam is a combination of beta-lactam and beta-lactamase inhibitor that can reach the gastrointestinal tract and have a broad spectrum against many gut anaerobes (Morjaria et al., 2019). The positive assocaition is not only apparent in adults allo-HCT recipients but also seen in an independent cohort of pediatric patients (Bekker et al., 2019). In the pediatric allo-HCT cohort, children who have received total gut decontamination (oral piperacillin/tazobactam) have much higher relative abundance of oral-typical bacteria in feces than those who have received selective gut decontamination (oral polymyxin/neomycin) (Fig. S7). Since the high oral bacterial fraction in feces indicates low bacterial biomass, piperacillin/tazobactam is very disruptive to gut microbiome density and should be prescribed with caution.

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**Figure 4. Relative enrichment of oral-typical bacteria in the feces of allo-HCT recipients.** (**A**)The total fractions of oral-typical bacteria and total bacterial loads of fecal microbiome samples with diverse microbiome compositions. (**B**) Population dynamics of relative and absolute abundances of oral and gut bacteria. Absolute abundance was computed by multiplying relative abundance with total bacterial loads (16S copies per gram of feces). Line and dots: mean; shading: 95% confidence interval (CI). (**C**) Negative association between proportions of oral-typical bacteria in feces and total bacterial loads across all samples (dots). Red line: best linear fit; shading and bars: 95% CI. (**D**) Association between antibiotic exposure and intestinal domination of oral-typical ASVs. Vertical line: hazard ratio; bar width: 95% CI. \*\*\*\*: P<0.0001; \*\*\*: P<0.001, \*: P<0.05. xx test.

**Ecological mechanisms of the marker hypothesis**

To elucidate the potential mechanisms of the marker hypothesis, we developed a mathematical model by considering two competing forces that regulate the density of oral bacteria in the intestine (Fig. 5A): antibiotics inhibit their growth but relieve them from the competitive inhibitions from the gut commensals (i.e., ecological release effect). The kinetic equations are shown below (see Supplementary Text for details)

Eq. (1)

Eq. (2)

Here and are the oral and gut bacterial loads respectively, is the oral-gut transmission rate due to saliva, food and liquid intake, is the rate constant of bacterial loss, and are the carrying capacities, and and are the maximum growth rates. The physiological values of these parameters were taken from the literature ( and ), predicted by theoretical modeling ( and ), or estimated by fitting human data ( and ); see Table S6 for their values and Supplementary Text for the details of parameter estimation.

Our findings from mouse and human data indicated that the oral bacterial populations in the intestine remain stable or decrease moderately. We simulated the dynamics of oral and gut bacterial densities under constant antibiotic exposure and tuned the growth-inhibitory levels of antibiotics by varying (susceptibility of oral bacteria) and (susceptibility of gut bacteria). The oral bacterial density can increase, remain nearly unchanged, or decrease, depending on the relative magnitude of and (Fig. S8). Particularly, a stable level of oral bacterial abundance is achieved when and have similar values (Fig. S8), reflecting similarity susceptibility of the two bacterial populations to antibiotics. In this case, antibiotic inhibition are stronger or and ecological release effect are balanced, thus explaining the observed stabilization of oral bacterial loads. A recent study has reported that the oral microbiome has more abundant but less diversity of antibiotic resistant genes than the gut microbiome. We do not find multi-drug resistant bacteria as a rule in the real data.

It is also interesting to note that the increase or decrease of oral bacterial density under dissimilar values of and are nonlinear and saturated, which is drastically different from the linear, unsaturated declines of gut bacteria. Our theoretical analysis showed that the minimum oral bacterial density was set by the basal influx/outflux ratio () and its maximum value is constrained by the carrying capacity of oral bacteria population in the gut (), which was estimated to be 20 fold less than the carrying capacity of gut bacteria . This is expected because gut bacteria adapt better (e.g., more efficient utilization of nutrients) in the environment they are associated with. Due to the ecological constraints, the increased oral bacterial density is relatively minor compared to declined gut bacterial density for the majority of parameters and this still falls in the regime of marker hypothesis (Fig. 5B). To show that quantitatively, we compared the deviation of oral bacterial fraction at any time point from values computed using pre-treatment oral bacterial loads or pre-treatment gut bacterial loads. If the deviation from the fixed oral bacteria is smaller than that from the fixed gut bacteria, it is closer to a marker hypothesis as the change in gut bacterial explains more than that of the oral bacteria. We found that the marker hypothesis dominates over the expansion hypothesis in most of the parameter space.

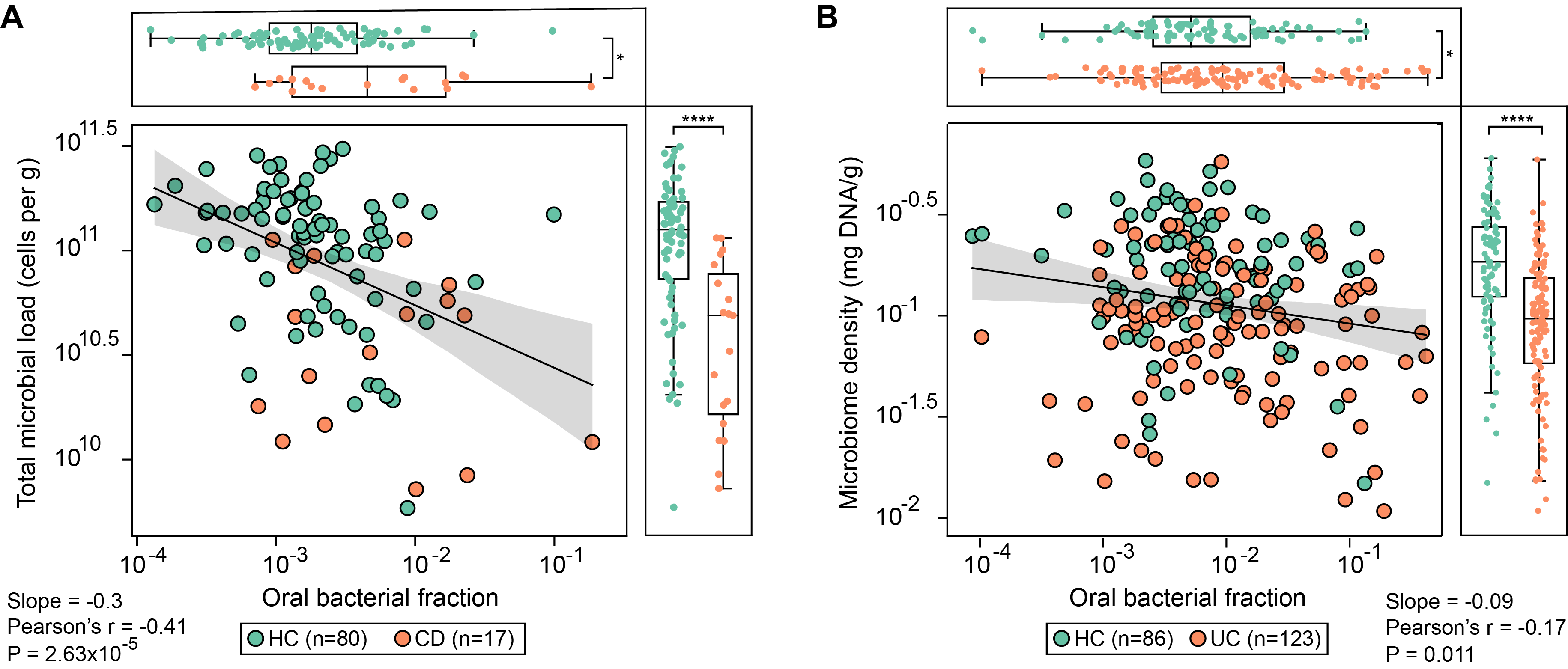
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**Fig. 5| Mathematical modeling reveals marker hypothesis.** (**A**) Microbiome ecology underlying the colonization of oral bacteria in the gut. The oral bacteria density is determined by two mechanisms with opposite directions: antibiotic directly inhibit growth of oral bacterial populations but indirectly promote their growth by releasing the inhibitions from gut resident bacteria. The red dashed line indicates that the inhibitions of oral populations on the gut bacteria are much weaker than the effects of gut bacteria on oral bacteria. (**B**) Simulation of relative enrichment of oral bacteria at different e. (**C**) Marker hypothesis dominates over the expansion hypothesis in a broader range of parameters. For the entire range of epsilon\_o, the enrichment is driven by marker hypothesis. We replaced the oral or gut bacterial loads at any timepoint by their respective values at the beginning and determine whether it is a by comparing the values to th real values.

**The marker hypothesis unifies biomarkers of IBD**

Relative enrichment of oral-associated bacterial species in the gut has long recognized as a characteristic of the gut microbiome in patients with IBD, including both Crohn’s disease (CD) and ulcerative colitis (UC). Using our method to quantify the total fractions of bacteria of the oral origin in feces, we confirmed that this association remains significant for all oral-typical bacteria as a whole (Fig. S10). Other than the enrichment of oral bacteria, it was revealed that both patients with CD and UC have low microbiome density compared to the healthy controls (Contijoch et al., 2019; Vandeputte *et al.*, 2017). The several biomarkers of IBD made it a puzzle how these different markers are independent and related. The marker hypothesis, if it can be generalized to IBD patients, would predict a negative association between the two signatures. By reanalyzing two public datasets (Contijoch *et al.*, 2019; Vandeputte *et al.*, 2017), we found that the oral bacterial fractions and total bacterial loads in the feces of patients with CD (Fig. 6A) and UC (Fig. 6B) were indeed negatively correlated in the log-log space. Since the two signatures are significantly different between the patients and their healthy controls in both studies, the marker hypothesis integrates the two signatures that each distinguishes IBD patients from the healthy individuals into a single mechanism. For the UC cohort, the association became insignificant (P=0.32) after excluding patients with prior antibiotics or an unclear antibiotic history, due to the loss of significant differences of oral bacterial fractions between the control and UC patients (P=0.15). However, relative enrichment of oral-typical bacteria was found in IBD patients without previous antibiotic exposure history. The heterogeneous results indicate a possibility that the relative enrichment of oral bacteria in IBD patients may be synergistically caused by antibiotics in IBD management and gut inflammation.



**Figure 4. Negative association between oral bacterial fractions and total bacterial loads in the feces of patients with Crohn’s disease (A) and Ulcerative Colitis (B).** Each circle represents a fecal sample. Lines: best linear fits; shading: 95% confidence intervals. Data sources: panel A (Vandeputte *et al.*, 2017); panel B (Contijoch *et al.*, 2019). Abbreviations: HC (healthy control); CD (Crohn’s disease); UC (ulcerative colitis). \*\*\*\*P<0.0001; \*P<0.05; Welch's t-test.

**DISCUSSION**

Except for a few quantitative studies (Contijoch *et al.*, 2019; Jian et al., 2020; Rao et al., 2021; Schluter et al., 2020; Vandeputte *et al.*, 2017; Vieira-Silva et al., 2019), most human gut microbiome research to date have focused on amplicon-based profiling of microbiome composition. Due to the limitation, gut microbiome dysbiosis has been mostly characterized by loss of biodiversity from the compositional data. Gut microbiome density, another fundamental parameter that impacts host-microbiome interactions (Contijoch *et al.*, 2019; Tang et al., 2019; Zarrinpar et al., 2018), has been much less studied. For example, the total microbial load in the gut regulates the proportion of mucosal RORγt+ Treg cells in mice (Britton et al., 2020). Notably, changes in gut microbiome density do not strictly reflect changes in the gut microbiome biodiversity (Contijoch *et al.*, 2019), suggesting that the two metrics describe different aspects of dysbiosis.

Both cell-based (e.g., flow cytometry) and molecular-based (e.g., qPCR, DNA mass) methods have been developed to measure absolute microbial abundances (Galazzo et al., 2020). In the fecal samples of healthy volunteers, the correlations of microbial loads between flow cytometry and qPCR are moderate (Pearson’s r close to -0.5) (Galazzo *et al.*, 2020; Vandeputte *et al.*, 2017). Although these methods all have their own limitations, we argued that qPCR is conceptually superior for our study due to its compatibility with next generation sequencing (Jian et al., 2021). To compute absolute abundance of any bacterial ASV, we should theoretically multiply its relative abundance by the total bacterial loads in the prepared samples before sequencing, rather than loads in the original samples (see Supplementary Text for mathematical explanataion). The technical biases introduced in the extraction, purification, and amplification steps of 16S amplicon sequencing are shared with qPCR, but not flow cytometry. Additionally, 16S qPCR measures bacterial loads (not microbial loads) and is compatible with 16S amplicon sequencing. Therefore, we used qPCR-based quantification to validate the marker hypothesis in the mouse and human experiments (Figs. 2-4).

Quantitative microbiome profiling bypasses compositionality effects and can thus calibrate the associations learned from relative microbiome profiling. One notable example is the negative association between *Bacteroides* and *Prevotella*, which is an artifact of relative microbiome analysis and disappears by taking absolute cell counts into accounts (Vandeputte *et al.*, 2017). Resonating with this finding, our study revealed another artifact of relative abundance-based association: the relative enrichment of oral-derived bacteria in the gut microbiome does not reflect the active expansion of the translocated oral populations, but simply indicates a depleted gut microbiome state. Therefore, the various bacterial species (e.g., *Streptococcus spp.*, *Veillonella parvula*, *Fusobacterium nucleatum*) found enriched in the intestine of IBD patients (Read *et al.*, 2021) may not be biologically different; they could be all biomarkers of declined gut bacterial density (Duvallet et al., 2017). Despite low biomass of orally derived bacterial populations in the gut, they may have functional impacts on human health. For example, the orally translocated *Klebsiella spp.* causes Th1 cell expansion in the mouse gut (Atarashi *et al.*, 2017).

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**AUTHOR CONTRIBUTIONS**

Conceptualization, C.L., T.R. and J.B.X; Mouse experiment: A.D.; Human Microbiome Project processing: H.B.L.; Public microbiome dataset analysis, C.L; Methodology, C.L.; Investigation, C.L. and T.R; Writing – Original Draft, C.L., T.R.; Writing – Review and Editing, T.M.H., J.B.X., J.U.P., B.Z., and M.R.M.v.d.B; Supervision, J.B.X. and T.M.H.

**DECLARATION OF INTERESTS**

J.U.P. reports research funding, intellectual property fees and travel reimbursement from Seres Therapeutics and consulting fees from DaVolterra, CSL Behring and from Maat Pharma. He has filed intellectual property applications related to the microbiome (reference nos. 62/843,849, 62/977,908 and 15/756,845). M.R.M.v.d.B. has received research support from Seres Therapeutics; he has consulted, received honorarium from or participated in advisory boards for Seres Therapeutics, WindMIL Therapeutics, Rheos, Frazier Healthcare Partners, Nektar Therapeutics, Notch Therapeutics, Forty Seven Inc., Priothera, Ceramedix, Lygenesis, Pluto Immunotherapeutics, Magenta Therapeutics, Merck & Co., Inc. and DKMS Medical Council (Board); and he has IP Licensing with Seres Therapeutics, Juno Therapeutics and stock options from Seres and Notch Therapeutics. T.M.H. has participated in a scientific advisory board for Boehringer-Ingelheim Inc.

**STAR METHODS**

**KEY RESOURCES TABLE**

|  |  |  |
| --- | --- | --- |
| **REAGENT or RESOURCE** | **RESOURCE** | **IDENTIFIER** |
| **Deposited Data** | | |
| Human Microbiome Project | Human Microbiome Project Consortium, 2012 | SRA study PRJNA48489 |
| MSK cohort | Liao et al., 2021; Yan et al., 2021 | SRA studies PRJNA394877, PRJNA607574, PRJNA606262, PRJNA548153, PRJNA545312 |
| PRISM, LifeLines DEEP and NLIDB cohorts | Franzosa et al., 2019 | SRA study [PRJNA400072](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA400072/) |
| UMH cohort | Schubert et al., 2014 | mothur.org/CDI\_MicrobiomeModeling/ |
| RISK cohort | Gevers et al., 2014 | SRA study SRP040765 |
| Belgium PSC/IBD cohort | Vieira-Silva et al., 2019 | European Genome-phenome Archive  EGAS00001003600 |
| PROTECT cohort | Schirmer et al., 2018 | SRA study PRJNA436359 |
| Mount Sinai cohort | Contijoch et al., 2019 | SRA study PRJNA413199 |
| LUMC cohort | Bekker et al., 2019 | SRA study PRJEB28845 |
| UPenn cohort | Lewis et al., 2015 | SRA study SRP057027 |
| **Databases** | | |
| SILVA v138 | Quast et al., 2013 | www.arb-silva.de |
| MicrobiomeHD | Duvallet et al., 2017 | [doi.org/10.5281/zenodo.840333](https://doi.org/10.5281/zenodo.840333) |
| **Softwares** | | |
| Python v3.7.9 | Python | python.org |
| Scikit learn v0.24.0 | Pedregosa et al., 2011 | scikit-learn.org |
| Non-negative matrix factorization | Lee and Seung, 1999 | scikit-learn.org |
| t-distributed stochastic neighbor embedding | van der Maaten and Hinton, 2008 | scikit-learn.org |
| Random Forest classifier | Ho, 1995 | scikit-learn.org |
| Lifelines v0.25.6 | Davidson-Pilon, 2019 | github.com/CamDavidsonPilon/lifelines |
| SourceTracker2 | Knights et al., 2011 | github.com/biota/sourcetracker2 |
| iRep | Brown et al., 2016 | github.com/christophertbrown/iRep |
| Cutadapt v3.4 | Martin, 2011 | github.com/marcelm/cutadapt |
| Naive Bayesian classifier | Wang et al., 2007 | rdp.cme.msu.edu |
| DADA2 | Callahan et al., 2016 | benjjneb.github.io/data2 |
| Bhatt lab workflow | Siranosian et al., 2021 | github.com/bhattlab/bhattlab\_workflows |
| MEGAHIT | Li et al., 2015 | github.com/voutcn/megahit |
| MetaBAT 2 | Kang et al., 2019 | bitbucket.org/berkeleylab/metabat/src/master/ |
| CONCOCT | Alneberg et al., 2014 | github.com/BinPro/CONCOCT |
| DAS Tool | Sieber et al., 2018 | github.com/cmks/DAS\_Tool |
| PATRIC | Davis et al., 2020 | www.patricbrc.org |
| Kraken2 | Wood et al., 2019 | github.com/DerrickWood/kraken2 |

**RESOURCE AVAILABILITY**

**Lead contact**

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Joao B. Xavier (xavierj@mskcc.org)

**Materials Availability**

This study did not generate new unique reagents.

**Data and code availability**

* All raw sequences of the microbiome datasets analyzed in this study are public available with accession numbers listed in the key resource table.
* All processed data supporting the findings of this study are available within the article and its supplementary materials.
* Customized Python scripts for all analyses included in this study are available on Github (https://github.com/liaochen1988/Source\_codes\_for\_oral\_microbiome\_contamination).

METHOD DETAILS

**Reference 16S rRNA gene sequences of oral-typical bacteria**

To detect oral-derived bacteria in the human gut, we reused the HMP data and defined oral-typical 16S rRNA gene ASVs based on the two criteria: (1) the mean relative abundance of each oral-typical ASV across all oral cavity samples is greater than 1e-4 and its relative abundance averaged across all gut samples is no bigger than the same threshold; (2) the prevalence of each oral-typical ASV across all oral cavity samples is greater than 0.05 and its prevalence averaged across all gut samples is smaller than or equal to the same cutoff value. Prevalence of an ASV is computed as the proportion of samples that contain the ASV at a relative abundance above 1e-3. Similar thresholds of relative abundance and prevalence were used in a previous study to identify oral-typical species from metagenomic profiles (Thomas *et al.*, 2019). The threshold of occurrence (or presence) of an ASV was also adopted from the literature (Machado et al., 2021). Importantly, we have shown that the estimation of total fractions of oral-typical bacteria is robust against variations of these cutoff thresholds (Fig. 5).

Due to the lack of large-scale paired oral-gut microbiome samples in mouse, we used the full-length 16S rRNA genes from the Mouse Oral Microbiome Database (MOMD) as the starting point. Similar approaches were implemented to identify oral bacteria in the human gut by profiling sequences against oral bacteria in the Human Oral Microbiome Database (HOMD) (Coker et al., 2018; Hu et al., 2022). However, the original MOMD contains sequences from bacteria that colonize both mouse oral cavity and gut. We thus filtered MOMD by excluding sequences that have exact matches in the Mouse Gut Microbial Biobank (Liu et al., 2020) and the Mouse Intestinal Bacterial Collection (Lagkouvardos et al., 2016). Both sources contain cultured gut microbes that also have full length 16S rRNA genes. To further expand the database, we profiled the sequences in a paired mouse oral-gut microbiome dataset and identified oral-typical ASVs using the same criteria mentioned above. For each oral-typical ASV that cannot be matched to any sequences in MOMD, we searched against the NCBI NT database for full-length 16S rRNA sequences that were isolated from mouse and contain the oral-typical ASV as a partial sequence.

The 16S rRNA sequences in the Mouse Oral Microbiome Database (MOMD) (Joseph et al., 2021) were used as the starting point for gathering oral-typical sequences (Fig. S1A). We filtered MOMD by removing gut-colonizing sequences closely matching to those in two large-scale mouse gut bacterial collections (Lagkouvardos *et al.*, 2016; Liu *et al.*, 2020) and added oral-typical sequences absent in the MOMD from a small but rare set (n=11) of paired oral-gut samples from pregnant mice (Theis *et al.*, 2020);.

The oral bacterial loads are computed by multiplying total bacterial loads with total fractions of oral-typical bacterial ASVs. The gut bacterial loads are thetotl bacterailloads struacted from oral bacterial loads. They represent the toal density of oral-atyocal ASVs, which may include ASVs that translocated from body sites other than oral cavity. Our study ignored sthis possibility and consider oral-atypical as gut-resident bacteria.

**Ecological modeling**

Our model (Eq. (1)-(2) in the main text) describes several key ecological processes associated with the oral-derived and gut-resident bacterial populations: transmission of oral bacteria to the gut lumen (zero-order kinetics), excretion of both populations in feces (first-order kinetics), population growth, and intra-/inter-population competition for resources (Lotka-Volterra-type (Stein et al., 2013)). The host effects such as gut inflammation are not explicitly modeled. We focus on antibiotic-induced perturbations and assume that antibiotics inhibit growth of both types of bacteria equally. If the averaged inhibitory effects between oral and gut bacteria are proportional and differ by a constant, the coefficients representing the unequal inhibitory effects (Carr et al., 2020) can be absorbed into the growth rates ( and ). Since oral cavity would be only briefly exposed to antibiotics during swallowing (Carr et al., 2020), we assume that ϵ does not affect . Antibiotics may also change dilution rate (e.g., inducing diarrhea) but this effect is indirect and generally mild (Polage et al., 2012). The details of model derivation and analytical solutions are available in **Supplemental Information**. Unless otherwise noted, the model parameters are assigned to their default values: , , , , , .

**Quantifying oral bacteria fraction in microbiome samples**

A similar approach using the Human Oral Microbiome Database (HOMD) has been previously employed to classify oral bacteria (Coker *et al.*, 2018; Hu *et al.*, 2022).

We model the bacterial community in any microbiome sample as a linear combination of five source communities, corresponding to the typical communities of the five bacterial habitats revealed by the HMP dataset in the tSNE plot. Our approach is based on two assumptions: (1) bacteria translocate among different body sites (O’Boyle et al., 1998) within individuals and (2) each human body site is a distinct habitat that selects for a particular microbiome composition, such that the microbiome composition at a specific body site in one person can be approximated by the typical composition shared among the entire population. The second assumption is supported by findings that microbiome compositions vary more between body sites within the same individuals than between the same body site but in different individuals (Chu et al., 2017; Human Microbiome Project Consortium, 2012; Segata et al., 2012).

We simultaneously obtained the typical compositions of these habitats () and their mixing proportions () in HMP samples by decomposing the HMP relative abundance table () using Non-negative Matrix Factorization (NMF). The following objective function was minimized by NMF under the constraints that both and are non-negative: , where is the element-wise L1 norm, is the Frobenius norm, and is the Kullback-Leibler distance. Both (a constant that multiplies the regularization term) and (a regularization mixing parameter) are set to zero because the decomposition error (i.e.,) is optimized without regularization (see **Fig. S1A**). We normalized such that each row sum equals to 1 (i.e., the relative abundance for each community sums up to 1) and is updated accordingly to keep their product () the same. Given the microbiome relative abundances of new test samples (), the mixing proportions of these samples () can be similarly solved by minimizing where is obtained in the previous step and fixed here. Unless otherwise specified, our approach sums up the fractions of oral bacteria from both oral cavity habitat 1 and 2 to compute “oral bacteria in feces”.

We choose to collapse bacterial taxa at the lowest classified level up to genus for two reasons: (1) the interindividual variability in gut microbiome is lower in higher taxonomic ranks (Eckburg et al., 2005; Human Microbiome Project Consortium, 2012) and (2) higher taxonomic rank reduces batch effects when comparing results across multiple studies (DeSantis et al., 2006). Since the test samples may be classified using any taxonomic database, its relative abundance table () needs to reconstructed to match the taxonomic labels assigned to HMP sequences () using the GreenGenes taxonomy (DeSantis et al., 2006). When a taxon is not a GreenGenes taxonomy, it will be mapped to the lowest classified taxonomy in GreenGenes along the lineage of the taxon until the rank kingdom. Non-bacterial sequences are removed from all relative abundance tables.

**Alternative oral bacteria quantification approaches**

We adopted two well-established microbial source tracking methods to validate our approach. A Random Forest classifier (1,000 trees with maximum tree depth 5) was trained on all HMP samples to predict sample collection body sites from bacterial composition. The prediction is probabilistic with probabilities calculated as the number of trees voting for each body site divided by the total number of trees in the forest. For new test samples, the proportion from each body site is therefore an average of the relative abundance of all bacterial taxa weighted by the probabilities that they come from the body site.

SourceTracker 2 (Knights et al., 2011) uses a Bayesian approach to estimate the mixing proportions of all source communities into a sink community. For any fecal sample as a sink, we used all other samples from different body sites of the same individuals as sources. Rarefaction is performed at 100 sequences/sample for both sources and sinks. The total fraction of oral bacteria in feces is the sum of mean proportions from all oral cavity samples (from different subsites).

**Identifying co-occurring ASVs**

The algorithm for computing co-occurrence is described in detail elsewhere (Machado et al., 2021). Briefly, it begins with ASV pairs and iteratively identifies co-occurring ASVs of larger combination sizes. In the next iteration, larger combinations are created by extending all co-occurring groups identified in the current iteration with one new ASV. The presence/absence of an ASV is determined by a relative abundance cutoff of 0.001. A combination of ASVs must satisfy the following criteria to be considered as co-occurring: (1) they must co-occur in at least 200 patients and 500 samples; (2) they must co-occur at least twice more than expected by chance, which is estimated by assuming each ASV is observed independently and its number of observation can be modeled by a binomial distribution; (3) the (false discovery rate) FDR-corrected *P*-values for the independent observation hypothesis must be less than 0.05. An oral group is a co-occurring group of oral ASVs. In this analysis, ASVs assigned to the following genera are considered to have an oral origin: *Actinomyces*, *Leptotrichia*, *Campylobacter*, *Fusobacterium*, *Neisseria*, *Corynebacterium*, *Rothia*, *Treponema*, *Veillonella*, *Prevotella*, *Streptococcus*, *Capnocytophaga*, *Haemophilus*. This list is a conservative enumeration based on the overlaps between principal bacterial genera found in the healthy oral cavity (Deo and Deshmukh, 2019) and the major signature taxa (relative abundance 1%) of the oral cavity habitat 1 and 2 of the HMP samples.

**16S rRNA gene amplicon sequencing data analysis**

Generally, we prefer to adopt the metadata, OTU/ASV abundances and taxonomy used in the original studies if provided. This helps us contrast our results with their findings by minimizing the technical differences in processing their 16S sequences. Sample metadata, OTU table (including taxonomy) and sequences of the HMP cohort were downloaded from the HMP website (https://www.hmpdacc.org/HMQCP/all/). Sample, subject and clinical metadata, taxonomy and counts of ASVs, 16S/18S quantitative PCR data, and binarized fungal CFU counts of the MSK cohort (Liao et al., 2021; Yan et al., 2021) were downloaded from Figshare (https://figshare.com/collections/Compilation\_of\_longitudinal\_microbiome\_data\_and\_hospitalome\_from\_hematopoietic\_cell\_transplantation\_patients/5271128). Subject and clinical metadata, and microbial species relative abundance profiles of the PRISM, LifeLines DEEP and NLIBD cohorts were downloaded from the supplementary material of Franzosa et al., 2019. Subject and clinical metadata, OTU taxonomy and counts of the UMH (Schubert et al., 2014) and RISK (Gevers et al., 2014) cohorts were downloaded from the MicrobiomeHD database (<https://zenodo.org/record/569601#.YTqb8y9h1TY>). Subject metadata and genus-level quantitative microbiome profiling matrix of the Belgium PSC/IBD cohort were downloaded from the supplementary material of Vieira-Silva et al., 2019. Sample, subject and clinical metadata as well as the OTU table of the PROTECT cohort were downloaded from the supplementary material of the associated publication (Schirmer et al., 2018). Sample, subject, clinical metadata as well as other microbiology data of the Mount Sinai cohort (Contijoch et al., 2019), the LUMC cohort (Bekker et al., 2019), and the UPenn cohort (Lewis et al., 2015) were downloaded from the supplementary material of the associated studies.

For samples whose relative microbiome profiles are not provided (the Mount Sinai cohort and LUMC cohort), their 16S reads were downloaded and analyzed using an in-house processing pipeline (Liao et al., 2021). Briefly, ASVs were identified using the Divisive Amplicon Denoising Algorithm (DADA2) pipeline including filtering and trimming of the reads (Callahan et al., 2016). Reads were trimmed to the first 180 bp or the first point with a quality score Q<2 (Martin, 2011). Reads were removed if they contained ambiguous nucleotides (N) or if two or more errors were expected based on the quality of the trimmed read. We assigned taxonomy to ASVs using the naive Bayesian classifier (Wang et al., 2007) and the SILVA 138 database (Quast et al., 2013).

**Shotgun metagenomic sequencing data analysis**

The Shotgun data in Lewis *et al.* 2015 was analyzed using the taxonomic classification services provided by the Pathosystems Resource Integration Center (PATRIC (Davis et al., 2020)). PATRIC can take the SRA accession number of each sample as an input without downloading or transferring the sequencing files, and output the taxonomic classification of metagenomic DNA sequences included in the sample. The default algorithm is Kraken2 (Wood et al., 2019), which uses exact k-mer matches to achieve high accuracy and fast classification speeds.

We used iRep (Brown et al., 2016) to determine the replication rates of *Streptococcus spp.* that dominate stool samples in the MSK cohort. We first identified 33 Shotgun samples with at least 10% ASV\_8 or ASV\_16 based on their relative abundances in the 16S samples. We adapted a recently published pipeline (Siranosian et al., 2021) to assemble the contigs from short reads using MEGAHIT (Li et al., 2015) and then bin the contigs into Metagenome-assembled genomes (MAGs) using two different methods: Metabat2 (Kang et al., 2019) and CONCOCT (Alneberg et al., 2014). The results were then aggregated to produce an optimized, non-redundant set of MAGs by DAS Tool (Sieber et al., 2018). The high-quality MAGs (75% complete, 175 fragments/Mbp sequence, and 2% contamination) classified as *Streptococcus spp.* by Kraken2 (Wood et al., 2019) were analyzed by iRep. The iRep value of a MAG represents the average number of replication events over different subpopulations of the MAG weighted by their relative abundances.

**Statistical analysis**

Pairwise Kruskal-Wallis test was used exclusively for multiple comparisons. The raw *P*-values were adjusted for multiple testing using Benjamini-Hochberg correction to control the FDR and associations with post-correction *P*<0.05 were considered significant. When different stratified fecal sample groups are compared for their oral bacteria fractions, we replaced values less than 0.034 with 0.034 in the test. This is because comparing oral bacteria fractions below this threshold is meaningless for detecting significant differences in the total bacterial density between the groups (see **Fig. 3D** in the main text). The Student’s t-test was used to assess the significance of slopes of linear fits in the scatter plots between bacterial relative abundance and total bacterial or microbiome density.

We used the Cox’s time-varying proportional hazard model to regress fecal dominations of oral bacteria (relative abundance30%) as a microbial endpoint of interest against antibiotic administration (aminoglycosides, aztreonam, piperacillin/tazobactam, linezolid, sulfamethoxazole/trimethoprim, cephalosporins, oral vancomycin, intravenous vancomycin, amoxicillin clavulanate, quinolones, metronidazole, doxycycline, clindamycin, macrolide derivatives, carbapenems, tigecycline, other antibiotics) as multivariable predictors. Although the enrichment of oral species in gut microbiome has been linked to liver cirrhosis (Dubinkina et al., 2017; Qin et al., 2014), liver dysfunction such as altered bile acid secretion is less likely a causal factor for allo-HCT patients since the collateral damage on liver caused by the pretransplant regimen is not as severe and patients with severe liver disease would not undergo transplantation. Next, we again examined domination of oral bacteria in fecal samples, this time as a univariable predictor of *Enterococcus* infection using the same time-varying Cox’s hazard model. Penalty was not added for all Cox hazard calculations. The oral domination states in missing samples were imputed as the same as their nearest preceding samples within 4 days and assumed false if their nearest preceding samples are 5 or more days earlier. For survival analysis of mortality, we looked at the most common likely origin of fecal samples collected between day 0 and engraftment, as the exposure. Outcome analysis started at engraftment. To compare overall survival Kaplan-Meier curves were plotted and between-group *P*-values were calculated using Wald test.

All statistical analyses were performed using Python v3.7.9. Information on the statistical tests and simulations can be found in the figure legends and in the corresponding Method Details.

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