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Enteric Populations of *Escherichia coli* are Likely to be Resistant to Phages Due to O Antigen Expression

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Abstract (250 words)

There is a surfeit of bioinformatic data showing that bacteriophages abound in the enteric microbiomes of humans. What is the contribution of these viruses in shaping the bacterial strain and species composition of the gut microbiome and how are these phages maintained over time? To address these questions, we performed experiments with *Escherichia coli* and phages isolated from four fecal microbiota transplantation (FMT) doses as representative samples of non-dysbiotic enteric microbiota and develop and analyze the properties of a mathematical model of the population and evolutionary dynamics of bacteria and phage. Our models predict and experiments confirm that due to production of the O antigen, *E. coli* in the enteric microbiome are likely to be resistant to infection with co-occurring phages. Furthermore, our modeling suggests that the phages can be maintained in the population due to the high rates of host transition between resistant and sensitive states, which we call leaky resistance. Based on our observations and model predictions, we postulate that the phages found in the human gut are likely to play little role in shaping the composition of *E. coli* at the strain level in the enteric microbiome in healthy individuals. How general this is for other species of bacteria in the enteric flora is not yet clear, although O antigen expression is common across many taxa.

Importance

Bioinformatic data shows that bacteriophages are ubiquitous in the gut, however little is known about the role these bacterial viruses play in shaping the bacterial species and strain composition in the human gut microbiome or how they are maintained over time in this dynamic environment. Here we show that *Escherichia coli* isolated from fecal samples are likely to be resistant to their co-existing phages due to production of the O antigen. Based on these results, we postulate that bacteriophages are likely playing little role of shaping the abundance and diversity of bacteria in the human gut microbiome in healthy individuals.

Main Text

Introduction

Within the last decade, research on the composition and function of the human microbiome has increased exponentially (1). The human microbiome is composed of trillions of microbes, including bacteria, viruses, fungi, protozoa, and archaea that are found within and upon our bodies (2). The enteric (gut) microbiome, in particular, has been tightly linked to human health and well-being (3-5). Many of greatest threats to human health such as cardiovascular disease, malignant carcinoma, malnutrition, and antimicrobial resistance have also been linked to the gut microbiome (6-12). It has been suggested that interventions modifying the enteric microbiome could address many of these issues. However, realizing the much-anticipated therapeutic potential of the enteric microbiome will require improved understanding of the microbial interactions that impact health and disease.

Most empirical studies of the human gut microbiome employ culture-independent, bioinformatic approaches (13, 14). These bioinformatic-based studies infer “who is there” and “what they are doing” from DNA sequence data (15). On the other hand, these studies provide little information about the population dynamics of bacteria and phage, especially about “who is infecting whom” and how that could determine and change the composition of species and strains of microbes within a given microbiome (16). Understanding these processes is critical to elucidating the mechanisms by which interventions may increase desirable and decrease less desirable microbiome functions.

One intervention that has shown clinical success in modifying microbiome states is fecal microbiota transplantation (FMT). FMT is performed by the enteric administration of intestinal microbiota from a healthy donor, typically in a recipient with a microbiome-associated disease. The most frequent clinical use of FMT is for treatment of recurrent *Clostridioides difficile* infection (RCDI) (17). Early-stage clinical trials have shown FMTs to also be highly efficacious at reducing colonization with multi-drug resistant bacterial organisms (MDRO) at large (18-20). It has recently been shown that FMTs may reduce recurrent MDRO infections as well as reduce the abundance of antibiotic-resistant bacteria colonizing the enteric microbiome (21). Beyond clinical use, FMT has also become an important model of microbial ecology and microbial competition within the context of human hosts. However, the mechanisms by which FMT may modify the microbiome states of recipients have just begun to be uncovered and the extent to which bacterial interactions fully account for the observed effects is unclear.

Metagenomic and limited experimental data suggest that the viral fraction of the enteric microbiota could contribute to the efficacy of FMT. For example, a small clinical trial with five patients receiving sterile filtrates of a donor’s microbiome was shown to be as effective as unfiltered FMTs (22), with other studies finding qualitatively similar results (23). Similarly, a study of fecal filtrate transfer in a piglet model prevented necrotizing enterocolitis (24). Could bacteriophage (phage), a filterable element of the gut microbiome, be responsible for changing the composition and structure of the recipient’s microbiome? Stated another way, could treating with an FMT be a kind of phage therapy? To address this question and understand the role phages may have in shaping the species and strain composition of the microbiome when transferred, we investigate the role of phages in determining the densities and distributions of *Escherichia coli* strains within the gut microbiome of four individual healthy hosts who have qualified as FMT donors (21) using bioinformatics, mathematical models, and culture-based infection assays. We focused on *E. coli* (25), due to its ubiquity in the human gut microbiome and because its phages have relatively large host ranges, unlike the phages of other enteric residents such as *Bacteroides spp (26).*

Results

E. coli and phage isolation from FMT doses

We first sought to isolate and characterize both major and minor populations of *E. coli* as well as the dominant *E. coli* bacteriophage populations from four FMT donors using the methods illustrated in Figure 1. Towards this end, to isolate the dominant, most frequent, population of *E. coli*, FMT samples were directly plated at a high dilution on *E. coli* selective media. To isolate minority populations of *E. coli*, FMT samples were plated on *E. coli* selective media containing different antibiotics.

We were not able to recover any bacteriophage via direct plating on lawns of either laboratory strains or fecal-derived *E. coli*, indicating that the density of phages in these FMT samples is likely low. Further, we were not able to obtain phage by simply adding Lysogeny Broth (LB) to the FMTs, which allowed for bacterial replication, and should have allowed the phage to replicate on the co-existing *E. coli.* This suggests that the phage do not grow well on the bacteria co-existing in the FMT. Moreover, phage could not be obtained when both LB and *E. coli* from other FMTs were added to each of the FMTs. This suggests that there is a property inherent to FMT-derived bacteria that do not make them a suitable host for phages. The hypothesis that FMT-derived *E. coli* are poor hosts for phage is further supported in that to obtain phages from FMTs, all FMT samples required the addition of both LB and a broadly phage-sensitive, non-lysogenic laboratory *E. coli* strain which lacks restriction modification systems. The results, taken together, show that there are phage in the FMTs, but that some property of the *E. coli* in the FMT is limiting the replication of the phage—a property which laboratory strains of *E. coli* do not possess.

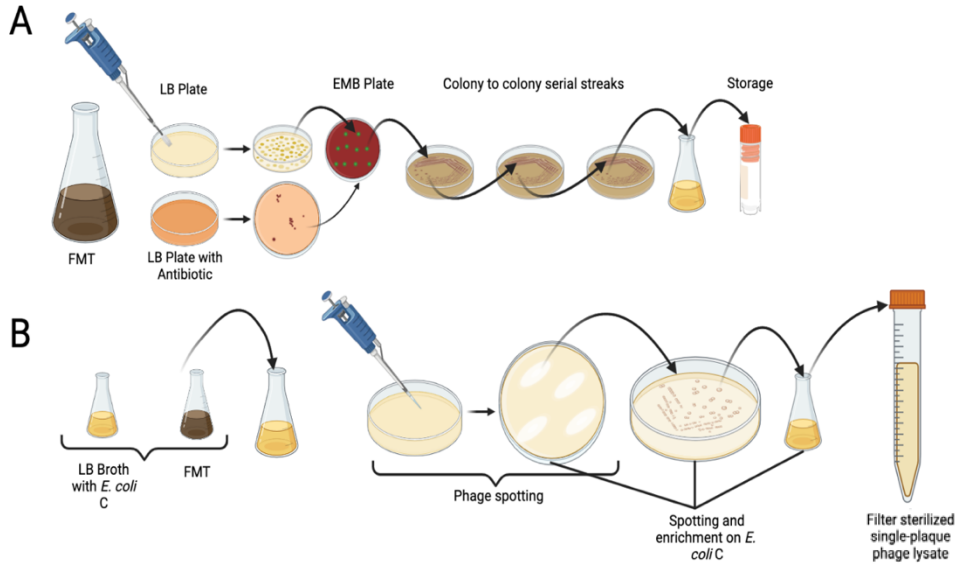

Figure 1. Diagram of bacteria and phage isolation protocol. (A) Method employed for isolating the dominant as well as minority populations of *E. coli* from the FMT samples. **(B)** Method employed for isolating bacteriophage from the FMT samples.

Genotypic analysis and diversity of the isolated *E. coli* and its phages

The virtue of culture-indepent methods for investigating the bacterial composition of the microbiome is the reduction of bias that is inherent to culture-dependent methods. To ensure that the isolates of both *E. coli* and its phage we obtained represent a sampling of the diversity that

exists in the enteric microbiome, we performed genotypic analyses of the isolates obtained above.

Via comparative genomic analysis, we identified an apparent dominant strain in each FMT (representing more than half of all *E. coli* isolated from a sample) and several minority strains of *E. coli* in each sample as shown in Figure 2. This demonstrates that we were successful in isolating distinct populations of *E. coli*. For each FMT donor, we assessed the diversity of recovered *E. coli* strains by alignment of predicted gene clusters, average nucleotide identity (ANI), *in silico* multi-locus sequence type (MLST), and *in silico* H and O serotype prediction. We chose to predict the H and O serotype *in silico* as the diverse expression of these antigens were traditionally a measure of *E. coli* diversity (27). When applying an ANI-based genomovar definition of $\geq$99.5% ANI, we identified a median of 2.5 (range 2-3) *E. coli* strains per donor (28). In general, classification by MLST was consistent with the ANI-based clustering. However, strains with similar ANI and of the same MLST displayed a great diversity in predicted O antigen type. These findings demonstrate that healthy individuals carry diverse *E. coli* strains as previously shown (29) and that our methods were readily capable of capturing that diversity.

We also were able to recover three unique phages each from three of the four donors for a total of nine FMT-derived phages; of note, we were not able to recover any phages that formed plaques from Donor A, even after enrichment with the lab strain of *E. coli*. Phylogenetic analysis of the phage genomes were conducted by generating an average amino acid identity (AAI) heatmap (Supplemental Figure 1) and consensus maximum likelihood phylogenetic trees based on the core phage genomes (Figure 2 Panel B). Based on AAI, we see a clear segregation between the *Peduovirus* genomes and those of the lambda and T-phages, which make up most of the genomes in the other two major clusters in the heatmap. The reference *Peduovirus* genomes, genomes isolated in this study, and the IMG/VR genomes shared AAI values of $\geq$90%, which reflects the high similarity in gene content between these phage. The consensus tree of these gene trees, estimated using Astral (30), is shown in Figure 2 Panel B. The tree was rooted using *Escherichia virus vB_eco_mar005P1*. The additional two genomes used as outgroups to root this tree were added manually. The phylogeny demonstrates that the genomes of donor D are only distantly related to those of B and C. The phages from donors B and C also appear to cluster separately, with the exception of a single phage, which is closely related to the genomes of donor B despite being isolated from donor C. This suggests that each FMT donor has multiple distinct phage populations and that the populations within each donor are distinct from the other donors.

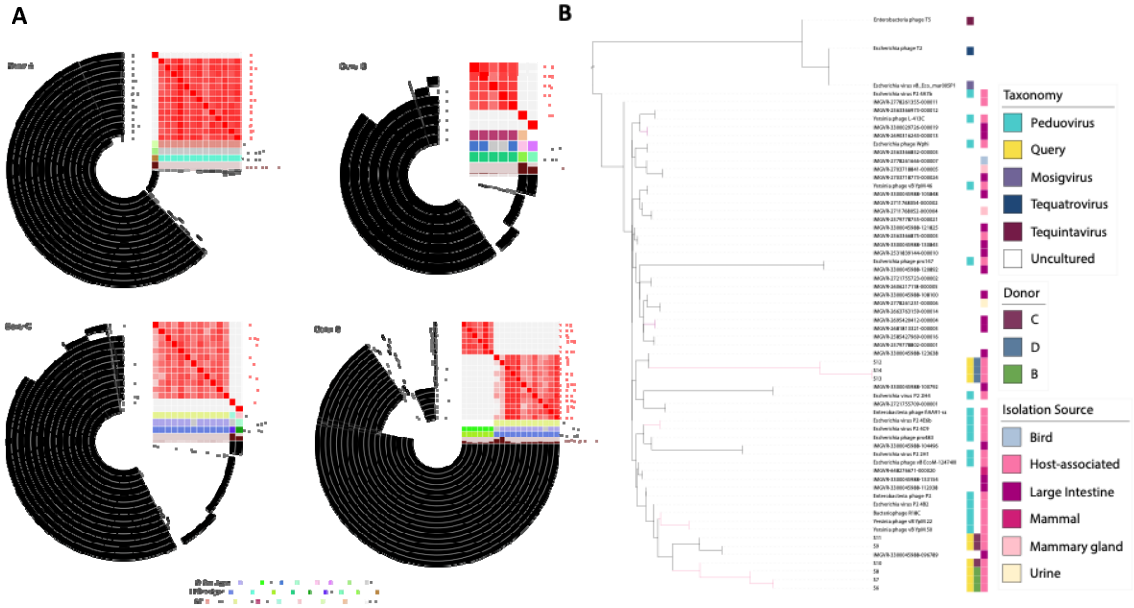

Figure 2. Diversity of isolated *E. coli* and phages. (A) Circular clustered gene alignments of *E. coli* isolated from four healthy donors show maintenance of minority populations that differ by average nucleotide identity (ANI, shown as red heat map scaled from 0.995 to 1.0), gene content (with singleton gene clusters ranging from 0 to 722), *in silico* multi-locus sequence type (MLST) classification, and *in silico* predicted O and H antigens. **(B)** Bacteriophage Consensus Maximum Likelihood phylogenetic tree based on core genes of the phage genomes in this study and close relatives from public databases**.** Thirty-two genes were shared in more than 55/60 of the genomes in the major amino acid identity (AAI) cluster of Supplemental Figure 1 and were used to build individual trees as described in the Methods Section.

<u>Susceptibility of *E. coli* isolates to phage</u>

For bacteriophages to shape the composition of an established enteric microbiota, a substantial fraction of the resident *E. coli* must be sensitive to those phages. To determine whether this is the case, we tested the susceptibility of the *E. coli* isolated above by spotting three classes of phages on lawns of each of the bacteria: i) five well-characterized laboratory phages not isolated from feces; ii) the co-existing phages isolated from the same donor; and iii) phages isolated from the other donors. In total, we tested 751 combinations of 54 bacterial isolates and 14 phage isolates. The laboratory strains of phages are expected to form clear plaques on lawns of sensitive bacteria, due to being obligated lytic. We find with the *E. coli* C lab strain that this is the case as all 14 tested lab and FMT-derived phage isolates produced clear plaques. As shown in Figure 3, this is not the case with the FMT-derived *E. coli* isolates, which give either no plaques (217/265, 81.9%) or turbid plaques (42/265, 15.8%) to the lab phages with one singular exception (T3 phage spotted on EC1D8; 1/265, 0.3%). When spotting FMT-derived phages on FMT-derived *E. coli* isolates, 392/477 (82.2%) combinations produced no plaques and 85/477 (17.8%) produced turbid plaques.

For phages in FMT doses to shape the composition of a recipient's enteric microbiota, the recipient's *E. coli* must be sensitive to FMT-derived phage. However, when comparing combinations of FMT-derived phage and *E. coli* isolated from the same (autologous, e.g. phages

from Donor B and *E. coli* from Donor B) or different donors (allogeneic, e.g. phages from Donor B and *E. coli* from Donor D) we do not find sensitivity. We found a significantly higher proportion of turbid plaques with autologous phage-*E. coli* combinations compared to allogeneic phage-*E. coli* combinations (29/117, 24.5% vs 56/360, 15.6%; chi-square p-value 0.033). Further analysis of these and the above pairs of bacteria and phage are described in Supplemental Table 1. These results, taken together, further the notion that there is something intrinsic to the FMT-derived bacteria that makes them less sensitive to phages—not just FMT-derived phages, but phages in general.

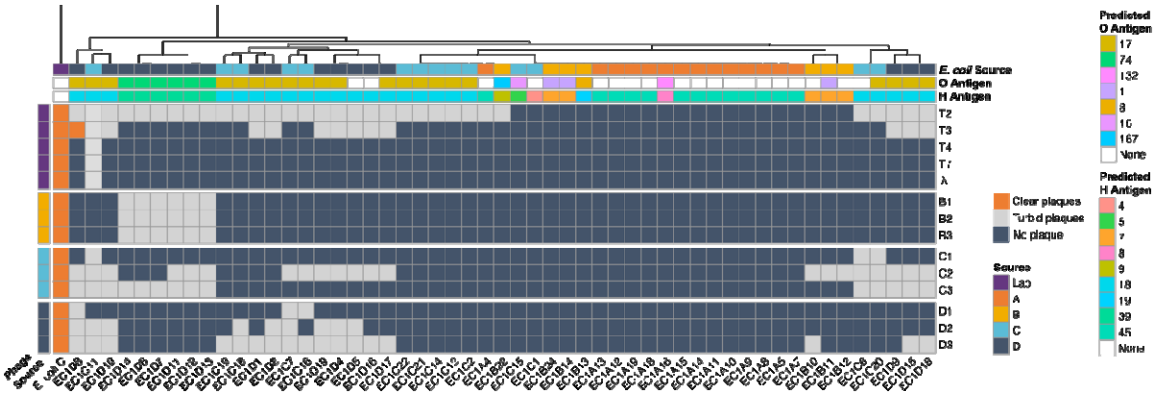

Figure 3. FMT isolated *E. coli* do not give clear plaques when spotted with laboratory or FMT-derived bacteriophages. Well-characterized laboratory and FMT-derived *E. coli* strains (Indicated by Source as Lab or A through D) were spotted with well-characterized laboratory (T2, T3, T4, T7, and a virulent mutant of phage λ) and FMT-derived bacteriophages (isolated from FMT donors B through D). Dark blue denotes no plaque formation, light grey denotes turbid plaque formation, and orange denotes clear plaque formation. Clear plaques were only produced by the tested phages on lawns of a laboratory strain of *E. coli* C indicating broad resistance or immunity of FMT-derived *E. coli* to phages from all sources.

<u>The O antigen as a mechanism for broad phage resistance</u>

One explaination for the broad level of phage resistance observed in the wild *E. coli* isolates is the display of the O antigen (31-33). This hypothesis is consistent with the fact that we can only isolate phage from the FMT samples by adding in both rich media and a broadly phage-sensitive lab strain of *E. coli*. Most lab strains, including *E. coli* C, do not express the O antigen, while the expression of the O antigen is nearly ubiquitous among wild *E. coli*. As evidence in support of this O antigen hypothesis, we performed spot assays with MG1655 (an O antigen negative lab strain), MG1655L5 (an isogenic mutant of MG1655 expressing a medium level of the O antigen), MG1655L9 (an isogenic mutant of MG1655 strongly expressing the O antigen), and the lab phage T7 (for further details on the bacterial strains and their sources see the Materials and Methods).

MG1655L9 does not support the production of plaques as is also seen with many of the FMT-derived *E. coli* strains. Importantly, lawns of MG1655L5 do support the production of plaques though those plaques are turbid (Figure 3). We interpret the turbidity of the plauqes found with the highly lytic phage T7 and MG1655L5 to support the notion that turbid plaques, which are traditionally associated with temperate phage, can occur with lytic phage when the dominant bacterial population within a plaque is resistant to the infecting phage. We have restricted the assays with our constructed O antigen strains to phage T7 for several reasons: i) this phage is known to have its receptor masked via steric inhibited due to the production and display of the O antigen; ii) bacteria require two genomic mutations to generate classical receptor site resistance to this phage, making the evolution of resistance unlikely in our assays (34); and iii) as shown in Figure 3, T7 forms no plaques on all but one of the FMT isolated *E. coli* and the plaques found on the exceptional isolate were turbid.

As additional evidence in support of the hypothesis that the O antigen is the reason for the broad level of phage resistance observed here, we repeated the phage isolation protocol described in Figure 1B using MG1655 and both of the above O antigen constructs, MG1655L5 and MG1655L9. MG1655, which lacks the O antigen, was able to enrich for phage in the FMTs while

neither of the O antigen expressing constructs were able to enrich for phages present in the fecal samples.

O antigen profile of enteric *E. coli*

We postulated that expression of the O antigen could account for the lack of clear plaques observed in Figure 3. To ascertain if *E. coli* isolated from FMTs bear the O antigen, we used the tool ECTyper to predict the O antigen type for each isolate (35). As shown in Table 1, we then serologically confirmed O antigen production by antiserum agglutination assay for the 34 strains that were annotated by this tool. While our tool did not annotate the O antigen type of all of our isolated strains, we believe that the strains which ECTyper could not predict are also O antigen positive as their dominant populations were found to be resistant, in similar fashion to the strains below in Table 1.

Table 1. O antigen type and production of FMT-derived *E. coli* isolates.

Strain	Predicted O antigen Type	Serological Confirmation
EC1A16	132	Positive
EC1B11	1	Negative
EC1B13	8	Positive
EC1B14	1	Positive
EC1B22	167	Positive
EC1B24	1	Positive
EC1C2	17/44	Positive
EC1C7	17/77	Positive
EC1C11	17/77/44	Positive
EC1C12	17/77	Positive
EC1C14	17/44	Positive
EC1C15	16	Positive
EC1C19	17/44	Positive
EC1C20	17/44	Positive
EC1C21	17/44	Positive
EC1C22	17/44	Positive
EC1C16	17/44	Positive
EC1C18	17/44	Positive
EC1D1	17/77	Positive
EC1D2	17/77/44	Positive
EC1D4	17/77/44	Positive
EC1D6	74	Positive
EC1D7	74	Positive
EC1D8	17/77	Positive
EC1D9	17/77	Positive
EC1D12	74	Positive
EC1D13	74	Positive
EC1D14	74	Positive
EC1D15	17/44	Positive
EC1D17	17/44	Positive
EC1D18	17/77/44	Positive
EC1D19	17/77	Positive
EC1D10	17/77	Positive
EC1D11	74	Positive

If the bacteria are resistant, how are the phages still present in the enteric microbiome?

Despite the observed resistance, we were still able to isolate bacteriophages from these FMT doses. How are these phages maintained if the dominant population is resistant? We postulate that phage maintenance occurs due to what we call “Leaky Resistance”, where there is a high transition rate from a resistant state to a sensitive state, providing a sufficient density of sensitive cells on which the phage can replicate. For this analysis, we adapted our model from (36). In Supplemental Figure 2, we present a diagram of our mathematical model of the population and evolutionary dynamics of lytic phage and bacteria. In the Supplemental Text, Supplemental Equations, and Supplemental Table 2, we detail two mathematical models and their parameters that could account for the presence of lytic and free temperate phages in the FMTs which are dominated by resistance. The results of the model with temperate phages (Supplemental Figure 3) are qualitatively similar to that with the lytic phage. The predictions of the model with lytic phage are presented in Figure 4.

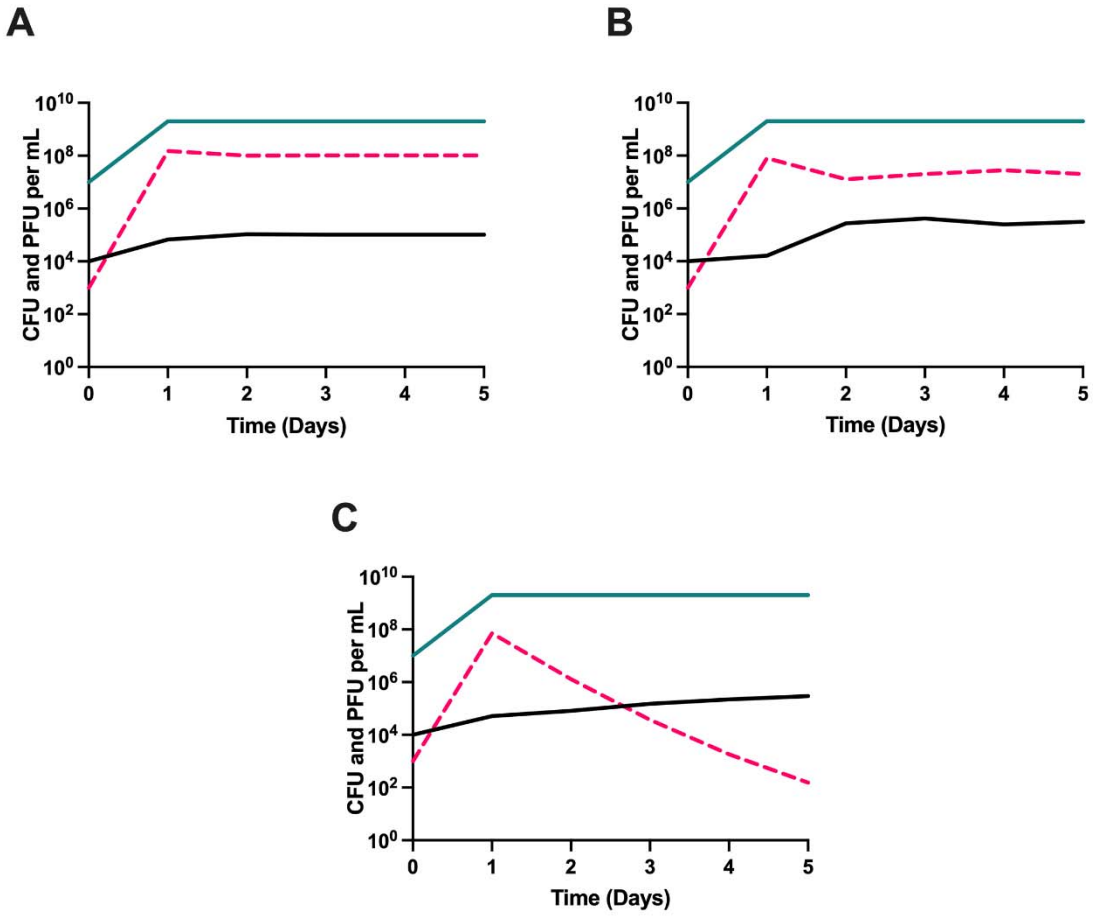

Figure 4. Predicted serial transfer dynamics of O antigen mediated resistance and a lytic phage. Simulation results, changes in the initial and end of transfer densities of bacteria and phage in serial transfer culture with three different transition rates between states. Where 1000 µg/ml of the limiting resource is added at each transfer. **A-** Transition rates from sensitive to resistant and from resistant to sensitive of 10^{-3} per cell per hour. **B-** Transition rates from sensitive to resistant and from resistant to sensitive of 10^{-4} per cell per hour, and **C-** Transition

rates from sensitive to resistant and from resistant to sensitive of 10^{-5} per cell per hour. O antigen expressing cells- turquoise, Sensitive cells- black, Phage- pink.

The mathematical model predicts that for the phage to be maintained on minority populations of sensitive cells, the transition rate between resistant and sensitive must be in excess of 10^{-5} per cell per hour. In Figure 5, we perform serial transfer experiments in parallel to those modeled in Figure 4. In Supplmental Figure 4 we consider the effect that the fitness cost of resistance and the effect that an unequal transition rate would have on these predicted dynamics. We find the relative fitness cost to have no qualitative effect on the predicted dynamics. Making the rate of transition to resistance greater than the rate to sensitivity has no effect on the dynamics, while, making the rate of transition to sensitivity greater than the transition rate to resistance allows the phages to be maintained at a greater range of transition rates (i.e. the phage are not loss at transition rates of 10^{-5} per cell per hour).

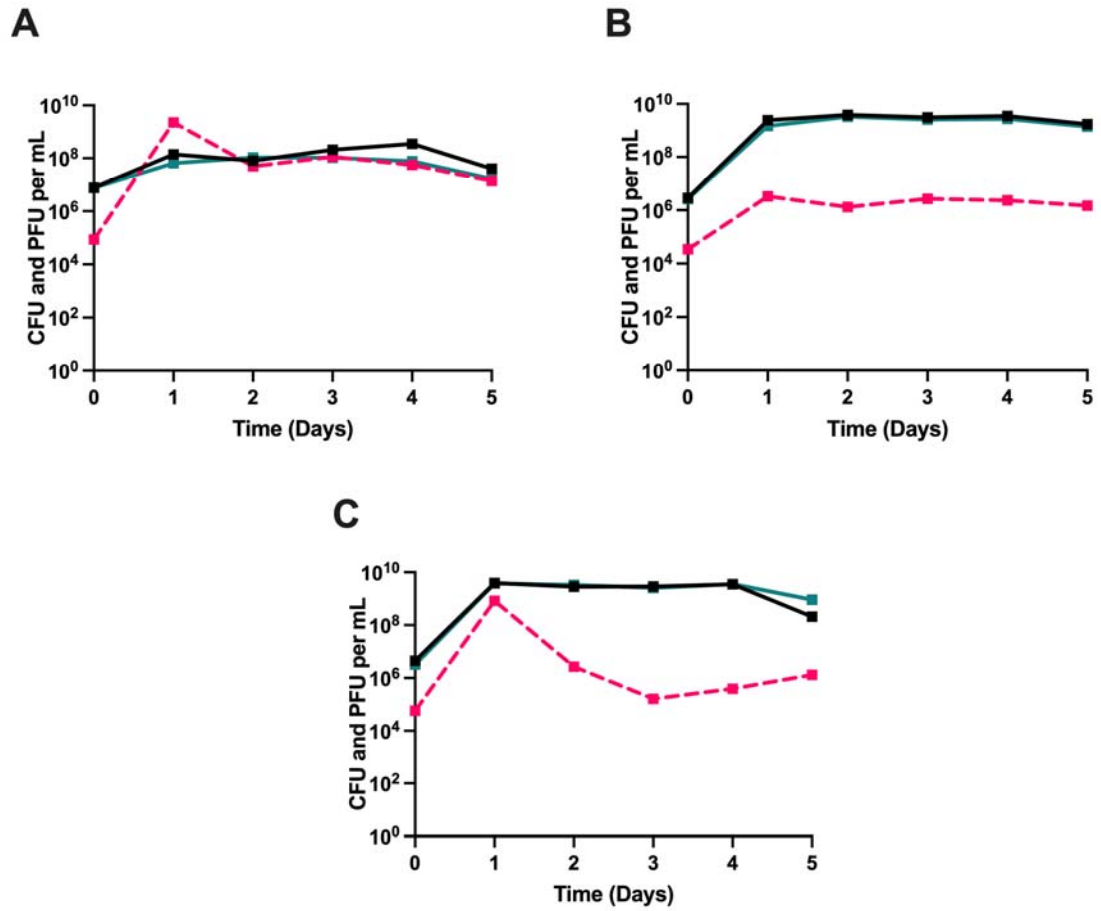

Figure 5. Serial transfer of T7 with MG1655 lacking and expressing the O antigen. Three variants of *E. coli* MG1655 serially transferred for five days in LB broth with the phage T7. Shown in solid lines are bacterial densities with the black line representing a phage free control. The broken lines represent phage densities. Shown is one of three biological replicates. **(A)** Wild type MG1655 not expressing the O antigen. **(B)** MG1655L5 expressing a medium amount of the O antigen. **(C)** MG1655L9 expressing a high amount of the O antigen. Two other, qualitatively similar, replicates are presented in Supplemental Figure 5.

Although we are unable to estimate the density of sensitive cells in our experiments, we find with MG1655, MG1655L5, and MG1655L9 that the phage are maintained, albeit at different levels.

This is consistent with the transition rate to sensitivity being at least 10^{-5} per cell per hour and demonstrates that leaky resistance is sufficient to independently account for how the phage are maintained despite the bacterial population being dominated by resistance.

Evolution of O antigen mediated phage resistance

Critical to the evolution of a novel phenotype is the ability of the genes encoding for that phenotype to increase when rare. Presumably the *E. coli* colonizing humans use the O antigen to protect against phagocytosis. This is one likely reason that naturally occurring *E. coli* bear and express the genes for the O antigen. The results of this study suggest there is an additional selective force for the evolution of the O antigen: bacteriophage predation. Consistent with this hypothesis is the observation that *in vitro* O antigen-expressing bacteria increase in frequency when rare in a population of O antigen-deficient cells only when bacteriophage are present (Supplemental Figure 6).

Discussion

We set out to elucidate the role of bacteriophages in shaping the strain and species composition of bacteria in the enteric microbiome. Our primary interpretation of the results presented in this report is that in the enteric microbiome of healthy individuals, bacteriophages are likely to play little role in determining the density, strain, and species composition of bacteria due to the high prevalence of phage resistance. In the case of *E. coli*, the expression of the O antigen is sufficient to confer phage resistance while allowing for the maintaince of phage due to the random transition between a phage-resistant and sensitive state, what we call leaky resistance. While there are several mechanisms that convey bacteriophage resistance (such as receptor site mutation and the carriage of phage resistance genes on prophage (37, 38)) we demonstrate that expression of the O antigen alone is sufficient to account for the resistance of *E. coli* to a broad swath of phages, including those to which they have never been exposed. Our models and experiments support the hypothesis that despite this resistance, phage will be maintained in communities of these O antigen expressing bacteria. Moreover, we expect that this same O antigen-mediated resistance phenomenon could be manifest in all *Enterobacteriaceae*. Stated another way, we postulate that resistance to co-occurring phages will be the dominant characteristic of bacteria in stable natural communities (39). This prediction is consistent with experimental studies of lytic and temperate bacteriophages *in vitro* (34, 40).

Resistant mutants almost always emerge and ascend to become the dominant population of bacteria in *in vitro* experiments with laboratory strains of bacteria and lytic phages. Nevertheless, phages are commonly maintained in these cultured populations that are dominated by resistant bacteria (40, 41). There are a variety of mechanisms by which the phage can be maintained, but their maintenance appears to play little role in regulating the densities of these cultures and these bacterial populations are more likely shaped by other proccessses such as resource availability. We postulate this could also be the case in the gut microbiome. While there are many other mechanisms by which phages can be maintained, such as physical refugia as a consequence of the structure of the gut or spontaneous temperate phage induction (Supplemental Figure 3), we have demonstrated that leaky resistance is sufficient to independently account for the results observed here. There is, however, a caveat to our purported mechanism for phage maintenance in that one prediction of our model is that the density of bacteria would exceed that of phage in natural populations. How common that is in natural communities is unclear as in marine settings it is thought that the density of phage far exceeds that of the bacteria (42, 43), though recent studies have questioned how high this ratio actually is (44). There is another caveat, in that we have restricted our study to human samples, because of the clinical relevance, we have however noted, studies by colleagues looking at the phage and *E. coli* interactions in the microbiome of other animals such as horses (45). However, we believe the mechanism proposed here happens broadly in nature and in the gut, though we cannot say how common this is, but could be answered by other investigators with culture-independent approaches.

We expect that the approach developed in this study also has broader utility for translational phage investigation. If phages are to be developed as therapeutics or as components of live biotherapeutic consortia, we expect that this will require complementary culture-based and culture-independent techniques that include experimental isolation, characterization, and strain-resolved analysis in the context of complex communities.

Fueled by the increasing incidence of antibiotic resistant infections in recent years there has been a resurrection of` interest in using bacteriophage to treat bacterial infections. When it comes to utilizing bacteriophages therapeutically, one would expect a tradeoff between this generalized resistance mechanism and bacterial virulence. This phenomenon was illustrated in a classical study by Smith and Huggins, where they utilized a phage targeting the K antigen of pathogenic *E. coli (46)*. Interestingly, the mechanism of resistance explored here, the O antigen, is known to be the target of some bacteriophages (47). If the results presented here for *E. coli* are at all general,

we would expect difficulties in isolating phage capable of lysing specific bacterial isolates. This anticipated result is echoed by investigators working on isolating phage for therapy where the rates of finding phage for an isolate are less than 50% for some pathogens (48-50). Here we provide one explanation for why this could be the case, resistance associated with production of the O antigen.

Materials and Methods

Fecal Microbiota Transplant Doses. Healthy human participants were recruited and provided informed consent to participate in a stool donor protocol approved by the Emory University Institutional Review Board (Protocol 00112302, PI Woodworth). Donors completed health and behavior questionnaires, blood, urine, and stool laboratory testing to screen for potential pathogens as described in the PREMIX trial (21). Each stool specimen from a single donor was divided by weight with 50-100g of stool in a Seward filter bag and suspended in 250mL USP sterile saline using a Seward benchtop stomacher at 200 rpm for 30 seconds twice. After removing the filter bag, the remaining suspension of stool in saline was transferred back to the 250mL saline bottle and delivered fresh without freezing for *E. coli* and phage isolation as described below. Additional aliquots with and without 10% glycerol by starting volume were prepared and stored at -80 °C.

Bacterial Strains. *E. coli* C was obtained from Marie-Agnès Petit at INRAE in Joy-en-Josas, France. MG1655 L9 and L5 were obtained from Douglas Browning at Aston University in Birmingham, UK (51). Briefly, these constructs were made by cloning either the *rfb* cluster or *wwbL* into isogenic MG1655. Wild *E. coli* were isolated from fecal microbiota transplantation doses as described below.

Bacteriophages. Phages Lambda^{VIR}, T2, T3, T4, and T7 were acquired from the Levin Lab's phage collection. Wild phages were isolated from fecal microbiota transplantation doses as described below.

Growth Media and Conditions. Unless otherwise noted, all bacteria were cultured in Lysogeny Broth (LB) at 37 °C. Lysogeny broth (LB) prepared according to manufacturer instructions (Difco, 244620). LB soft agar made with 0.7% w/v agarose, and LB plates made with 1.6% agarose, LB phage plates prepared as LB plates supplemented with 20 mM $CaCl_2$.

Isolating Bacteria from the FMTs. Serial dilutions of FMT in 0.85% NaCl solution was followed by plating on Davis Minimal (DM) plates (Sigma-Aldrich, 15758) supplemented with 0.4% w/v lactose (Sigma-Aldrich, L3750). Individual colonies were randomly chosen and picked with a sterile stick and simultaneously streaked onto an EMB plate and a DM plate. Both were incubated at 37 °C overnight. Isolates colored green on the EMB plate were picked from their its respective minimal plate and serially streaked from the DM lactose plate onto another DM lactose plate and incubated at 37 °C overnight. Each isolate was labeled, stored, and frozen.

Isolating Phage from the FMTs. 0.1 mL of FMT suspension was inoculated into 10mL LB broth with ~1e^{7} CFU/mL *E. coli* C. These flasks were grown with shaking at 37 °C overnight. The next day, samples were centrifuged and filtered through a 0.22-micron filter (Basix, 13-1001-06). These rough lysates were then plated for single plaques. Individual plaques were picked with a sterile stick and resuspended as described above to obtain clonal phage lysates.

AAI heatmap. We identified the closest relatives to the genomes of this study (query genomes) using whole-genome searches against public genome databases. The final set of genomes used in subsequent analyses consisted of 98 genomes – including the 9 query genomes, 34 genomes from the IMG/VR v4 database (52), and 55 genomes that are publicly available on NCBI. Prodigal v2.6.3 (53) was used for gene prediction with default settings, and pairwise all versus all Average Amino acid Identity (AAI) was calculated using the aai.rb script from the Enveomics collection (54) using default settings except for decreasing the number of minimum hits to 20. The final heatmap was constructed using the pheatmap package in R. Accession numbers of the public genomes are labelled in the figure.

Core gene tree. The subset of genomes that make up the major cluster in the AAI heatmap were used for gene clustering at 30% identity with MMseqs2 v 13.4511 (55). The resulting clusters were used to identify the core genome – the genes that are found in 55/60 (~90%) of the genomes. The 32 core genes were extracted using seqtk-1.4 and individual gene alignments were generated using MUSCLE v3.8.31 (56) with default settings. Each alignment was used to generate a maximum likelihood gene tree in MEGA11 (56) with default parameters. These gene trees were concatenated and passed into Astral 5.7.8 (30) to generate the final consensus tree as Astral has a built-in algorithm to deal with any missing data in the input gene trees. The final tree was visualized and annotated in iTol (57) and is shown in Figure 2B.

Phage Susceptibility Testing. ~$1e^6$ of each phage lysate was spotted onto lawns of the bacteria using the double-layer soft agar technique as described in (58). Phage lysates were diluted 1:100 prior to spotting to minimize lysis from without.

Sampling Bacterial and Phage Densities. Bacteria and phage densities were estimated by serial dilutions in 0.85% NaCl solution followed by plating. The total density of bacteria was estimated on LB (1.6%) agar plates. To estimate the densities of free phage, chloroform was added to suspensions before serial dilutions and double-layer soft agar enumeration.

Serial Transfer Experiments. All serial transfer experiments were carried out in 10 mL LB broth cultures grown at 37 °C with vigorous shaking. The cultures were initiated by 1:100 dilution from 10 mL overnight cultures grown from single colonies. Phage was added to these cultures to reach the initial density of~10^7 PFU/mL. At the end of each transfer, 0.1 mL of each culture was transferred into flasks with fresh medium (1:100 dilution). Simultaneously,0.1 mL samples were taken for estimating the densities of CFUs and PFUs as described above.

Antibiotics and Their Sources. Streptomycin (S6501) was obtained from Sigma-Aldrich; Tetracycline (T1700) from Research Products International; Gentamicin (BP918-1) from Fisher BioReagents; Chloramphenicol (C5793) from Sigma-Aldrich; Ciprofloxacin (A4556) from AppliChem); Ceftriaxone (C5793) from Sigma-Aldrich; Meropenem (QH-8889) from Combi-Blocks; Azithromycin (3771) from Tocris; Fosfomycin (P5396) from Sigma-Aldrich; and Colistin (C4461) from Sigma-Aldrich. All antibiotic Sensi-Discs were obtained from Becton Dickinson.

O Antigen Antisera Assay. *E. coli* O antigen specific antisera was obtained from SSI Diagnostica and used as detailed in their protocol for the slide agglutination assay. A known O antigen negative lab strain (MG1655) and its construct bearing the O antigen (MG1655L9) were used as negative and positive controls respectively.

Whole-Genome Sequencing. Samples were sent to MIGS (Pittsburgh, USA) as bacterial colonies grown on an agar plate or as sterile phage lysates for extraction and Illumina sequencing. Individual colonies and lysates were extracted per the manufacturer's protocol using Zymo's DNA miniprep bead beating lysis kit. Sample libraries were prepared for Illumina sequencing using Illumina's DNA Prep kit and IDT 10bp unique dual indices and sequenced on an Illumina NovaSeq 6000, producing paired end 151bp reads. Demultiplexing, quality control, and adapter trimming was performed with bcl-convert (v4.1.5). Illumina reads were quality filtered using Trimmomatic (59). *E. coli* C host sequences were depleted from phage lysate sequencing data by mapping to a closed *E. coli* C genome with bowtie2 (60). After trimming and host decontamination (for phage lysates), remaining reads were and assembled *de novo* using SPAdes v3.13 (61). Pairwise comparisons of average nucleotide identity on the assembled genomes were performed with the Mashmap method using fastANI v1.32 as in (62). Gene sequences were predicted with Prodigal v2.6.3 (53) and annotated with Prokka v1.14.6 (63). O and H antigens were predicted *in silico* with ECtyper (35). Bacterial pangenome figures were created with anvi'o v.7.1 (64).

Numerical Solutions (Simulations). For our numerical analysis of the coupled, ordered differential equations presented (Supplemental Equations 1-8) we used Berkeley Madonna using the parameters presented in Supplemental Table 1. Copies of the Berkeley Madonna programs used for these simulations are available at www.eclf.net.

Statistical Analysis. Statistical tests of difference in proportions of phage plaquing results tabulated as a contingency table were performed with the chisq.test function in stats package version 4.3.0 in R version 4.3.0 using the R studio interface version 2023.06.0. Resulting p-values <0.05 were considered statistically significant.

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Data Availability

The Berkeley Madonna program used for the simulations are available at ECLF.net. All raw sequence data for the *E. coli* and phage genomes shown have been deposited in the sequence read archive (SRA, NCBI, Bethesda, MD, USA) and can be found with bioproject accession PRJNA1028583.

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