Modeling the Infection Dynamics of Bacteriophages in Enteric *Escherichia coli*: Estimating the Contribution of Transduction to Antimicrobial Gene Spread

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Animal-associated bacterial communities are infected by bacteriophages, although the dynamics of these infections are poorly understood. Transduction by bacteriophages may contribute to transfer of antimicrobial resistance genes, but the relative importance of transduction among other gene transfer mechanisms is unknown. We therefore developed a candidate deterministic mathematical model of the infection dynamics of enteric coliphages in commensal *Escherichia coli* in the large intestine of cattle. We assumed the phages were associated with the intestine and were predominantly temperate. Model simulations demonstrated how, given the bacterial ecology and infection dynamics, most (>90%) commensal enteric *E. coli* bacteria may become lysogens of enteric coliphages during intestinal transit. Using the model and the most liberal assumptions about transduction efficiency and resistance gene frequency, we approximated the upper numerical limits (“worst-case scenario”) of gene transfer through specialized and generalized transduction in *E. coli* by enteric coliphages when the transduced genetic segment is picked at random. The estimates were consistent with a relatively small contribution of transduction to lateral gene spread; for example, generalized transduction delivered the chromosomal resistance gene to up to 8 *E. coli* bacteria/hour within the population of 1.47×10^8 *E. coli* bacteria/liter luminal contents. In comparison, the plasmidic *bla*_{CMY-2} gene carried by ~2% of enteric *E. coli* was transferred by conjugation at a rate at least 1.4×10^3 times greater than our generalized transduction estimate. The estimated numbers of transductants varied nonlinearly depending on the ecology of bacteria available for phages to infect, that is, on the assumed rates of turnover and replication of enteric *E. coli*.

Animal-associated bacterial communities, including enteric bacteria, are infected by bacteriophages, and the infection dynamics are poorly understood (1, 2). Bacteriophages can transfer genes encoding bacterial antimicrobial resistance (AMR) laterally between bacteria. The magnitude of the contribution of this transfer mode to the resistance distribution *in vivo* is unknown.

A DNA phage can transfer a bacterial gene, which is not a part of the phage’s genome, if the bacterial genome segment containing the gene is picked up accidentally from the lysed bacterium due to an error in phage replication and is transferred by the phage to another bacterium, a process called transduction. Once a temperate phage infects a bacterium, it could proceed to either a direct lytic cycle or a lysogenic cycle. In the latter case, the phage incorporates into the bacterium’s genome in the form of prophage for the duration of the lysogenic cycle; the phage commences the lytic cycle upon prophage induction. The term “specialized gene transduction” is used when the prophage mistakenly excises with the adjacent genome segment containing the gene; the segment size may be as large as the prophage size (3, 4). The genetic material of the resulting transducing virion (Table 1) may insert into the genome of the next infected bacterium, transferring the carried segment. The term “generalized gene transduction” is used when the gene is transferred by a transducing particle (*tp*) (Table 1). A *tp* is a nonreplicative virion that may be formed during a lytic cycle that either followed prophage induction or was direct. In this case, the accidentally packaged segment may be from anywhere in the genome—any segment of appropriate size (which varies between phages), including plasmid DNA (4, 5). In the bacterium receiving the genetic material of the *tp*, only a part of the transferred segment is incorporated into the genome (6).

From the standpoint of the dynamics of gene transfer, the transduction events of the two types vary in two main respects. The first is the probability of the gene in the lysed bacterium being picked. This probability depends on the size of the accidentally packaged genome segment and also, in the case of “specialized” transduction, on the location of the segment relative to the prophage. The second is the probability of the delivered segment being incorporated into the genome of the receiving bacterium, which depends on whether the gene vehicle is a transducing virion (in the case of specialized transduction) or a *tp* (in the case of generalized transduction).

Available evidence shows that bacteria of animal intestines have unique phage communities (1, 7–9); the phages appear to be associated with the intestinal habitat and to be DNA phages (1). (The RNA phages observed in the intestinal contents are likely from the ingested plants [1].) The enteric phages appear to be mostly temperate, and the majority of virions in feces are from prophage inductions (1, 2, 8, 10–14). A prophage can be induced *in vitro*, spontaneously or with the use of inducing agents, in 67% to 98% of *Escherichia coli* strains and in 77% to 100% of *Salmonella enterica* serovar Typhimurium strains from different sources

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TABLE 1 Terminology used in the developed model of infection dynamics and transduction by enteric phages

Term	Definition	Role in transduction
Virion	A phage virion that can self-replicate in the next infected bacterium and does not carry a bacterial genetic segment picked up during the last replication cycle	Can help a transducing virion if this coinfects the next bacterium
Transducing virion	A phage virion that can replicate in the next infected bacterium, either autonomously or if helped by a coinfecting virion, and that carries a bacterial genetic segment picked up during the last replication cycle	In the next bacterium, with a certain probability, the bacterial genetic segment can be incorporated into the chromosome.
Transducing particle (<i>tp</i>)	A particle composed of the phage's capsid and the bacterial genetic segment picked up during the last replication cycle and capable of injecting the genetic segment into the next bacterium	In the next bacterium, with a certain probability, (a part of) the bacterial genetic segment can be incorporated into the chromosome if the segment was chromosomal or establish as a plasmid if the segment was a plasmid.
Multiplicity of infection (MOI)	The ratio of the no. of virions and <i>tp</i> of enteric phages circulating in the intestine to the no. of live bacteria in the system at a given time	These quantities inform the "single-hit" mathematical model which links the availability of phages, expressed as the MOI, to the probability of infection for replicating bacteria susceptible to infection.
"Single-hit" probability of infection	The probability that the genetic material of a virion or <i>tp</i> that encounters a live bacterium is injected into the bacterium	
Direct lytic cycle	A lytic cycle that immediately follows infection of the bacterium	Lytic cycles of both types add to the bacterial population death rate and thus impact the rate of bacterial replication and, hence, susceptibility to new phage infections. If the bursts contain transducing virions or <i>tp</i> , and these are internalized by the next bacteria, segments of the lysed bacteria's genomes may be transduced to the next bacteria.
Lytic cycle after prophage induction	A lytic cycle that follows induction of the prophage at the end of the lysogenic phage cycle in the bacterium	

(15, 16). Of the phages released by *E. coli*, 24% to 69% (depending on the strain source), and of those released by salmonellae, 94% to 100% are capable of generalized transduction *in vitro* (15, 16). For example, phages isolated from bovine salmonellae transduce the *bla*_{CMY-2} gene encoding resistance to third-generation cephalosporins among these bacteria (17). However, some studies report the dominance of virulent (i.e., not capable of a lysogenic cycle) phages' virions in horse feces (18) and isolation of mostly virulent phages from bovine feces (8).

The enteric phage communities are highly diverse. There are an estimated 1,000 to 2,000 phage genotypes in human feces (12) and several hundred morphologically distinct phages in horse feces (19). Genomic differentiation indicates from 700 to 1,500 coliform strains in horse feces (18). Published data show that the most abundant phage contributes only 2% to 7% of the virions in human feces (12) and 5% to 11% in horse feces (18, 19). A coliphage from horse feces has been experimentally shown to infect 2% to 4%, and is probabilistically estimated to infect at most 8%, of the coliform strains present, with any given strain sensitive to 1 or 2 of the phages (2, 18). The diversification of the bacterial host susceptibility is thought to be a part of its evolutionary response to phage infections (20), and the bacterial strain diversity may itself have been driven by phage-delivered genomic contents (21, 22). Hence, any given prophage in mammalian enteric *E. coli* is likely infrequent, and any given phage is unlikely to individually make a significant contribution to gene transfer among these bacteria. If we aim to evaluate the enteric phage contribution to gene transfer in the enteric *E. coli*, considering them an exemplar enteric bacterial population, we need to approximate the overall infection dynamics of these phages in these bacteria.

We used the available data on the overall enteric phage ecology and phage biology and developed a candidate deterministic mathematical model of the infection dynamics of enteric phages in the exemplar system of commensal *E. coli* in the cattle large intestine. The model structure and parameters were chosen to reproduce the dominance of temperate phages. We then used the model to approximate the upper numerical limits of transduction of a hypothetical AMR gene among enteric *E. coli* bacteria by the enteric phages. The transfer limits were estimated by assuming that all the phages were capable of either specialized or generalized transduction of chromosomal genes or of generalized transduction of a 100-kbp plasmid, as well as that all *E. coli* bacteria carried one copy of the AMR gene in the chromosome or on the plasmid, respectively. We then repeated the estimations, assuming that only the most abundant phage (the prophage present at random in 10% of enteric *E. coli* bacteria) could transduce the gene. Finally, turning to a specific example, we previously developed a mathematical model of the plasmidic *bla*_{CMY-2} gene transfer through plasmid conjugation in the cattle enteric *E. coli* (23). Here, we assumed *bla*_{CMY-2} was carried on a 100-kbp plasmid that was also transducible by all the enteric phages and compared gene transfers to gene-free enteric *E. coli* through generalized plasmid transduction versus plasmid conjugation. Our results provide a quantitative perspective on the potential contribution of transduction to the lateral spread of AMR genes in enteric bacteria.

MATERIALS AND METHODS

Model formulation. (i) Bacteria. The overall dynamics of enteric phages was modeled. A schematic and complete model flowcharts are provided in Fig. 1. At a given time, N_{tot} was the total number of enteric commensal

FIG 1 Schematic (A) and complete (B) flowcharts of the mathematical model of infection dynamics of enteric phages, with estimation of bacterial AMR gene transduction (generalized transduction is shown on the example of a plasmid). ST, specialized transduction; GT, generalized transduction.

TABLE 2 Parameters for the mathematical model of infection dynamics of enteric phages, with estimation of bacterial AMR gene transduction

Parameter	Definition, unit	Value	Source(s) of value
Flows			
Phage infection and ecology			
ph_br_n	Burst size per lysed bacterium	250	29
ph_fr_tp	Fraction of tp^a in burst	0.02	6
ph_d	Fractional extracellular deactivation rate of phage virions/ tp , per h	0.0745	29
ph_cir_n	No. of circulating virions/ tp		Link variable determined by relevant variable values during model simulations
MOI	No. of circulating virions/ tp per bacterium		Link variable determined as ph_cir_n/N_{tot}
pr_inf_hit	Probability of virion/ tp injecting the carried genetic material into the bacterium given absorption	0.8	Assumed
pr_inf	Probability of infection for a growing susceptible or lysogen not immune to other enteric phages		Link variable determined as $1 - \exp(-MOI \times pr_inf_hit)$ (6, 24)
$b_fr_inf_lys$	Fraction of new infections going into lysogenic cycle	0.9	Estimated through model simulations ^b
b_fr_limm	Fraction of lysogens cross-immune to other enteric phages	1	Estimated through model simulations ^b
α_{phimm}	Fitness cost of phenotypical immunity to enteric phages	0.05	Assumed based on idea in references 2, 10
γ	Fractional outflow rate of virions and tp/h	0.01	Same as for bacteria
$b_fr_lys_in$	Fraction of lysogens in inflowing bacteria	0.013	27, 28
$b_fr_pimm_in$	Fraction of inflowing bacteria phenotypically immune	0.0001%	Estimated through model simulations ^{b,c}
$b_fr_lys_en$	Fraction of lysogens in enteric bacteria (start)	0.9	Assumed based on data in references 15, 16
$b_fr_pimm_en$	Fraction of enteric bacteria phenotypically immune (start)	0.0001%	Equal to $b_fr_pimm_in$
Bacterial population			
k	Maximum fractional replication rate, h	0.34	26
d	Fractional death rate, h	0.17	26
γ	Fractional inflow/outflow rate, h	0.01	23, 35
N_{max}	Maximum no. of bacteria/ml luminal contents	5.5 log units	35
Specialized transduction			
g_n	No. of AMR gene copies per bacterium	1	Chosen scenario
pr_gpick_sptr	Probability virion picks adjacent genome segment, per prophage induction	1.00×10^{-6}	3
$pr_g_nb_ph_chr$	Probability prophage in the chromosome is adjacent to genome segment containing AMR gene	0.0202020	Approximated: $(2 \times 100)/(100 \times 99)^d$
Generalized transduction			
g_n	No. of AMR gene copies per bacterium	1	Chosen scenario
$pr_gpick_gntr_chr$	Probability tp picks the chromosomal segment containing AMR gene, per lytic cycle per bacterium	0.0192234	Approximated: $(1/51) \times (1 - 1/51)^e$
pr_tp_gin	Probability the bacterial chromosomal gene carried by received tp becomes part of the recipient bacterium's chromosome	0.02	6
$pr_gpick_gntr_pl$	Probability tp picks plasmid bearing AMR gene, per lytic cycle per bacterium	0.0196078	Approximated: $(1/51)^e$
$pr_tp_pl_est$	Probability transduced plasmid establishes in the recipient bacterium	1	Chosen scenario
$ph_frAMRtp$	Fraction of tp with AMR gene of total virions/ tp		Link variable determined by relevant variable values during model simulations
Timing			
t_newi	Newly infected bacteria, h	1	Assumed
t_tp	Bacteria process tp , h	1	Assumed
t_ltcl_v	Direct lytic cycle, h	0.3833	29
t_ltcl_t	Lytic cycle after prophage induction, h	0.9583	3, 29
t_lscl	Lysogenic cycle, h	100	Estimated through model simulations ^b

^a tp , transducing particle.^b These parameter values allowed reproduction of the data and reported trends in enteric phage ecology (1, 2, 8, 10–17).^c For the model of the most abundant temperate enteric phage, $b_fr_pimm_in = 86.3\%$.^d The probability of the prophage adjoining the segment containing the AMR gene copy was approximated by assuming random cooccurrence of the 50-kbp prophage with the 50-kbp segment in the chromosome of 5,000 kbp.^e The probability that a tp picks the chromosomal genome segment or plasmid containing the AMR gene copy was approximated by assuming that the tp picks 100 kbp of the bacterial genome at random, the chromosome contains 5,000 kbp, the plasmid contains 100 kbp, and hence the total bacterial genome contains 5,100 kbp.

E. coli bacteria. The bacteria were in one of six states with respect to infection by enteric phages: phenotypically immune (N_{pimm}); susceptible (N_{sus}); newly infected (N_{newi}); lysogens (N_{lys}); those that received and were now processing tp (N_{prtp}), after which they returned to the susceptible state; or in the lytic cycle (N_{lyt}). When replicating in the intestine, the susceptibles and lysogens that were not immune to other enteric phages were infected by the circulating virions or received tp of enteric phages. Of new infections by virions, a fraction ($b_fr_inf_lys$) progressed to the lyso-

genic cycle; the rest were lytic. Bacteria were also counted in their three transient states: bursting (N_{bs}), acquiring the AMR gene through specialized transduction (N_{st_AMR}), and acquiring the AMR gene through generalized transduction (N_{gt_AMR}). N_{st_AMR} represented bacteria entering the lysogenic cycle after being infected by transducing virions (which were assumed always to incorporate into the genome in the form of prophages) that carried the chromosomal gene copy. N_{gt_AMR} represented bacteria finishing the processing of tp that carried either the plasmid with the gene

TABLE 3 Matrix of hypothetical scenarios modeled and estimated AMR gene transduction in commensal *E. coli* of the cattle large intestine

Scenario of AMR gene copy distribution in enteric <i>E. coli</i>	Transduction for enteric phage ecology where ^a :					
	All phages temperate and transduce chromosomal segment containing gene		Most abundant temperate phage (in 10% of <i>E. coli</i> bacteria) transduces chromosomal segment containing gene		All phages temperate and transduce plasmid containing gene (GT)	Most abundant temperate phage (in 10% of <i>E. coli</i> bacteria) transduces plasmid containing gene (GT)
	ST	GT	ST	GT		
1 (in all on chromosome)	0.02	8	0.003	1		
2 (in all on a 100-kbp transducible plasmid)					418	53
3 (in 1.8% ^b on a 100-kbp plasmid transferable through conjugation and transduction)					8 (7×10^{-4})	1 (9×10^{-5})

^a For scenarios 1 and 2, the entry is the number of *E. coli* bacteria in a liter of the luminal contents/hour receiving the gene *de novo*. For scenario 3, the entry is the number of gene-free *E. coli* bacteria in a liter of the luminal contents/hour acquiring the gene. The ratios to the number of gene acquisitions through plasmid conjugation are in parentheses. ST, specialized transduction; GT, generalized transduction.

^b The gene frequency and transfer rate through plasmid conjugation were from an earlier model for the exemplar *bla*_{CMY-2} gene (23).

copy (the plasmid assumed to always become established in the recipient) or the chromosomal gene copy and the gene became part of the recipient's chromosome. At a given time, a fraction (*b_fr_lys_en*) of enteric *E. coli* bacteria were lysogens of and a fraction (*b_fr_pimm_en*) were phenotypically immune to enteric phages; a fraction (*b_fr_AMR_en*) had an AMR gene copy. The definitions and values of the rest of the model parameters are given in Table 2 or are provided below.

The enteric *E. coli* bacteria mixed randomly and homogeneously, and while in all six nontransient states they died at the same fractional population death rate, *d*, from other causes than lysis induced by temperate enteric phages. (A contribution of virulent phages to the bacterial deaths was assumed to be a part of the death rate, *d*.) The bacteria flowed out with feces and the ingested *E. coli* flowed into the intestine at similar fractional rates. The inflow was composed of *E. coli* susceptible, phenotypically immune, and already lysogens of enteric phages. A fraction (*b_fr_AMR_in*) of the inflow had a copy of the AMR gene. The density-dependent bacterial population growth in the intestine, to the maximum possible number, *N*_{max} (the size of the realized niche available to *E. coli* within the enteric microbiome), was modeled with a logistic growth model. The bacteria in the newly infected state or processing *tp*, and those in the lytic cycle, did not replicate. The susceptible bacteria, phenotypically immune bacteria, and lysogens replicated. The susceptible bacteria and lysogens replicated at the maximum fractional population replication rate, *k*. The phenotypically immune bacteria experienced a fitness cost (based on the idea of a competitive disadvantage of phage resistance [2, 10]), which manifested as an α_{pimm} fractional reduction in their replication potential (i.e., to *k* − α_{pimm}).

(ii) **Phage dynamics.** The virions/*tp* of enteric phages circulating in the intestine were distributed homogeneously and were randomly and homogeneously mixed with the enteric *E. coli*. The virions/*tp* flowed out with feces at the same fractional rate as the bacteria (Table 2). The number of virions/*tp* in intestinal circulation further decreased due to their extracellular deactivation, or when internalized by the bacteria (Fig. 1). The extracellular deactivation rates for both transducing and nontransducing virions, and for both virions and *tp*, were equal. The numbers of circulating virions/*tp* were replenished by virions released from the lysed bacteria. At any given time, the ratio of circulating virions/*tp* to *N*_{tot} (the total number of bacteria in the nontransient states with respect to phage infection) was estimated in the model and designated MOI (multiplicity of infection) (Table 1). The virions/*tp* were absorbed ("hit" on bacterium) equally well by the growing susceptible (nonlysogen) bacteria and by ly-

sogens not immune to other enteric phages; the probabilities of internalization given the absorption of a virion or *tp* were equal (*pr_inf_hit*). We also assumed that all phages acted as independent infectious agents. With these assumptions, the probability of infection for a growing susceptible bacterium or non-cross-immune lysogen bacterium was approximated using the "single-hit" model (6, 24) as follows: *pr_inf* = 1 − exp(− MOI × *pr_inf_hit*).

The prophage was inherited vertically, i.e., there was "clonality" of lysogens; similarly, bacteria that were susceptible or phenotypically immune to infection by enteric phages also passed their properties to the immediate progeny. Lysis upon induction was the only mechanism resulting in prophage loss by *E. coli* (25). The possibility of AMR gene transfer through genetic recombination between phages coinfecting a bacterium was ignored.

(iii) **Transduction.** There was one copy, either chromosomal or plasmidic, of the hypothetical AMR gene per bacterium. Recall that a fraction (*b_fr_AMR_en*) of enteric *E. coli* bacteria had the gene; i.e., the probability of the gene's presence in the lysed bacterium was *b_fr_AMR_en* and depended on the gene distribution. In the modeled scenarios, this probability was constant over time.

For specialized transduction of a chromosomal gene copy, the probability that progeny virions leaving the lysed bacterium carried the chromosomal segment containing the AMR gene copy, if present, was as follows: *pr_g_nb_ph_chr* × *pr_gpick_sptr*, where, *pr_g_nb_ph_chr* was the probability that the prophage in the chromosome (nondirectionally) was adjacent to the segment containing the gene copy and *pr_gpick_sptr* was the probability of the recombination error during prophage excision that resulted in the virion acquiring the adjacent segment. It was assumed that the transducing virion was either fully functional or, if not, that there was always a functional virion able to "help" the transducing virion infect the next bacterium (because at the simulated system's dynamic equilibrium, the intestinal virion quantities were sufficient to infect all the replicating bacteria susceptible to infection). Hence, if the infection by the transducing virion was lysogenic, the acquired gene segment became part of the lysogenized bacterium's genome. Therefore, the probability that a virion infecting a bacterium transduced the AMR gene was as follows: *ph_prAMR* = *b_fr_AMR_en* × *pr_g_nb_ph_chr* × *pr_gpick_sptr*.

For generalized transduction of a chromosomal gene copy, the probability that *tp* leaving the lysed bacterium had packaged the chromosomal segment containing the AMR gene copy, if present, was *pr_gpick_gntr_chr*. The probability of recombination of the gene into the genome of

the next bacterium receiving the tp was pr_tp_gin . Hence, given the assumed constant $b_fr_AMR_en$, the probability that a tp received by a bacterium transduced the AMR gene was as follows: $ph_prAMRtp = b_fr_AMR_en \times pr_gpick_gnt_chr \times pr_tp_gin$.

For generalized transduction of a plasmid, the probability that tp leaving the lysed bacterium had packaged the plasmid, if present, was $pr_gpick_gnt_pl$, and the probability of plasmid establishment in the next bacterium receiving the tp was $pr_tp_pl_est$. In the relevant modeled scenarios (Table 3), the AMR gene copy, if present in the bacterium, was always on the plasmid. Further, the assumed fraction of enteric *E. coli* bacteria carrying the plasmid, $b_fr_AMR_en$, was constant over time (the plasmid was either present in all bacteria or, for the exemplar plasmidic gene, the system was at dynamic equilibrium with respect to the plasmid distribution). Therefore, the probability that a tp received by a bacterium transduced the plasmid with the AMR gene was as follows: $ph_prAMRtp = b_fr_AMR_en \times pr_gpick_gnt_pl \times pr_tp_pl_est$.

Model equations. The continuous-time deterministic mathematical model was represented by ordinary differential equations for the numbers of enteric commensal *E. coli* bacteria in each of the six nontransient states with respect to infection by enteric phages (equations 2 to 8), and three transient states of lysis and transduction of a hypothetical AMR gene (equations 9 to 11). The final equation (equation 12) was for the number of virions/ tp of these phages circulating in the intestine. These equations and the supportive algebraic equation 1 are provided below.

Total living bacteria:

$$N_{tot} = N_{pimm} + N_{sus} + N_{newi} + N_{prtp} + N_{lys} + N_{lyt} \quad (1)$$

Phenotypically immune bacteria:

$$\begin{aligned} \frac{dN_{pimm}}{dt} = & \underbrace{(k - \alpha_{pimm}) \left(1 - \frac{N_{tot}}{N_{max}}\right) N_{pimm}}_{\text{Replication}} \\ & + \underbrace{\gamma \times b_fr_pimm_in \times N_{tot}}_{\text{Inflow}} - \underbrace{dN_{pimm}}_{\text{Death}} - \underbrace{\gamma N_{pimm}}_{\text{Outflow}} \end{aligned} \quad (2)$$

Susceptible bacteria (including lysogens not immune to other enteric phages):

$$\begin{aligned} \frac{dN_{sus}}{dt} = & \underbrace{(1 - pr_inf) \times k \left(1 - \frac{N_{tot}}{N_{max}}\right) N_{sus}}_{\text{Probability infected}} + \underbrace{(1/t_prtp) N_{prtp}}_{\text{Finished processing } tp} \\ & - \underbrace{dN_{sus}}_{\text{Death}} - \underbrace{\gamma N_{sus}}_{\text{Outflow}} + \underbrace{(1/t_newi) N_{newi} \times (1 - ph_fr_tp)}_{\text{New lysogens without cross-immunity}} \\ & \times \underbrace{b_fr_inf_lys \times (1 - b_fr_limm)}_{\text{New lysogens without cross-immunity}} \\ & + \underbrace{\gamma \times (1 - b_fr_pimm_in - b_fr_lys_in)}_{\text{Inflow}} \times \underbrace{b_fr_limm \times N_{tot}}_{\text{Inflow}} \end{aligned} \quad (3)$$

Newly infected bacteria:

$$\begin{aligned} \frac{dN_{newi}}{dt} = & \underbrace{pr_inf \times k \left(1 - \frac{N_{tot}}{N_{max}}\right) N_{sus}}_{\text{Infection}} - \underbrace{(1/t_newi) N_{newi}}_{\text{Flow to next state}} \\ & - \underbrace{dN_{newi}}_{\text{Death}} - \underbrace{\gamma N_{newi}}_{\text{Outflow}} \end{aligned} \quad (4)$$

Lysogens (immune to other enteric phages):

$$\begin{aligned} \frac{dN_{lys}}{dt} = & \underbrace{k \left(1 - \frac{N_{tot}}{N_{max}}\right) N_{lys}}_{\text{Replication}} + \underbrace{(1/t_newi) N_{newi} \times (1 - ph_fr_tp)}_{\text{New lysogens with cross-immunity}} \\ & \times \underbrace{b_fr_inf_lys \times b_fr_limm}_{\text{New lysogens with cross-immunity}} \\ & + \underbrace{\gamma \times b_fr_lys_in \times b_fr_limm \times N_{tot}}_{\text{Inflow}} - \underbrace{dN_{lys}}_{\text{Death}} - \underbrace{\gamma N_{lys}}_{\text{Outflow}} \end{aligned} \quad (5)$$

Bacteria processing tp :

$$\begin{aligned} \frac{dN_{prtp}}{dt} = & \underbrace{(1/t_newi) N_{newi} \times ph_fr_tp}_{\text{Start processing } tp} - \underbrace{(1/t_prtp) N_{prtp}}_{\text{Finished processing } tp} \\ & - \underbrace{dN_{prtp}}_{\text{Death}} - \underbrace{\gamma N_{prtp}}_{\text{Outflow}} \end{aligned} \quad (6)$$

Bacteria in the lytic cycle were composed of those (lyt_v) in direct lytic cycles and (lyt_t) in lytic cycles following prophage induction: $N_{lyt} = N_{lyt_v} + N_{lyt_t}$ where

$$\begin{aligned} \frac{dN_{lyt_v}}{dt} = & \underbrace{(1/t_newi) N_{newi} \times (1 - ph_fr_tp) \times (1 - b_fr_inf_lys)}_{\text{Start direct lytic cycle}} \\ & - \underbrace{(1/t_lsc_v) N_{lyt_v}}_{\text{Bursting}} - \underbrace{dN_{lyt_v}}_{\text{Other death}} - \underbrace{\gamma N_{lyt_v}}_{\text{Outflow}} \end{aligned} \quad (7)$$

and

$$\begin{aligned} \frac{dN_{lyt_t}}{dt} = & \underbrace{(1/t_lsc) N_{lys}}_{\text{Prophage induction}} - \underbrace{(1/t_ltcl_t) N_{lyt_t}}_{\text{Bursting}} \\ & - \underbrace{dN_{lyt_t}}_{\text{Other death}} - \underbrace{\gamma N_{lyt_t}}_{\text{Outflow}} \end{aligned} \quad (8)$$

Bacteria in a transient state of bursting:

$$N_{bs} = \underbrace{(1/t_lsc_v) N_{lyt_v}}_{\text{Burst after direct lytic cycle}} + \underbrace{(1/t_ltcl_t) N_{lyt_t}}_{\text{Burst after prophage induction}} \quad (9)$$

Bacteria in a transient state of acquiring a copy of the AMR gene through specialized transduction:

$$\begin{aligned} N_{st_AMR} = & \underbrace{(1/t_newi) N_{newi} \times (1 - ph_fr_tp)}_{\text{New lysogens}} \times \underbrace{ph_prAMRvr}_{\text{Probability virion transduced AMR gene}} \end{aligned} \quad (10)$$

Bacteria in a transient state of acquiring a copy of the AMR gene through generalized transduction:

$$N_{gt_AMR} = \underbrace{(1/t_prtp) N_{prtp}}_{\text{Finished processing } tp} \times \underbrace{ph_prAMRtp}_{\text{Probability } tp \text{ transduced AMR gene}} \quad (11)$$

Circulating virions/ tp :

$$\begin{aligned} \frac{dph_cir_n}{dt} = & \underbrace{ph_br_n \times N_{bs}}_{\text{From bursts}} - \underbrace{N_{newi}}_{\text{Loss to new infections}} - \underbrace{ph_d \times ph_cir_n}_{\text{Deactivation}} \\ & - \underbrace{\gamma \times ph_cir_n}_{\text{Outflow}} \end{aligned} \quad (12)$$

Model parameterization. (i) Bacteria. The model time step was 1 h. A published model (23) was adopted to reproduce aspects of the ecology of enteric commensal *E. coli* in cattle other than infection by enteric phages; the parameters and their values are included in the bacterial population section of Table 2. (This included adopting the number of enteric *E. coli* bacteria as the starting value for simulating the infection dynamics.) One modification was made to the adopted model to connect it to the dynamics of phage infection: the maximum possible hourly *E. coli* net population fractional growth rate in that model was as follows: $r = 0.17$ (equivalent to a 4-hour *E. coli* doubling time during the exponential phase of growth [26]). There were no empirical data to support an informed partitioning of r into the fractional rates of bacterial replication, k , and death, d ; we assumed that r was equal to $k - d$ and assigned k a value of 0.34 and d a value of 0.17.

(ii) Phage dynamics. The phages infecting *E. coli* bacteria in the luminal contents were associated with the enteric habitat (1, 7–9). The cattle ingesta are composed mostly of water and feed; we assumed that the phages ingested with water and feed were plant associated and noninfec-

tious for enteric *E. coli*. However, an estimated 1.3% of the ingesta is the mixture of cattle feces and soil from their close environment (27). The environmental *E. coli*, in particular those in the floor manure slurry and water trough sediments, are known to harbor prophages of the cattle enteric *E. coli* phages (28). We assumed that all the *E. coli* bacteria ingested from the close environment of the cattle were lysogens of the enteric phages.

The durations of the direct lytic cycle and of the lytic cycle following prophage induction, as well as the burst size and extracellular deactivation rate of virions/*tp* (Table 2), were estimated as medians of the data for the *E. coli* temperate phages *in vitro* (29).

(iii) **Specialized transduction.** One AMR gene copy per bacterium was assumed. An *E. coli* gene averages 1 kbp (<http://www.ncbi.nlm.nih.gov/>). A conservative estimate of the total bacterial genome, 5,100 kbp, was taken to be the sum of the size of the average *E. coli* chromosome, 5,000 kbp (<http://www.ncbi.nlm.nih.gov/>), and the size of the average bacterial conjugative plasmid, 100 kbp (30).

The prophage of a specialized transducing phage in the chromosome was assumed to be 50 kbp (31) and, in the case of an excision recombination error, was assumed to pick up an adjacent chromosomal segment of the same length (3). The probability of the error was 1/1,000,000 (3). The probability of the 50-kbp prophage being adjacent to the 50-kbp genome segment containing the AMR gene copy, if present in the chromosome of 5,000 kbp, was approximated by assuming random cooccurrence (ignoring directionality) of the prophage and segment as $(2 \times 100)/(100 \times 99)$. Of the burst, 98% were virions and the rest were *tp* (based on available estimates for *E. coli* phages [6]).

(iv) **Generalized transduction.** A *tp* of *E. coli* was assumed to carry 100 kbp of the accidentally packaged bacterial DNA (~ 0.02 of the total bacterial genome, based on available estimates for *E. coli* phages [6]). The target site for phage replication was nonspecific; hence, the probability that a *tp* randomly acquired the genome segment containing the AMR gene copy, if present in the lysed bacterium, assuming the gene copy number was 1, depended on the size of the segment picked (100 kbp) and the total genome size (5,100 kbp). Hence, the probability of a *tp* acquiring the segment containing the AMR gene was approximately 1/51; the segment could be chromosomal or the plasmid assumed to be present in *E. coli*. For a chromosomal segment only, this probability was adjusted to $(1/51) \times (1 - 1/51)$ (that is, by subtracting the chance of the acquisition of the plasmid).

In the next bacterium receiving the *tp* that carried the chromosomal segment containing the AMR gene, recombination of the gene into the bacterium's genome was assumed to occur with a probability of 0.02 (based on available estimates for *E. coli* phages [6]). If the *tp* transduced the plasmid, the plasmid was assumed to always establish in the recipient bacterium.

(v) **Exemplar plasmidic gene.** The gene copy was on a 100-kbp plasmid that was assumed to be transferable through both conjugation and transduction; furthermore, it could be transduced by all enteric phages. The number of plasmid-bearing enteric *E. coli* bacteria was N_{pl+} , and that of plasmid-free enteric *E. coli* bacteria was N_{pl-} . A published model for the plasmidic *bla*_{CMY-2} encoding resistance to the cephalosporin ceftiofur (23) was adopted as the exemplar to reproduce the dynamics of gene transfer through plasmid conjugation. Of enteric *E. coli*, 1.8% bear the plasmid with *bla*_{CMY-2} at the system's dynamic equilibrium (i.e., $b_{fr_AMR_en} = 0.018$) (23). There was no plasmid loss by the bacteria (23, 32). The plasmid-bearing bacteria experienced a fitness cost represented by a fractional reduction of 0.05 (23, 33) in the maximum growth rate, r . Plasmid transfer was a frequency-dependent transmission process; the number of plasmid-free *E. coli* bacteria acquiring the gene through plasmid conjugation was $\beta(N_{pl+} \times N_{pl-}/N_{tot})$ (23). The value of β was 0.004/hour and corresponded to the dynamics of transfer of the exemplar *bla*_{CMY-2} (23). The number of plasmid-free enteric *E. coli* bacteria acquiring the gene through plasmid conjugation per hour was estimated in the published model (23) and compared to the number acquiring the gene through plasmid generalized transduction, as estimated in the present

FIG 2 Infection dynamics of enteric phages in commensal *E. coli* of the cattle large intestine. In each panel, the data are from 1,000 model simulations of the system at its dynamic equilibrium. (A) Distribution of enteric phage lysogen, susceptible, and phenotypically immune *E. coli* bacteria, depending on the fraction of lysogens cross-immune to other enteric phages. (B) Fraction of enteric phage virions in the luminal contents originating from prophage inductions versus direct lytic cycles and the total of circulating virions, depending on the average lysogenic cycle duration when all lysogens were cross-immune. The dashed line indicates the parameter value in the baseline model; the rest of the model's parameter values are given in Table 2.

model. Here, in addition to universal plasmid establishment in the transductants, we assumed nonspecific phage attachment to the bacteria expressing and not expressing the conjugative pili.

Model implementation and software. The model was implemented in Vensim PLE Plus software (Ventana Systems Inc., Harvard, MA, USA); solutions of the differential equations were approximated numerically by the embedded fourth-order Runge-Kutta method. The dependency of the model outputs on the value of a model parameter (presented in Fig. 2 to 4) was investigated by running 1,000 model simulations, obtaining the parameter's values from a uniform distribution by Latin Hypercube sampling (34). Figure 1 was made in Microsoft Office PowerPoint 2007 software (Microsoft, Redmond, WA, USA). The rest of the figures were made in SigmaPlot software (Systat Software, San Jose, CA, USA).

RESULTS

Mathematical model of phage dynamics in enteric *E. coli*. We developed a deterministic mathematical model of the infection dynamics of resident enteric phages in free-living commensal *E. coli* in the luminal contents of the cattle large intestine (Fig. 1 and

FIG 3 Sensitivity of the estimated numbers of specialized and generalized transductions to parameters of enteric *E. coli* ecology and phage infection ecology. The transductions are by all enteric phages of the chromosomal AMR gene in the commensal *E. coli* in a liter of the luminal contents of the cattle large intestine/hour. In the baseline model, 90% of infections went into the lysogenic cycle of 100 h, and all lysogens were cross-immune to infection by other enteric phages. The ingested bacteria (98.7% of which were free of enteric phages) flowed in, and bacteria and virions/tp flowed out with feces at a fractional rate of 0.01/hour to those present. In each panel, the data are from 1,000 model simulations of the system at its dynamic equilibrium. The dashed line indicates the parameter value in the baseline model; the rest of the model's parameter values are given in Table 2. (A) Varying inflow/outflow rate. (B) Varying bacterial replication rate (the maximum possible replication rate was varied). (C) Varying fraction of lysogens cross-immune to infection by other enteric phages. (D) Varying fraction of infections going into the lysogenic cycle.

Table 2). The bacteria were assumed to be in one of six states with respect to infection: phenotypically immune, susceptible, newly infected, lysogens, processing tp, or in the lytic cycle. Lysogens could be cross-immune or not cross-immune to other enteric phages. The bacterial population experienced turnover due to ingestion and excretion. Ingested *E. coli* bacteria were susceptible to (98.7%) or lysogens of (1.3%) the cattle enteric phages, and a few (0.0001%) were phenotypically immune to infection. Both the bacteria and phage virions/tp flowed out with feces. While in the luminal contents, bacterial population growth was density dependent. When replicating, the susceptible bacteria and the lysogens without cross-immunity became infected, with a probability that depended on the number of circulating virions/tp of enteric phages per enteric *E. coli* bacterium.

Based on empirical data (15–17), 95% of *Enterobacteriaceae* carry one or more prophages, 99% of which are capable of generalized transduction. Considering these to be independent proba-

bilities, the probability of a bacterial strain carrying a prophage of a transducing phage is therefore 0.95×0.99 , or 0.94. The developed model showed how, given the bacterial ecology and infection dynamics, 93% of *E. coli* bacteria in the luminal contents of the cattle large intestine at a given time could be lysogens of enteric phages, with 91% of fecal virions originating from prophage inductions. The estimates, therefore, were consistent with the current data and understanding. This outcome occurred when we assumed that 90% of the infections resulted in lysogeny of 100 hours and that the newly created lysogens were cross-immune to other enteric phages. From the approximately 1/4 daily population turnover of the enteric *E. coli* bacteria through ingestion and excretion (23, 35), the average *E. coli* intestinal residency time can be expected to be on the order of 100 hours. Hence, a 100-hour average lysogenic cycle duration was sufficiently short for a large fraction of the lysogens created in the intestine ($\sim 2/3$) to be lysed while still in the intestine, and the rest were excreted in feces.

FIG 4 Sensitivity of the estimated numbers of specialized and generalized transductions to average characteristics of enteric phages. The transductions were by all enteric phages of the chromosomal AMR gene in the commensal *E. coli* bacteria in a liter of the luminal contents of the cattle large intestine/hour. In the baseline model, 90% of infections went into the lysogenic cycle of 100 h, and all lysogens were immune to infection by other enteric phages. The ingested bacteria (98.7% of which were free of enteric phages) flowed in, and bacteria and virions/tp flowed out with feces at a fractional rate of 0.01/hour to those present. In each panel, the data are from 1,000 model simulations of the system at its dynamic equilibrium. The dashed line indicates the parameter value in the baseline model; the rest of the model's parameter values correspond to the baseline model, with all enteric phages transducing. (A) Varying burst size. (B) Varying lysogenic cycle duration. (C) Varying fractional rate of extracellular deactivation of virions/tp. (D) Varying probability of injection of the genetic material carried by virion/tp given absorption on the bacterium (in the single-hit model of infection probability; the value in the baseline model was 0.8).

Given the high density of enteric *E. coli* bacteria, the probability of infection was modeled using the single-hit model and MOI (Table 2) (with the burst size parameterized from available literature, at the system's dynamic equilibrium, the estimated MOI was >25). A modification of this approach to infection probability may be necessary for *in vivo* systems with lower bacterial densities (36). This model formulation was adopted as the baseline to estimate the transductions by enteric phages of a hypothetical AMR gene in *E. coli* in the luminal contents of the large intestine.

In the baseline model, with the phage burst size and extracellular deactivation rate set as determined for *E. coli* temperate phages *in vitro*, the estimated intestinal virion quantities were sufficient to infect all the replicating bacteria susceptible to infection. The number of circulating virions was estimated to be on the order of 10^6 /ml luminal contents. For comparison, others have reported totals of 10^7 to 10^{10} phage virions/ml in the ruminal contents of cattle and sheep (2), 10^8 /ml in the intestinal mucosa of healthy humans (37), and 10^{10} to 10^{11} /ml in horse feces (1, 38). However, the estimates in horses vary through time to a low of 10^3 /ml, and as low as 10/ml has been reported in pig feces (8).

Considering alternative model structures. We investigated whether other assumptions for the enteric phage ecology—differing from those adopted in the baseline model formulation—could reproduce the empirical data. We considered two alternative model structures. First, we relaxed the assumption of lysogen cross-immunity and assumed absence of ingested *E. coli* with phenotypic immunity to enteric phages. This scenario resulted in a high rate of lytic cycles; only 12% of enteric *E. coli* bacteria were lysogens of enteric phages at the system's dynamic equilibrium, and 74% were prophage-free susceptible bacteria. Second, when we further allowed for just 0.0001% of the ingested *E. coli* bacteria to be immune to enteric phages, these bacteria came to dominate the intestinal niche, expanding to 63% of the total enteric *E. coli* bacteria at the system's dynamic equilibrium, while lysogens were rare (4%). These scenarios were considered unlikely because they were inconsistent with the current data and understanding: the expected 94% *E. coli* lysogens and the majority of fecal virions from prophage inductions. Figure 2A illustrates how the distribution of enteric phage lysogens and susceptible and phenotypically immune *E. coli* bacteria at the system's dynamic equilibrium de-

pendent on the assumption regarding lysogen cross-immunity to other phages. When all lysogens were assumed to be cross-immune, the average lysogenic cycle duration determined what fraction of the virions circulating at a given time originated from lytic cycles after prophage inductions versus direct lytic cycles (Fig. 2B).

Estimated AMR gene transfer in enteric *E. coli* through specialized transduction. The estimate of specialized transductions of the chromosomal AMR gene was the number of *E. coli* bacteria entering lysogeny after infection by transducing virions carrying a gene copy (Fig. 1). Since the upper limits of transduction were the focus, in the modeled scenario, all enteric *E. coli* bacteria were assumed to carry the gene, and 93% contained prophage of a specialized transducing phage (the baseline model formulation was used). The prophage and the segment containing the AMR gene copy were assumed to be 50 kbp each and to be carried in the 5,000-kbp chromosome. With the presence of the 100-kbp plasmid, the total bacterial genome was 5,100 kbp. The approximate probability that the prophage adjoined the segment containing the AMR gene is given in Table 2. The probability of an excision recombination error was taken to be 1/1,000,000 (3). If the prophage was adjacent to the segment containing the AMR gene and the error occurred, the prophage excised with the segment. Two percent of the burst were *tp*, and 98% were virions (6). With these conditions, an estimated 0.02 *E. coli* bacterium per hour of the total 1.47×10^8 *E. coli* bacteria in a liter of the luminal contents at the system's dynamic equilibrium acquired the gene *de novo* through specialized transduction by all enteric phages (Table 3).

Estimated AMR gene transfer in enteric *E. coli* through generalized transduction. The generalized transductions of the chromosomal AMR gene were estimated in the baseline model, assuming that *E. coli* bacteria that had internalized the gene-containing genome segment that was carried by a *tp* experienced recombination of the gene into their genome with a probability of 0.02 (6). At prophage induction, a 100-kbp bacterial genome segment, approximately 0.02 of the genome (6), was randomly picked up and packaged into *tp*. The approximate probability that the packaged segment contained the AMR gene copy is given in Table 2. Again, all enteric *E. coli* bacteria were assumed to carry the chromosomal AMR gene, and 93% contained prophage of a generalized transducing phage. With these conditions, an estimated 8 *E. coli* bacteria per hour of the total 1.47×10^8 *E. coli* bacteria in a liter of the luminal contents at the system's dynamic equilibrium acquired the AMR gene *de novo* through generalized transduction by all enteric phages (Table 3).

For generalized plasmid transduction, we assumed that the plasmid always established in the recipient bacterium and enumerated the *E. coli* bacteria after the internalized genetic material of *tp* was processed (assuming a 1-hour processing time) (Table 2 and Fig. 1). The plasmid was 100 kbp, which is the average size of a bacterial conjugative plasmid (30). All enteric *E. coli* bacteria were assumed to carry the plasmidic AMR gene, and 93% contained prophage of a generalized transducing phage. The probability that the 100-kbp transduced segment was the plasmid was approximated as indicated in Table 2. With these conditions, an estimated 418 *E. coli* bacteria per hour of the total 1.47×10^8 *E. coli* bacteria in a liter of the luminal contents at the system's dynamic equilibrium acquired the gene *de novo* through plasmid transduction by all enteric phages (Table 3).

For the final, comparative scenario we used the example of *bla*_{CMY-2} carried on a 100-kbp plasmid transferable through both

conjugation and transduction. At the modeled system's dynamic equilibrium (here in terms of both phage infection dynamics and plasmid distribution), the plasmid with the gene was present in 1.8% of enteric *E. coli* bacteria (23). The assumptions on generalized plasmid transduction were the same as described above; we further assumed nonspecific phage attachment to the bacteria whether or not they expressed the conjugative pili. With these conditions, an estimated 8 gene-free *E. coli* bacteria/liter of luminal contents/hour acquired the gene through generalized plasmid transduction by all enteric phages. This number represented a very small proportion (7×10^{-4}) of the number of *E. coli* bacteria acquiring the gene through plasmid conjugation. The latter was estimated using an earlier published model, where a plasmid-bearing enteric *E. coli* bacterium produced 4×10^{-3} transconjugants/hour (23).

The numbers of gene transductions depended on the ecology of enteric *E. coli* and its phages. The estimated numbers of transductants depended nonlinearly on parameters of enteric *E. coli* ecology: turnover and replication rates in the baseline model (Fig. 3A and B). A higher turnover could supply more phage-free *E. coli*. A higher replication rate of those in the intestine resulted in more *E. coli* bacteria available for enteric phages to infect, and hence, both more new lysogens and more lytic cycles. The bacterial growth was density dependent; hence, phage-induced deaths in turn opened the niche for more replication of bacteria susceptible to phage infection; however, these bacteria had to compete to fill the *E. coli* enteric niche with the lysogens replicating at the same rate. The net effect was saturation, with higher replication rates leading to somewhat lower numbers of transductions (Fig. 3B).

The numbers of transductants also depended nonlinearly on the fraction of lysogens assumed to be cross-immune to infection by other enteric phages (Fig. 3C) and, for specialized transduction, linearly on the fraction of phage infections resulting in lysogenic versus direct lytic cycles (Fig. 3D). The numbers of transductants were not highly sensitive to enteric phage characteristics, such as the average burst size and extracellular deactivation rate, within the ranges of values considered (Fig. 4).

DISCUSSION

The infection dynamics of phages in bacterial populations *in vivo* and the contribution of transduction to the lateral spread of bacterial AMR genes are poorly understood. We developed a deterministic mathematical model of the infection dynamics of enteric phages in commensal *E. coli* bacteria in the luminal contents of the cattle large intestine, assuming that phages were temperate and associated with the enteric habitat. Using this model, we estimated the upper numerical limits of transductions of a hypothetical AMR gene among the enteric *E. coli* bacteria by assuming universal gene presence in the bacteria, that all enteric phages were capable of gene transduction, and guaranteed establishment of the transduced plasmid. The estimated limits of the contribution of either specialized or generalized transduction to lateral spread of an AMR gene in this *in vivo* system were low in absolute terms (Table 3). Although we focused on a hypothetical AMR gene, the results are generalizable to any *E. coli* gene, assuming for specialized transduction that the gene is not physically linked to predilection sites of phage insertion and for generalized transduction that packaging of bacterial genomic DNA occurs randomly. To adjust the transduction estimates for those cases when this is not true, one would require data on the physical distribution of the

prophages or the target sites for phage replication, respectively, compared to the physical distribution of the AMR gene of interest in the bacterial genome.

In another modeled scenario, we compared the estimated contribution of generalized plasmid transduction to that of plasmid conjugation for the lateral spread of an exemplar plasmidic AMR gene (Table 3). Using the most liberal assumptions for transduction efficiency, transduction contributed on the order of 1,000 times less to the gene lateral transfer to new *E. coli* bacteria than conjugation.

Our estimations of gene transductions between *E. coli* bacteria used two approximated probabilities for the phage to serve as a gene transfer vehicle. The first probability was that of a transducing virion or *tp* picking up the chromosomal segment or the plasmid containing the gene copy from the bacterium being lysed. The second probability was that of stable establishment of the gene copy in the next bacterium infected by the virion or receiving the *tp*. The numbers of transductants depended linearly on these probabilities. Hence, it is straightforward for the reader to adjust the transduction estimates we report should his/her approximations of these probabilities differ from ours. As new data on the event frequencies in bacterial populations become available, e.g., a proposed theory for the establishment mechanisms (39), modeling transduction can be taken to another level of precision. Other useful data would include better information on the fraction of enteric phages that are temperate and are capable of specialized or generalized transduction, the sizes of the bacterial genome segments that can be transduced, the variation in the burst size and the *tp* fraction of the burst, the comparative rates of extracellular survival of virions and *tp*, and the loss of virions and *tp* from intestinal circulation due to ineffective absorption. The same linear relationship with numbers of transductions applies to the AMR gene prevalence, because the probability of the gene being picked up from the lysed bacterium is a linear function of the gene prevalence. In the scenarios we modeled (Table 3), gene prevalence was constant, and hence, the variation in time in the fractions of virions and *tp* carrying the gene was irrelevant. The constant gene prevalence itself may result from dynamic processes, e.g., for our exemplar plasmidic gene, from the balance between plasmid conjugations in the intestine and the replacement of intestinal *E. coli* bacteria through ingestion by those with a lower frequency of plasmid carriage (23). For the systems where the gene prevalence changes over time (e.g., during antimicrobial treatment in the animal), the model can be extended to capture the variation in gene carriage by the virions and *tp*.

In this study, we aimed to approximate the upper numerical limits of transduction; hence, we used liberal assumptions about the length of the segment transduced. For specialized transduction, the segment was the same size as the prophage, 50 kbp. This was supported by the data on the size of the transduced segment relative to the prophage for an *E. coli* phage (3) and the size of the prophage of a DNA phage in ruminant intestines, which tends to be 35 to 50 kbp (31). For generalized transduction, we assumed a 100-kbp segment or ~ 0.02 of the total genome, based on available estimates for *E. coli* phages (6).

We considered transduction between enteric *E. coli* bacteria of an AMR gene already carried by the bacteria. This gene transfer mode, based on our results, may make a quantitatively minimal contribution to the total lateral gene spread between enteric *E. coli* bacteria. However, transduction can also potentially transfer

AMR genes across bacterial taxa. These genes can then spread within taxa through transfer modes that are more quantitatively important, e.g., plasmid conjugation. This perspective was not addressed in our model. It is also useful to clarify that, for an episome (a plasmid capable of temporary incorporation into the chromosome), in our model, the transduction rate would be similar to that of a chromosomal segment for the period of chromosomal residency and to that of a plasmid for the period of extra-chromosomal residency, and the relative durations of these periods would need to be known for accurate estimation of the resulting net transduction rate.

Crucial to understanding the role of transduction in lateral gene spread is knowledge of the ecology of enteric bacteria and their phages. This gene transfer mode is a by-product of phage infection dynamics. The developed model of the dynamics of enteric phages in the cattle enteric commensal *E. coli* population showed that the numbers of transductants depended nonlinearly on the rates of *E. coli* turnover and replication (Fig. 3A and B). Ingestion supplied bacteria free of enteric phages, and bacterial intestinal replication made them susceptible to the infections. The phage-induced deaths, in turn, increased the replications because of density dependence of bacterial population growth. The potential for increased growth, however, was limited, because *E. coli* is a facultative anaerobe. Still, despite the growth limitations and turnover with largely phage-free replacement, assuming 90% of the infections were lysogenic, most of the enteric *E. coli* bacteria carried prophage of an enteric phage at the system's dynamic equilibrium. It could not be all enteric *E. coli* bacteria because the bacteria ingested by cattle with water and feed were free of the animal's enteric phages. Our estimations were based on the burst size and extracellular phage survivorship as measured in *E. coli* temperate phages *in vitro* (29). If these approximate well those in the intestine, the model outputs supported the theories that phages may be uniquely associated with the enteric habitat (1, 7–9), that temperate enteric phages are common, and that a large fraction of enteric bacteria carry prophages of enteric phages (1, 2, 10–14), including transducing phages (15–17).

The numbers of transductions also depended nonlinearly on what fraction of lysogens created in the intestine were cross-immune to other enteric phages (Fig. 3C). The model reproduced the empirical observation that a large fraction of enteric *E. coli* bacteria are lysogens of enteric phages when we assumed that cross-immunity for the enteric phage community was the norm. We also assumed the community was associated with this particular enteric habitat. Indeed, the DNA phage profile of feces is relatively stable within an individual but varies significantly between closely related individuals in human families (11), and that of rumen contents varies between cohoused small ruminants (40). On the other hand, commensal *E. coli* bacteria can circulate between their animate (mammalian intestines) and nonanimate habitats (35, 41). Given inheritance of the prophage throughout bacterial generations, this circulation between the intestines might explain how, despite the unique enteric phage communities in individuals and cross-immunity of new lysogens to other phages within a given community, it is possible to observe *E. coli* strains carrying prophages of multiple enteric phages. Polylysogeny (cocarriage of prophages) is reported in salmonellae of bovine origin (17) and in *E. coli* of vertebrates (15, 22). Developing a mathematical model for this hypothesis would require a good empirical understanding of the relative distributions of related prophages in the genomes of

E. coli strains from the intestinal contents of different animals and species. Further, the bacterial host ranges of individual phages, the degree of cross-immunity of lysogens (42), the relative durations of the lysogenic cycles, and the specifics of competition between coinfecting phages (43) would all need to be considered in the development of a model detailing the impact of polylysogeny on the ecology of enteric bacteria and the transduction of their genes.

Some phages attach specifically to the bacterial conjugative pilus (44); however, we could not locate published data on the distribution of these phages among enteric phages. It has been suggested that mammalian feces are not a habitat of these phages (8). However, in another study, the estimated mean number of pilus-specific *E. coli* phages in cattle feces is 95/g (45). In the current model, the simulated total number of *E. coli* phage virions/*tp* was approximately 3.8×10^6 /ml luminal contents in the cattle large intestine. Of *E. coli* pilus-specific phages in sewage effluents, 90% are RNA phages and 10% are DNA phages (46). Merging these data, we approximated the fraction of DNA pilus-specific phages among the cattle enteric coliphages as follows: $[95/(3.8 \times 10^6)] \times 0.10 = 2.5 \times 10^{-6}$. Further, for this phage group to contribute to lateral gene transfer from plasmid-bearing to plasmid-free bacteria, the phages need to be able to infect the latter, which would not generally be expected to express pili. Accordingly, it has been demonstrated that generalized transduction by pilus-specific phages is several times less likely from “male” (pilus expressing) to “female” than from “male” to “male” *E. coli* bacteria (47). We therefore ignored pilus-specific transducing phages in the current model.

This modeling study provided a quantitative perspective on the possible contribution of transduction to AMR gene lateral spread within a bacterial species *in vivo*. A model of infection dynamics of enteric phages in commensal enteric bacteria was developed. The model can be exploited and extended in the future to test hypotheses on enteric phage ecology and phage circulation between its animate and nonanimate habitats or on the effects of phages on the ecology and genetic makeup of the bacteria.

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