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Competition between *Escherichia coli* Populations with and without Plasmids Carrying a Gene Encoding Extended-Spectrum Beta-Lactamase in the Broiler Chicken Gut

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ABSTRACT Extended-spectrum-beta-lactamase (ESBL)/AmpC-producing *Escherichia coli* strains are widely found in *E. coli* isolates from broiler feces, largely due to the presence of the $bla_{CTX\text{-}M\text{-}1}$ gene on IncI1 plasmids. Plasmid carriage is theorized to cause fitness loss and thus should decrease under conditions of reduced antibiotic use. However, *in vitro* studies showed plasmid carriage to increase in the absence of antimicrobials, due to plasmid conjugation. We investigated whether this translates to increased levels of plasmid in the gastrointestinal tracts of chickens, where conjugation rates may be different and subtle differences in growth rates may have a larger impact on colonization. Eight groups of five chickens were orally inoculated at 4 days of age with a 0.5-ml volume containing 10^6 CFU/ml *E. coli* cells, of which 0%, 0.1%, 10%, or 100% carried the IncI1 plasmid with the gene $bla_{CTX\text{-}M\text{-}1}$. At 13 time points during 41 days, fecal samples were taken from each chicken. *E. coli* strains with and without plasmids were quantified. Trends in *E. coli* subpopulations were analyzed using generalized linear mixed models, and population dynamics were studied by fitting to a mechanistic model. Trends in *E. coli* subpopulations were different between groups rather than between individual chickens, suggesting substantial levels of *E. coli* exchange between chickens in a group. The IncI1 plasmid carrying $bla_{CTX\text{-}M\text{-}1}$ was transferred with conjugation coefficients at levels higher than those observed *in vitro*. Across groups, the plasmids disappeared or were established independently of the initial fraction of plasmid-carrying *E. coli*, but no major increase occurred as observed *in vitro*. Differences in growth rates were observed, but competitive exclusion of plasmid-carrying variants was counteracted by conjugation.

IMPORTANCE Bacteria that produce extended-spectrum beta-lactamases are resistant to an important class of antimicrobials in human and veterinary medicine. Reduction in antibiotic use is expected to decrease the prevalence of resistance. However, resistance genes often lie on plasmids which can be copied and transferred to other bacteria by conjugation, so *in vitro* resistance was observed to increase in the absence of antimicrobials. We sought to determine whether this also occurs in the chicken gut and if competitive exclusion by similar *E. coli* variants without the resistance occurred. We studied the excretion of *E. coli* carrying IncI1 plasmids with the $bla_{CTX\text{-}M\text{-}1}$ resistance gene in small groups of broiler chickens, after inoculating the chickens with *E. coli* suspensions containing different fractions of plasmid-carrying cells. Our results showed little variation between chickens within groups but large differences between groups that were independent of the ratio of variants with and without the plasmid and with persistence or extinction of the plasmid. However, there was no major plasmid increase as observed *in vitro*. We conclude that *in vivo*

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studies with sufficient independent replications are important for intervention studies on plasmid-mediated antimicrobial resistance.

KEYWORDS Bayesian model, antibiotic resistance, challenge, conjugation, experiment, poultry

The prevalence of extended-spectrum-beta-lactamase (ESBL)-producing bacteria in poultry rose steeply from the beginning of the 21st century in the European Union (1–3), but high prevalence among poultry is also reported worldwide (see, e.g., references 4–6). This high prevalence of ESBL-producing bacteria is a concern for veterinary public health. Although most ESBL genes in the general human population are not shared with poultry, some ESBL genes from poultry have been isolated from humans. Moreover, poultry farmers more often carry ESBL genes that are identical to the ones found in chickens than members of the general human population (7). Reduction of the use of antibiotics in livestock is promoted to decrease the selection pressure for antibiotic resistance, which is indeed observed in The Netherlands and Denmark (8, 9).

ESBL genes are often carried by conjugative plasmids (8, 9). Of the 235 ESBL-producing *E. coli* in broiler cecal samples collected at slaughter in The Netherlands in 2015, 46.8% of the ESBL-producing *E. coli* carried the $bla_{\text{CTX-M-1}}$ gene (8). Previous studies examining this gene on IncI1 plasmids in *E. coli* showed that plasmid carriage did not impose a demonstrable fitness cost on its bacterial host *in vitro*, eventually resulting in most bacteria carrying the plasmid (10). When the rate of plasmid gain by conjugation were to outweigh the rate of plasmid reduction due to fitness costs *in vivo*, IncI1 plasmids carrying $bla_{CTX\text{-}M\text{-}1}$ would expand and persist in the gut microbiome (11, 12) in the absence of antibiotics. That would greatly hinder efforts to reduce antibiotic resistance by decreasing antimicrobial usage. On the other hand, if fitness costs are high enough *in vivo*, plasmid-carrying bacteria should ultimately disappear by competitive exclusion. Competitive exclusion has earlier been shown effective at decreasing the levels of ESBL-producing *E. coli* in broilers that received cultures containing several different species (13–15). The issue then arises of whether this is caused by direct competition between ESBL-producing *E. coli* populations and ESBL-free *E. coli* populations.

We studied colonization of *E. coli* harboring IncI1 plasmids carrying $bla_{CTX\text{-}M\text{-}1}$ in the gut of freshly hatched chickens, after inoculation with a mixture of two strain variants of *E. coli* that differed only with respect to plasmid carriage. The aim of these experiments was to find out whether plasmid carriage would increase by conjugation or whether plasmid-carrying *E. coli* variants would be outcompeted by plasmid-free *E. coli* variants, i.e., representing competitive exclusion. Eight groups of five chickens were inoculated with two variants of the same *E. coli* strain in different ratios; one *E. coli* variant harbored conjugative IncI1 plasmids carrying $bla_{CTX\text{-}M\text{-}1}$ (TE75.01) and one variant did not (E75.01). The TE75.01 variant was obtained from a previous study (10) by mating strain E75.01 with a plasmid donor (E38.27). To emphasize that the two variants differ only in the carriage of the conjugative IncI1 plasmids carrying $bla_{CTX\text{-}M\text{-}1}$, we named these variants "E75.01" and "TE75.01," whereas in a previous study (10), variant TE75.01 was called T38.27. Variant E75.01 was resistant to ciprofloxacin (the resistance locus was on the bacterial chromosome), whereas TE75.01 was resistant to both ciprofloxacin (on the chromosome) and cefotaxime (on the plasmid). The chickens were sampled 13 times in the follow-up period of 41 days, during which *E. coli* variants were quantified in the presence and absence of antibiotics to distinguish between different subpopulations.

RESULTS

In our experiments, we distinguished six *E. coli* subpopulations, indicated by origin (*I* [inoculum population], *C* [colonizer population], or *R* [resident population]) and plasmid carriage (+ or −). Populations I^+ and I^- consisted of the inoculated bacteria that had not yet colonized the gut and were assumed not to grow. When they

TABLE 1 Setup of experimental groups

Treatment	n^a	No. of replicates	Inoculum (CFU/ml)	
			Without plasmid (E75.01)	With plasmid (TE75.01)
0%	5	2	10^6	0
0.1%	5	2	10^6	10^3
10%	5	2	10^6	10^5
100%	5	2	0	10^6

[a]Data represent the number of birds per group per replicate.

colonized the gut, they turned into populations C^+ and C^- (colonizing bacteria), which also consisted of all offspring of the colonized inoculum. Finally, populations R^+ and R^- consisted of the *E. coli* strains that were resident (*R*) (i.e., naturally present) at the time of inoculation and of their offspring. Only populations C^+, C^-, R^+, and R^- are excreted; populations C^+ and C^- are ciprofloxacin resistant; populations C^+ and R^+ are cefotaxime resistant. On the molecular level, populations I^+ and C^+ represent the TE75.01 variant and populations I^- and C^- the E75.01 variant.

General results. The design of the experiment is presented in Table 1, and the quantification data are presented in Fig. 1. One chicken (group 4, treatment 0.1%, replicate B) died during the experiment after day 11. The data from this chicken that had been collected up to that time point were included in the calculations (for data from each chicken, see Fig. S1 in the supplemental material).

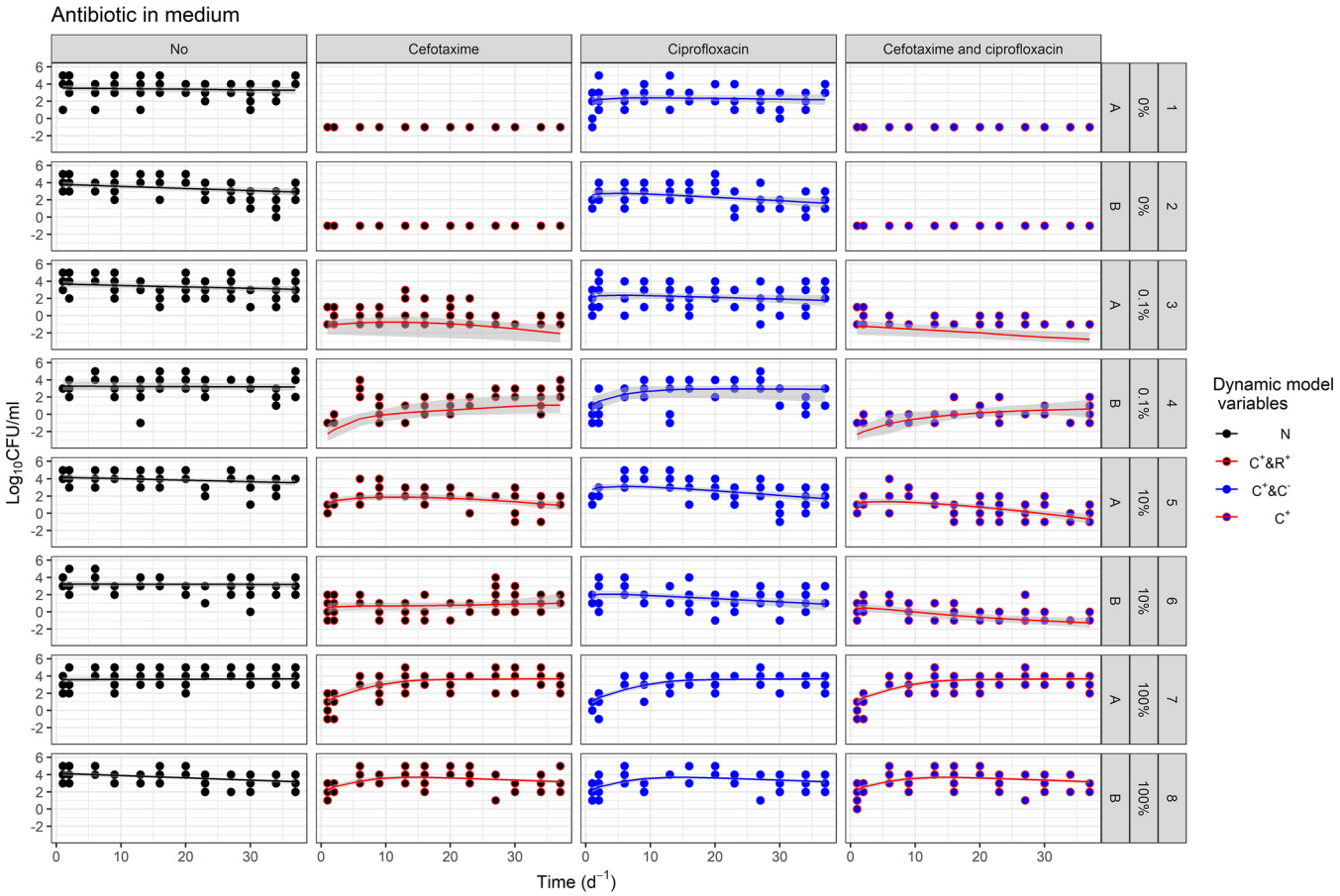

FIG 1 Log_{10} of the CFU counts per milliliter in the fecal sample of the inoculum variant (C^+ plus C^-) (resistant to ciprofloxacin) of *E. coli* with plasmids (R^+ plus C^+) which was resistant to cefotaxime and of the inoculum variant with plasmid (C^+) which was resistant to both ciprofloxacin and cefotaxime. The measurements are indicated with dots and the fitted models with lines. Each panel gives the results from five chickens per group. "A" and "B" represent replicate experiments. "0%," "0.1%," "10%," and "100%" refer to the inoculation and represent the fraction of plasmid-carrying *E. coli* in the inoculum that was given to the chickens in the group.

TABLE 2 Intraclass correlation coefficients for models of trends in the total *E. coli* population, the inoculum variant, or the number of plasmid-carrying bacteria[a]

Random effect	Correlation coefficient											
	Total *E. coli*			Plasmid			Inoculum strain			Inoculum strain with plasmid		
	Isolator	Animal	Inoculum	Isolator	Animal	Inoculum	Isolator	Animal	Inoculum	Isolator	Animal	Inoculum
Isolator	0.083			0.576			0.167			0.775		
Animal		0.094			0.664			0.207			0.716	
Inoculum			0.132			0.698			0.271			0.804
Isolator + Animal	0.095	0.026		0.662	0.009		0.204	0.019		0.776	0.001	
Isolator + Inoculum	0.077		0.095	0.251		0.471	0.123		0.190	0.116		0.692
Animal + Inoculum		0.051	0.114		0.056	0.673		0.048	0.279		0.007	0.796
All	0.070	0.026	0.092	0.250	0.006	0.477	0.115	0.018	0.192	0.106	0.001	0.702

[a]Linear models were fitted to data representing log-transformed numbers of bacteria.

The members of the negative-control groups (i.e., groups 1 and 2 [0% A and 0% B]) did excrete the ciprofloxacin-resistant E75.01 variant but were consistently cefotaxime resistance negative, showing 0.00% cefotaxime resistance (Fig. 1). The fraction of positive samples in the negative-control group was thus 0.00% in 120 samples (99% credible interval, 0.00 to 0.04) (Fig. 1). The members of the other groups did excrete cefotaxime-resistant bacteria, which were either the TE75.01 variant (which were also ciprofloxacin resistant) or resident *E. coli* (which had acquired the cefotaxime resistance by conjugation).

In most samples, the total number of plasmid-carrying bacteria (cefotaxime resistant, C^+ and R^+) was higher than the number of plasmid-carrying bacteria originating from the inoculum (both cefotaxime and ciprofloxacin resistant, C^+). From this we can conclude that the resident *E. coli* population in the gut of the chickens had acquired the plasmid by conjugation (Fig. 1).

Trends in excreted bacteria. For each univariable model, the intraclass correlation (ICC) value was well above 0 for each grouping variable ("group," "chicken," or "treatment"). However, combining the grouping variables, the ICC data for chicken decrease to values near 0 (Table 2). Therefore, further analyses were done on the level of the group, and data from individual chickens were considered independent observations of a group.

The total concentration of *E. coli* decreased during the experiments in 7 of 8 groups. Trends in the levels of cefotaxime-resistant *E. coli* bacteria, ciprofloxacin-resistant *E. coli* bacteria, and bacteria that were both cefotaxime resistant and ciprofloxacin resistant differed from group to group. Both an increase followed by a decrease and stable establishment of the colonizer were observed (Fig. 1).

Population dynamics. The population dynamics of C^+, C^-, R^+, and R^- were inferred by fitting a dynamical mathematical model to the count data (Fig. 2). The inferred dynamics show a high level of diversity in the dynamics of the colonizer and plasmid across the groups, with no clear relation to the inoculation (see Fig. 3 for graphs of the dynamics and Table 3 for inferred parameter values). After a transition period, the colonizer (C^+ and C^-, blue in Fig. 3) came to represent an almost stable proportion of *E. coli* bacteria in some groups. In both 10% inoculation groups, the proportion continued decreasing after an initial growth phase but the bacteria were still present at the end of the experiment.

We did not observe extinction of plasmid-carrying variants in any of the inoculation groups (0.1%, 10%, or 100%), although in group 3, 0.1%-A plasmid levels were low and possibly still decreasing at the end (Fig. 1). This is reflected in the estimated effect on the higher growth rate of the bacteria without plasmids (see parameter α data in Table 3). This difference was also present in groups 5 and 6 (10%) but was not present in group 4 (0.1%) and groups 7 and 8 (100%).

We did not observe an increase in plasmid carriage in any of the groups, unlike the increase in plasmid carriage to approximately 5% of the population via conjugation

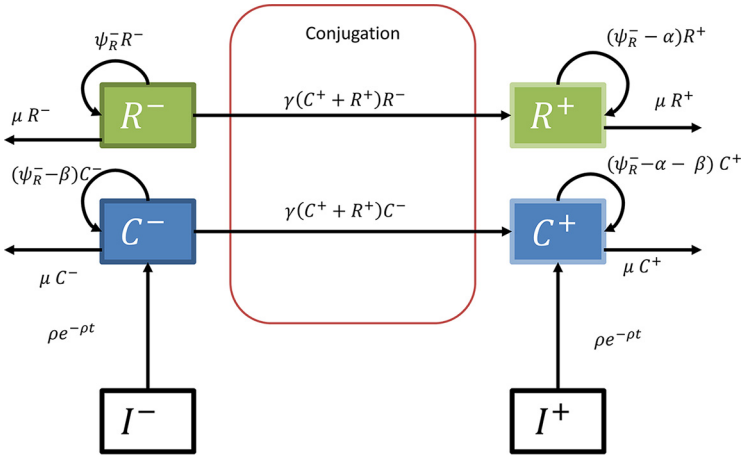

FIG 2 Diagram of the dynamical mathematical model used to infer different populations from the quantification based on the running drop method. In this flow diagram, "*R*" represents the resident *E. coli* population and "*C*" the colonizers descending from inoculum *I*. Superscript plus and minus signs indicate populations with or without the IncI1 plasmid carrying ESBL gene $bla_{\text{CTX-M-1}}$. "$I^- + C^-$" populations thus represent variant E75.01, and "$I^+ + C^+$" populations represent variant TE75.01. Inoculum *I* (the initial value is denoted by I_0) turns into colonizing bacteria *C* with a delay following an exponential distribution with mean $1/\rho$. The excreted bacteria are assumed to be either resident or colonizer bacteria; therefore, the implicit assumption is that the bacteria in the inoculum either change into colonizers or die. The populations with plasmid take part in conjugation with coefficient γ (number of conjugations per bacterium per hour). Bacterial populations grow with growth rate ψ and are flushed out with rate μ. *R* and *C* subscripts define parameters for the resident population and the colonizers, respectively.

that was observed *in vitro* (10). Nonetheless, in three groups (group 4 [0.1% B] and groups 5 and 6 [10% A and B]), the resident strains had acquired the plasmid, resulting in their forming a substantial fraction (i.e., more than 1 in 10,000 CFU per gram feces) of the population.

These observations are reflected in the estimated log conjugation coefficients, i.e., the log per-bacterium transfer rates of the plasmid per unit of time (parameter $\log_{10}\gamma$ in Table 3), which were much higher in groups. Overall, they were also higher than the previously calculated values of −8.0 to −12.2 for this bacterium-plasmid combination *in vitro* (10) and were especially higher in the groups for which we observed plasmid carriage in a substantial fraction of the resident population (group 4 [0.1% B] and groups 5 and 6 [10% A and B]).

DISCUSSION

We studied the dynamics of *E. coli* with and without IncI1 plasmids carrying $bla_{\text{CTX-M-1}}$ in groups of five chickens. On the basis of the results of earlier *in vitro* experiments, a rapid increase in plasmid carriage could have been expected, but we hypothesized that, alternatively, competition between plasmid-carrying and noncarrying variants could have led to extinction of the plasmid by competitive exclusion (16). The results were highly variable: in one of the six groups (0.1%-A), the plasmid seemed on its way to extinction. In three groups, conjugation to the resident *E. coli* was inferred and plasmid concentrations slowly increased, whereas in two groups, stable coexistence of the C^+ and R^- strains had been reached.

The *E. coli* population within one group of chickens could be considered to represent one population in the dynamic model, because the level of intraclass correlation between chickens was very low, while marked differences were found between groups. Cohousing in groups reduces heterogeneities in the dynamics between chickens, suggesting a high level of fecal-oral exchange of bacteria. Similar effects were found for *Campylobacter jejuni*, in that individually housed chickens showed a more gradual increase in probability of colonization with increasing challenge dose than group-housed chickens, which showed an all-or-nothing response. In the group-housed birds, the level of variation in the concentration of *C. jejuni* in the

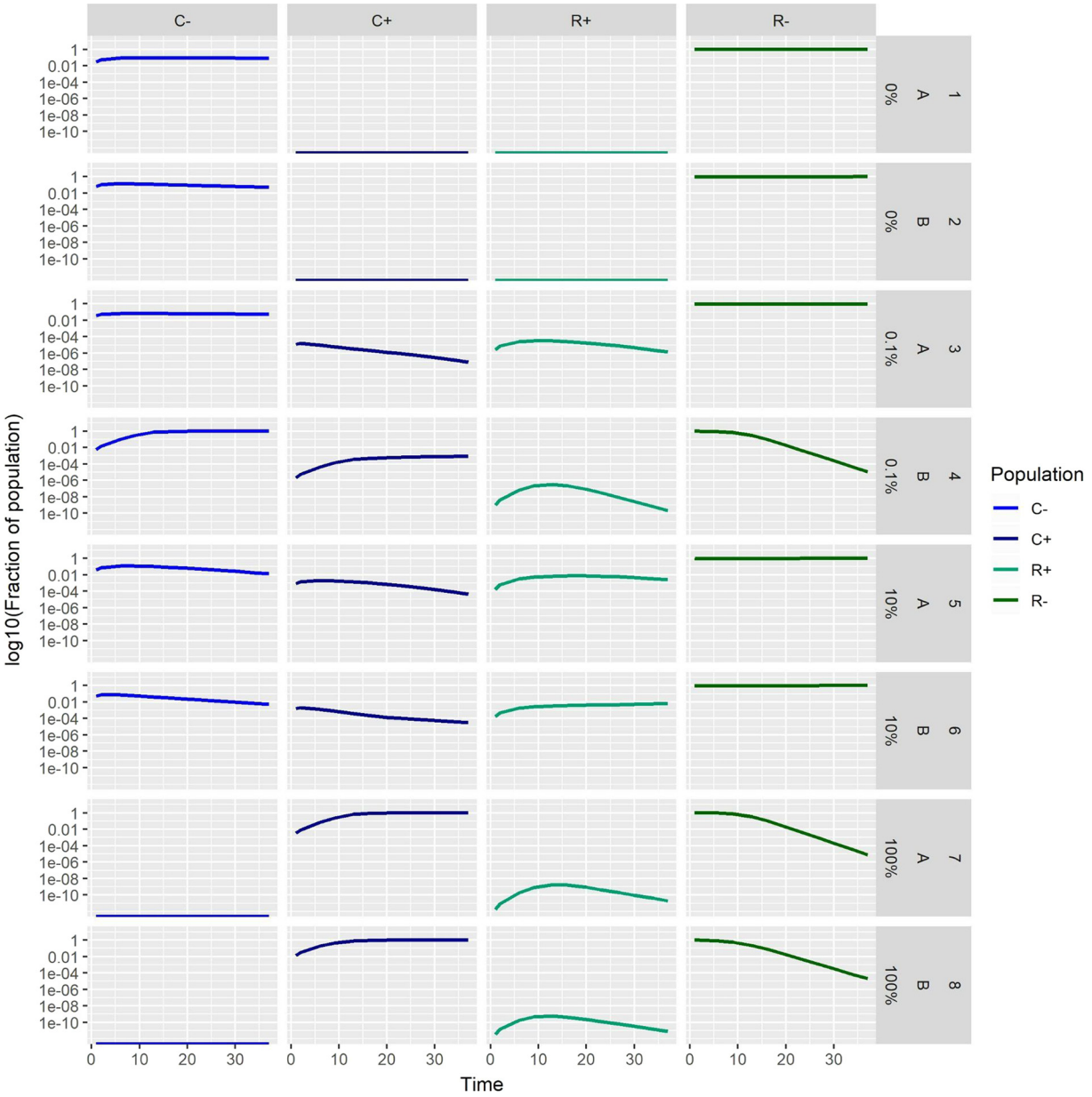

FIG 3 Fractions of the total concentration of *E. coli* inferred by fitting the bacterial count data to the dynamical mathematical model. Populations represent resident *E. coli* without the plasmid (R^-), resident *E. coli* with the plasmid (R^+), colonized *E. coli* without plasmid (C^-), and colonized *E. coli* without plasmid (C^+) in the fecal samples of five chickens for each of the groups. "0%," "0.1%," "10%," and "100%" refer to the fraction of plasmid-carrying *E. coli* in the inoculum.

cecum was low at day 7, with a standard deviation of 0.57 $\log_{10}$ CFU/g on average (17). Experiments with *Salmonella enterica* serovar Typhimurium and the effect of competitive microbiota did show reduced variation within replicate groups rather than between replicates in one case (18), while this was not the case in other experiments with a similar setup (19).

In each group, ciprofloxacin-resistant bacteria could be recovered, indicating that viable inoculum had reached the gut and was excreted. The levels of success of these colonizing variants were, however, not the same in all of the groups. In six of eight groups, the fraction of the *E. coli* population consisting of the colonizer variants remained stable after a certain period, but in groups 5 and 6 (10% A and B), the colonizer populations decreased after a transient increase during the first days. It is unclear whether this reflected a temporary residence in the gut without permanent

TABLE 3 Median and credible interval of parameter values fitted for the dynamical model for each of the eight groups separately[a]

			Value(s) (credible interval)							
Group	Treatment	Replicate	α	β	$\log_{10}\gamma$	ρ	I_0	$\frac{I_0^+}{I_0^+ + I_0^-}$	$\log_{10} n_0$	be
1	0%	A		0.01 (−0.09–0.09)		0.40 (0.08–3.51)	0.10 (0.03–0.36)	0[b]	3.54 (3.19–3.90)	−0.01 (−0.02–0.01)
2	0%	B		0.04 (−0.03–0.13)		0.04 (0.09–3.5)	0.19 (0.06–0.54)	0[b]	3.83 (3.48–4.16)	−0.02 (−0.04–0.01)
3	0.1%	A	0.29 (0.04–1.34)	0.01 (−0.06–0.09)	−4.09 (−20.90–3.44)	0.64 (0.08–4.70)	0.08 (0.02–0.30)	3.9×10^{-4} (1.8×10^{-6}–3.4×10^{-3})	3.69 (3.3–4.06)	−0.02 (−0.03–0.00)
4	0.1%	B	−0.03 (−0.16–1.71)	−0.43 (−1.70–0.10)	−6.32 (−27.60–3.10)	0.15 (0.03–1.22)	0.03 (0.00–0.22)	3.7×10^{-4} (4.1×10^{-5}–0.003)	3.29 (2.76–3.87)	−0.00 (−0.02–0.01)
5	10%	A	0.18 (0.03–0.56)	0.10 (0.03–0.216)	−4.65 (−5.22–4.13)	0.19 (0.09–0.48)	0.26 (0.09–0.61)	0.02 (6.1×10^{-3}–0.08)	4.2 (3.9–4.5)	−0.02 (−0.03–0.00)
6	10%	B	0.15 (−0.25–0.63)	0.09 (0.02–0.79)	−3.94 (−5.91–3.36)	0.73 (0.09–4.52)	0.12 (0.03–0.60)	0.03 (6.1×10^{-3}–0.17)	3.21 (2.89–3.61)	−0.00 (−0.02–0.01)
7	100%	A	−0.23 (−0.39–0.07)	−0.23 (−0.39–0.07)	−12.6 (−31.40–3.97)	0.10 (0.03–0.43)	0.03 (0.01–0.10)	1[b]	3.57 (3.26–3.89)	0.00 (−0.01–0.01)
8	100%	B	−0.20 (−0.36–0.04)	−0.20 (−0.36–0.04)	−13.50 (−30.60–4.64)	0.19 (0.05–0.86)	0.06 (0.02–0.22)	1[b]	4.16 (3.89–4.43)	−0.03 (−0.04–−0.02)

[a]These values and the credible intervals that were used to infer the population sizes for the data are also shown in Fig. 3. Parameters are as follows: α, difference in growth rates of bacteria with and without plasmid; β, difference in growth rates of resident and colonizer; $\log_{10}\gamma$, $\log_{10}$ conjugation coefficient; ρ, change rate for inoculum to colonizer; I_0, fraction of resident population that was replaced by the inoculum after change to colonizer; $\frac{I_0^+}{I_0^+ + I_0}$, fraction of inoculum carrying the plasmid; $log_{10}n_0$, $\log_{10}$ total *E. coli* population at time zero; be, population growth rate.

[b]The proportion of plasmid-carrying *E. coli* in the inoculum was set to either 0 for treatment 0% or 1 for treatment 100%.

colonization or a fitness disadvantage due to competition after further maturing of the gut microbiota. If there was indeed a fitness disadvantage for the colonizer variants, it is unlikely that this was mediated by the plasmid, as both group 7 and group 8 with the 100% plasmid-carrying variant showed persistent colonization.

The conjugation coefficient (γ) of a donor is lower *in vitro* (10) than *in vivo*. This difference in conjugation coefficient levels might be caused by differences in *E. coli* concentrations between the gut and *in vitro* experiments (20, 21). Another explanation could be that conjugation in the gut might partly take place on the epithelium, and there is a known difference in conjugation efficiency between liquid and surface media (22–24). Furthermore, the coefficient is estimated based on the concentration of both resident and colonizer variants, and resident strains might be in less extensive contact with the donor, due to prior niche colonization in the gut. Finally, differences in the intestinal environment, such as presence or absence of the immune response (21), can alter the transfer of plasmids.

It is possible that the dynamics of plasmids in *E. coli* populations are also affected by the specific *E. coli* resident strains, which we did not type because of the limited availability and high costs of genomic typing methods at the time of the experiments. However, it is not likely that these were very different between the groups in our experiment, because all chickens came from the same specific-pathogen-free (SPF) facility, were transported together to the experimental facility, and received the same tap water and sterilized food in the isolator housing. Also, given knowledge of the group-specific microbiomes, the low number of independent observations (8 groups) would make it impossible to draw any conclusions beyond spurious associations. It is unlikely that differences were caused by contamination with cefotaxime-resistant *E. coli* in the SPF facility or during transport, because both control groups remained negative throughout the experiment in 120 samples. Additionally, offspring from the same flock were used in more recently performed studies, where ESBL negativity was confirmed (13). The use of an uninoculated negative-control group was not considered necessary, because all the purposes of such control group might serve were addressed otherwise. First, the possibility of the presence of resistant bacteria in the resident flora was excluded by testing prior to inoculation and by results from other studies performed with chickens from the same SPF facility. Second, the possibility of contamination between groups was excluded by the use of the 0% plasmid groups, which remained cefotaxime sensitive during the experiment. Therefore, efforts, resources, and experimental animals were saved by not including a negative-control group.

A limitation of our study was that we used phenotypic plating for the typing of the bacteria and therefore could not directly observe the *E. coli* populations of interest—e.g., we cannot measure the resident *E. coli* population separately from the total *E. coli* population [Fig. 4]—but we were required to infer these by the use of mathematical models to quantify these populations from the plate counts. This approach might have introduced a bias as a consequence of differential growth on plates with no antibiotics and on plates with cefotaxime, ciprofloxacin, and the combination of cefotaxime and ciprofloxacin. In addition, the phenotype was not confirmed by molecular typing, which increases the uncertainty of findings, although we think that this is of minor importance due to the setup of the experiment and the unlikeliness of introduction of other antimicrobial-resistant *E. coli* bacteria. In conclusion, this experiment provided evidence that competitive exclusion of ESBL-producing bacteria might be caused by differences in growth rates compared to conspecific bacteria concurrently taken up by the chicken or to resident bacteria already present in the gut. The transfer of the ESBL gene through conjugation can, however, counteract the competition by growth. Interventions aiming at reducing levels of plasmid-mediated antimicrobial resistance should thus take into account that a competitive advantage in growth may be futile if it is overcome by conjugation. Furthermore, our results showed considerable between-cage variation, even with ESBL-producing bacteria disappearing without intervention. This shows that intervention trials should be carried out with independent replicates of each treatment or should be preceded by experiments to determine the between-cage variation.

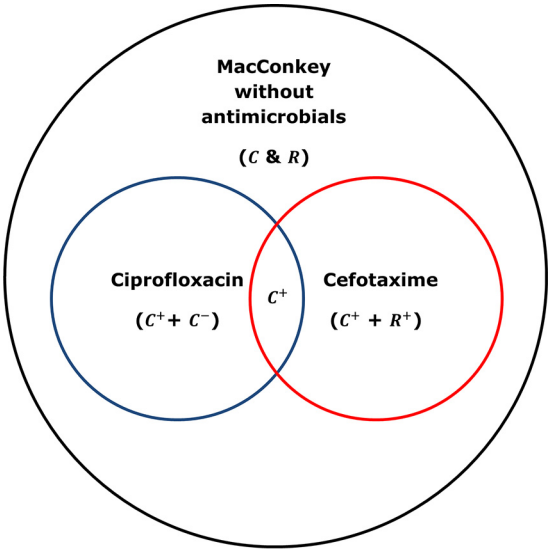

FIG 4 Schematic overview of the identification and quantification of *E. coli* populations in the experiments. Quantification was done for each sample on MacConkey agar without antimicrobials, or with ciprofloxacin, or with cefotaxime, or with both ciprofloxacin and cefotaxime. "*C*" indicates colonizing variants E75.01 and TE75.01 and "*R*" the resident *E. coli*. The symbols "+" and "–" indicate whether the bacteria contained the IncI1 plasmid carrying the ESBL $bla_{CTX\text{-}M\text{-}1}$ gene.

MATERIALS AND METHODS

Experimental protocol. A total of 61 broiler chickens were obtained from a specific-pathogen-free parent flock, a crossbreed of Cobb, Hybro, and Ross broiler breeders, from GD-Animal Health (Deventer, The Netherlands). The parent flock was free of ESBL-carrying bacteria, as confirmed by cloacal swabs tested according to the protocol described below. The chickens were obtained from an SPF facility, at which parent flocks are tested repeatedly to confirm that they are ESBL free (13). The results for the control chickens remained negative, indicating that the probability of transmission of ESBL from the parents was negligible even if the parents were ESBL positive, which they most likely were not. After hatching (day 0), individual chickens were tagged for identification in a flow cabinet and transported in an air-filtered cardboard transport box to the research facility. Chickens were randomly divided over eight HEPA-filtered, negative-pressure isolators (Beyer and Eggelaar, Utrecht, The Netherlands), which were cleaned and disinfected. The wired floor surface of 0.9 m^2 was partly covered with a sterile cloth during the first week of the experiment.

At day 4, five chickens were randomly selected from each group and excess chickens were euthanized. Each chicken was inoculated orally using a syringe without a needle with 0.5 ml of a solution with 10^6 CFU/ml *E. coli* with one of four different proportions of IncI1 plasmid carriage: 0% (negative control), 0.1%, 10%, or 100%(positive control). These four inoculations were replicated (Table 1). Replicates are denoted replicate A and replicate B throughout this paper. Fecal samples were taken by the use of cloacal swabs just before inoculation (day 4), and samples were taken after 24 h (day 5) and at days 6, 10, 13, 17, 20, 24, 27, 31, 34, 38, and 41 after inoculation.

A single broiler feed ration (Research Diet Services, Wijk bij Duurstede, The Netherlands), without antibiotic or coccidiostatic drugs and sterilized by gamma irradiation at 21 kGy (Synergy Health Ede B.V., Ede, The Netherlands), was used for the entire experiment. Feed and tap water from the same source were provided *ad libitum*. At day 41, the chickens were euthanized by cervical dislocation.

All experimental protocols were approved by the Animals Experiments Committee of Utrecht University (Utrecht, The Netherlands) and were carried out in accordance with the Dutch Experiments on Animals Act and EU directive 86/609/EEC.

Inoculum. The inoculum consisted of one or a mixture of two *E. coli* variants, variant E75.01 and variant TE75.01, originally derived in the Dutch national monitoring program. Both variants were used in a previous study by Fischer et al. (10). Variant E75.01 was used as recipient and was resistant to ciprofloxacin, due to mutations on the chromosome. TE75.01 was obtained by mating of strain E75.01 and strain E38.27 from the Dutch national monitoring program (10). TE75.01 was thus resistant to ciprofloxacin, due to mutations on the chromosome, and carried an IncI1 plasmid of sequence type 7, carrying ESBL gene $bla_{CTX\text{-}M\text{-}1}$ conferring resistance to cefotaxime obtained from strain E38.27. The IncI1 plasmid contained additional factors coding for toxin-antitoxin (TA) systems (25). The inoculum was a mixture of dilutions of E75.01 and TE75.01 variants that included a 0%, 0.1%, 10%, or 100% concentration of variant TE75.01 (see Table 1).

Quantification of bacterial populations. Quantification was performed for each sample on four different plates prepared as follows: without antibiotics for all *E. coli* isolates, with cefotaxime for plasmid-carrying *E. coli* isolates, with ciprofloxacin for both plasmid-free *E. coli* isolates and transconjugants, and with both cefotaxime and ciprofloxacin for *E. coli* isolates carrying the plasmid.

A semiquantitative method was applied to quantify the bacterial populations in cloacal samples modified from the track-dilution method described previously by Siragusa (26). Cloacal samples were obtained using sterile dry cotton swabs, which were individually weighed before and after sampling to measure the amount of collected feces. These cloacal swabs were processed individually by suspending the feces in 1 ml of saline solution from which 10-fold dilution series were made to quantify the number of CFU. From each dilution series, a 10-μl volume was inoculated into 9-cm^2 MacConkey agar plates with no antimicrobials, with cefotaxime, with ciprofloxacin, or with both cefotaxime and ciprofloxacin. The plates were positioned in an angle of about 45°, allowing the 10 μl to run down the plate, and were incubated overnight at 37°C. The highest dilution from which *E. coli* growth was observed was used to determine the number of CFU per milliliter in the feces suspension. Using the weight of the individual cloacal samples, the CFU level per gram of feces was calculated.

Data analysis. The purpose of this study was to infer the population dynamics of variants E75.01 and TE75.01 as well as of transconjugants among the members of the resident *E. coli* population. By fitting a dynamical mathematical model of these populations (E75.01, TE75.01, and the resident *E. coli* population), the population dynamics could be inferred from the bacterial counts.

To fit the bacterial count data to a mathematical model of the population dynamics in the gut, the following classifications were used (symbols representing variables are indicated between brackets): all *E. coli* (*n*), ciprofloxacin-resistant *E. coli* (C^+ plus C^-), cefotaxime-resistant *E. coli* (C^+ plus R^+), and ciprofloxacin-resistant and cefotaxime-resistant *E. coli* (C^+). Variables *C* and *R* were used to distinguish between the E75.01 and TE75.01 variants that had colonized the gut (indicated by a "*C*") and the resident *E. coli* population (indicated by an "*R*"). The plus and minus signs in the superscript indicate plasmid carriage and lack of plasmid carriage, respectively (Fig. 2).

Trend analyses. The aim of the trend analyses was to assess the extent to which chickens differed for each group, for the groups within each treatment group, and for the treatment groups with respect to each other. This was done by calculating intraclass correlation coefficients (ICCs) with chicken, group, and treatment as the grouping variables. ICCs were calculated by fitting the bacterial count data (log[CFU per gram]) to the sampling day (of days 6 to 41) with Bayesian linear regression models, using weakly informative priors on the regression coefficients (standard normally distributed) and the standard deviation (Cauchy [0.5]). The results were used to decide at which level to group data in the dynamical model analysis.

Dynamics. In all chickens, the intestinal *E. coli* population of size *n* consisted of one of the following fikkiwubg subpopulations: colonizers with plasmid (C^+), colonizers without plasmid (C^-), resident bacteria with plasmid (R^+), and resident bacteria without plasmid (R^-). To account for the delay between inoculation and start of excretion, we conceptually distinguished between inocula with and without plasmid ($I = I^+ + I^-$) and inocula that cannot multiply and colonizers (*C*) that can multiply, with the inoculum slowly turning into colonizers which were able to multiply. The colonizers thus represent a combination of the settled inoculum and offspring of the settled inoculum. For our analysis, we chose to measure only colonizers (C^+ or C^-) and residents ($R+$ and R^-), although they are enumerated as all *E. coli* colonizers ($n = C^+$ plus C^- plus R^+ plus R^-), ciprofloxacin-resistant *E. coli* bacteria (C^+ plus C^-), cefotaxime-resistant *E. coli* bacteria (C^+ plus R^+), and ciprofloxacin-resistant and cefotaxime-resistant *E. coli* bacteria (C^+).

The dynamics of the four *E. coli* subpopulations were analyzed with a dynamical model of bacterial growth, conjugation, and shedding. A diagram of this model is presented in Fig. 2, and its details are provided as supplemental material. The model describes a process by which inoculum *I* becomes colonized population *C* with an exponentially distributed delay of mean $\frac{1}{\rho}$. The colonized and resident populations *C* and *R* grow and flush out, ending up in the samples. The model incorporates competition by analysis of differences in growth rate ($\Delta\psi_c$) between the colonized and resident populations *C* and *R* and between bacteria with and without plasmids ($\Delta\psi_p$).

We described the process of conjugation with a density-dependent transfer with conjugation coefficient γ. The term "Density-dependent transfer" means that the intensity of conjugation increases with the number of bacteria that can donate the plasmid and the number that can receive the plasmid. Conjugation coefficient γ scales with intensity and was defined as the number of conjugations occurring per plasmid donor and recipient pair per unit of time. This definition of conjugation coefficient is essentially different from the frequency of transconjugants among the final population at the end of an assay. In our model, the colonized population with the IncI1 plasmid (C^+) could conjugate with rate coefficient γ with non-plasmid-carrying colonizers (C^-) and with the same rate coefficient γ with resident non-plasmid-carrying *E. coli* (R^-). The latter conjugation created the fourth group of resident *E. coli* bacteria with the IncI1 plasmid (R^+). The R^+ population also conjugated with C^- and R^- populations again with the same coefficient γ. The data calculated as $x = [C^+, C^+$ plus R^+, C^+ plus $C^-, n]$ represented the counts of ciprofloxacin-resistant and cefotaxime-resistant *E. coli* bacteria, ciprofloxacin-resistant *E. coli* bacteria, ciprofloxacin-resistant *E. coli* bacteria, and all *E. coli* bacteria. These data were fitted to the model by Hamiltonian Monte Carlo Bayesian analysis using the following likelihood function equation:

$$\mathrm{L}(\theta|x) = \sum_{T} \sum_{i=1}^{4} \left[H(x_i - \nu)\, f\left(x_i(T)|\{\log[E(\theta)_i], \sigma_i\}\right) + \left[1 - H(x_i - \nu)\right] \int_0^{\nu} f\left(y|\{\log[E(\theta)_i], \sigma_i\}\right) dy \right]$$

In this function, θ is the vector of model parameters and initial values, *f* is the probability density function of the normal distribution where the mean $E(\theta)$ was calculated by numerically solving the

dynamical mathematical model, and H is the Heaviside function, being 0 if $x_i - v = <0$ and 1 otherwise. The latter was used to account for a detection limit v, which was set to 10^{-1}, allowing the use of "zero" observations (no bacteria observed) by use of the cumulative density in the second term of the likelihood function. Marginal posterior and prior density distributions for the fitted parameters are provided in the supplemental material (see Table S1 and Fig. S2 to S9).

All calculations were performed using R 3.4.3 (27) and calling Stan from R (28) and using package rstanarm (29) for linear Bayesian regression. Scripts are provided in the supplemental material.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AEM.00892-19.

SUPPLEMENTAL FILE 1, PDF file, 1.1 MB.

SUPPLEMENTAL FILE 2, CSV file, 0.01 MB.

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We declare that we have no conflicts of interest.

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