Within-host dynamics shape antibiotic resistance in commensal bacteria

Nicholas G. Davies, Stefan Flasche, Mark Jit and Katherine E. Atkins

Antibiotic-resistant infections tend to be more common in populations that consume more antibiotics. The explanation seems obvious: greater antibiotic use selects for more resistance. However, capturing this pattern in an explicit model of bacterial transmission has been notoriously difficult. The problem is that empirical observation suggests a gently rising, roughly linear relationship between consumption and resistance, with both resistant and sensitive (that is, non-resistant) strains coexisting over a 4- to 20-fold range of antibiotic treatment rates. In contrast, simple models of person-to-person bacterial transmission predict competitive exclusion; that is, they predict that resistant strains will either disappear completely or spread to fixation, depending on the rate of antibiotic consumption in a population. Although potential explanations for this discord between theory and observation have been proposed, a generalizable, biologically explicit mechanism that accounts for widespread coexistence has yet to be identified. In short, despite the global public health threat of antibiotic resistance, we do not fully understand how resistance spreads in human populations.

We propose that within-host competition shapes resistance evolution and can promote widespread coexistence in commensal bacteria—that is, in species that are normally part of the host microbiota, but which occasionally cause disease when they invade sterile sites. Mathematical models of resistant strain transmission routinely overlook within-host interactions between different bacterial strains, but commensal bacteria regularly cohabit with genetically and phenotypically distinct strains of the same or different species. Laboratory experiments have shown that resistant and sensitive microbes inhibit each other’s growth when co-colonizing the same host, suggesting that these distinct strains engage in exploitative competition for host resources. Meanwhile, theory developed for malarial parasites has proposed that within-host competition between co-colonizing resistant and sensitive strains may interact with antimicrobial treatment to generate frequency-dependent selection for resistance at the population level, promoting coexistence. We develop this theory, arguing that population-level coexistence can be promoted by any phenotypic diversity that mediates competition between co-colonizing strains. Accordingly, we expect co-colonization to promote coexistence not only between resistant and sensitive bacteria, but also among other diverse microbes exploiting the same host niche, such as pneumococcal serotypes.

We develop a ‘mixed-carriage’ model that mechanistically captures within-host competition in an explicit model of bacterial transmission. This stochastic individual-based model—which can be approximated using deterministic ordinary differential equations (ODEs) for analytical simplicity—observes the key requirement of structural neutrality; that is, it avoids systemic biases that non-mechanistically promote (or inhibit) coexistence. When fit to data reported by 30 European countries, the model provides a parsimonious and generalizable explanation for empirical patterns of resistance across four pathogen–drug combinations. We also show how within-host competition can help to explain observed patterns of resistance and antigenic diversity among competing serotypes of the commensal bacterium Streptococcus pneumoniae.

Results

Co-colonization creates frequency-dependent selection for resistance. Frequency-dependent selection is known to promote diversity among competitors in animals and plants and microbes. In the classic scenario, a rare mutant invades a population by exploiting some weakness of wild-type individuals, but gradually becomes a victim of its own success by displacing the competitors it relies on to exploit. Stable coexistence between types can result if mutants tend to increase in frequency when they are rare (because there are ample wild-type individuals to exploit) but decrease in frequency...
when they are common (because there are too few wild-type individuals to exploit). Extending a hypothesis suggested by Hastings for malarial parasites \(^4\), we suggest that frequency-dependent selection for resistant bacteria is created by within-host competition among co-colonizing strains.

The mechanism works as follows. Suppose that a small group of resistant cells could colonize one of two hosts. One host already carries sensitive bacteria, while the other carries resistant bacteria. All else equal, the resistant cells would benefit more by colonizing the sensitive-cell carrier, because if that host were to subsequently take antibiotics—eliminating the resident sensitive cells—the newly arrived resistant cells could multiply to fully exploit the host niche, thereby increasing their potential to be transmitted to new hosts. Indeed, in vivo studies have shown that in co-colonized hosts harbouring both sensitive and resistant cells, the resistant pathogens increase in abundance when their sensitive competitors are killed by antibiotic treatment \(^5\). In other words, treatment results in competitive release for the resistant cells. By contrast, co-colonizing the resistant-cell carrier offers no such benefit to resistant cells, because later antibiotic use gives no advantage to the invading bacteria over the resident bacteria. This disparity creates frequency-dependent selection for resistance (Fig. 2a) because—a resistant cell is more likely to find itself co-colonizing a sensitive-strain carrier when resistance is rare.

Although originally phrased in terms of competition between malarial parasites mediated by antibiotic treatment and resistance \(^4\), this mechanism has broader applicability. First, other forms of within-host competition—not just treatment-mediated competitive release—can promote coexistence. For example, in vitro studies \(^2\), and in vivo \(^3\) studies have shown that in the absence of antibiotics sensitive cells often exhibit greater within-host growth relative to resistant cells—consistent with resistance carrying a fitness cost \(^1\). When \(r/u > c/(1-c)\), either strain can persist in isolation, but only one strain persists when both are present, with the sensitive strain prevailing when \(r/u > c/(1-c)\). The average resistance prevalence in Europe has hardly changed in recent years, suggesting that the observed coexistence is stable rather than a transient state on the way to competitive exclusion.

**Implicit versus explicit models of within-host dynamics.** Models that do not account for within-host competition will fail to capture this source of frequency-dependent selection for resistance (Fig. 2c). Nonetheless, existing models that incorporate co-colonization have not convincingly reproduced empirically observed coexistence \(^4\). We suggest that these models may have fallen short not because within-host competition is a weak driver of coexistence, but because they feature unrealistic assumptions concerning within-host dynamics. To illustrate this point, we compare two models of resistant pathogen transmission: an existing model \(^4\), which we refer to as the ’knockout model’, and a new ’mixed-carriage model’. These models share the same population-level dynamics, but differ in how they capture within-host dynamics, resulting in a substantial disparity in population-level patterns of resistance.

The shared assumptions of both models are as follows. There are two co-circulating bacterial strains: one resistant and one sensitive.
Hosts mix randomly, with each colonized host infecting other hosts at rate $\beta$, transmitting a ‘germ’ to a randomly selected host. A germ contains cells of one strain, chosen randomly in proportion to the number of cells of each strain carried by the transmitting host. All colonized hosts, including those carrying multiple strains, are assumed to be equally infectious. Resistant germs fail to transmit with probability $c$, where $c$ is the transmission cost of resistance. Additionally, transmission only succeeds with probability $k$ if the recipient is already a carrier, where $k$ is the efficiency of co-colonization relative to primary colonization. Finally, each host is naturally cleared of all strains at rate $\alpha$, and cleared of sensitive cells by antibiotic treatment at an additional rate $\tau$.

Starting from this common framework, the two models make divergent assumptions about within-host dynamics. First, the existing ‘knockout’ model assumes that hosts can be treated as though they contain two subcompartments of equal size (Fig. 3a). When a germ is transmitted to an uncolonized host, the invading strain fills the entire host niche, occupying both subcompartments. If, instead, germs are successfully transmitted to an already colonized host, the invading strain ‘knocks out’ and replaces the contents of one of the two subcompartments at random. These assumptions allow the knockout model to be implemented using only four host states—namely, X hosts are uncolonized; S hosts carry the sensitive strain only; R hosts carry the resistant strain only; and SR hosts carry both strains, one in each subcompartment (Fig. 3b). In the Methods, we describe how these model dynamics may be analyzed either using a stochastic individual-based framework or by integrating systems of ODEs.

As shown by Lipsitch et al., the knockout model is perhaps the simplest mathematical model that allows co-colonization without exhibiting systemic biases that artificially promote coexistence (this lack of bias is known as structural neutrality). Nonetheless, a mechanistic interpretation of a host’s two equally sized subcompartments, as posited by this model, is challenging. For example, they could represent two physically distinct but ecologically equivalent niches, but the identity of these two niches would be unclear, and it is known that bacteria of different strains can readily occupy the same host niche. Alternatively, the two subcompartments may be a way of representing a single host niche (for example, the nasopharynx or the gut), but it is unclear why a group of invading cells should replace either all resistant cells or all sensitive cells from an SR carrier rather than replacing cells from either strain at random. In addition to these conceptual difficulties, the knockout model predicts coexistence only across a narrow range of treatment rates that does not reflect the wide range over which coexistence is observed empirically (Fig. 3c).

To overcome these issues, we propose a new ‘mixed-carryage’ model that explicitly tracks within-host strain frequencies without splitting the host niche into two subcompartments. As in the knockout model, when a host is newly colonized, the invading strain is assumed to immediately occupy the entire host niche, reaching the host’s carrying capacity (Fig. 3d). However, when new cells enter, they are simply added to the cells that are already being carried. The carrying capacity is then immediately reimposed by eliminating excess cells at random, rather than by eliminating all cells from a given subcompartment containing only one strain. That is, following co-colonization, the host niche contains a fraction $\frac{1}{1+c}$ of the ‘old’ cells (an unbiased sample of the host’s carriage before co-colonization) and a fraction $\frac{c}{1+c}$ of the ‘new’ cells, where $c$ is the ‘germ size’ (that is, the relative size of an invading group of cells compared with the host’s carrying capacity). Because this model allows hosts to carry an arbitrary mix of cells of different strains, it requires keeping track of a large number of host states, which our stochastic individual-based implementation achieves. However, under the simplifying assumption that germ sizes are small ($c$ is much smaller than 1), the model is well approximated using a system of ODEs with only five host states (Fig. 3e), for a similar mathematical tractability to the knockout model (see Supplementary Note 1 for details). Strikingly, the mixed-carryage model supports much more coexistence than the knockout model, suggesting that a more explicit model of within-host dynamics may more readily explain observed patterns of resistance (Fig. 3f).

Because it specifically tracks within-host strain frequencies, the mixed-carryage model can serve as a starting point for more complex models. To illustrate this, we add differential within-host growth to the model, such that sensitive cells gradually increase in abundance relative to resistant cells sharing the same host (Fig. 3g). Accordingly, we assume that the sensitive strain grows exponentially relative to the resistant strain at rate $w$, eliminating the resistant strain completely if its relative within-host frequency drops below a critical threshold $f_{\text{crit}}$—while the overall carriage remains fixed at the host’s carrying capacity. Again, this differential growth requires tracking a large number of host states, which can either be accounted for directly with an individual-based model implementation or approximated using a finite number of mixed-carryage states in a system of ODEs, with the number of states depending on the desired degree of concordance with the idealized dynamics.
of within-host growth (Fig. 3h and Supplementary Note 1). Differential within-host growth tends to gradually eliminate resistant cells from co-colonized carriers, partially reducing the frequency-dependent benefit associated with resistant cells co-colonizing sensitive-strain carriers. However, it also introduces an additional frequency-dependent advantage for sensitive cells co-colonizing resistant-strain carriers, which, overall, can further expand the potential for coexistence (Fig. 3i).

In each model, the potential for coexistence depends on the prevalence of co-colonization, which is partly governed by the parameter $k$: while setting $k = 0$ eliminates co-colonization and recovers competitive exclusion, allowing co-colonization ($k > 0$) promotes coexistence. In Supplementary Note 2, we identify the key processes that inhibit coexistence in the knockout model and promote coexistence in the mixed-carriage model, showing how the extent of coexistence depends crucially on the prevalence of hosts carrying both sensitive and resistant strains.

### Structural neutrality of the knockout and mixed-carriage models

A structurally neutral model is one in which, when the biological differences between two strains are stripped away, pathogens of either strain are not treated differently from one another. The aim of structural neutrality is to ensure that the predicted outcome of competition between strains—whether it is coexistence or competitive exclusion—is attributable to identifiable, biological differences between the strains, rather than hidden assumptions embedded in the model structure. The knockout model meets the mathematical criteria for structural neutrality proposed by Lipsitch et al., but we argue that it violates the spirit of neutrality nonetheless. Specifically, the knockout model assumes that when a host carrying pathogens of two different strains is invaded by a new strain, the invading strain completely replaces one of the two resident strains while leaving the other untouched—even if the two resident strains differ only by a neutral, biologically meaningless label. This property artificially depletes within-host strain diversity, inhibiting coexistence by reducing the scope for within-host competition. In contrast, the mixed-carriage model avoids this artificial loss of diversity, while adhering to both the spirit and the letter of structural neutrality. In Supplementary Note 3, we demonstrate the structural neutrality of the mixed-carriage model, and discuss how a model’s adherence to within-host neutrality depends on the interpretation of within-host states.

Explicitly capturing within-host dynamics reproduces widespread coexistence. We used Bayesian inference via Markov chain Monte Carlo (MCMC) to fit both the knockout and mixed-carriage models to consumption and resistance data reported by 30 European countries across two common drug classes for the commensal pathogens *Escherichia coli* and *S. pneumoniae*. We assumed that countries differ only in their rate of antibiotic consumption, while other epidemiological parameters are shared across countries and are constrained to be consistent with empirically observed ranges.

---

**Fig. 3 | Models of within-host dynamics. a.d.g.** An example sequence of transmission events illustrating within-host dynamics for each model. b.e.h. Host states and transitions for each model; some transitions from intermediate states (dark grey circles in h) are omitted for clarity. c.f.i. Equilibrium resistance prevalence (the probability that a randomly selected pathogen from a randomly selected host is resistant) as a function of the antibiotic consumption rate $r$ and the efficiency of co-colonisation $k$ (shown is $k = 0, 0.25, 0.5,$ and $1.0$). Coexistence increases with $k$ and is more widespread under the mixed-carriage model (f), particularly with differential within-host growth (i). Parameters are $\beta = 5 \text{month}^{-1}$ and $\omega = 1 \text{month}^{-1}$, with particular values of $c \approx 0.07–0.12$ (c and f) and $w_{1} = 14–34$ (i) chosen so that resistance prevalence passes through 0.5 when $r = 1 \text{yr}^{-1}$ (Supplementary Note 1).
for carriage prevalence and average duration of carriage. Due to the limited range of coexistence predicted by the knockout model, we find that it cannot capture observed patterns of resistance\(^4,6\) (Fig. 4a). However, the empirical data are better captured by the mixed-carriage model (Fig. 4b), particularly when differential within-host growth is introduced (Fig. 4c). Using the Akaike information criterion (AIC) to select the most parsimonious model, we find that the mixed-carriage model with differential within-host growth has the most statistical support across all bacteria–drug combinations (Fig. 3). Frequent co-colonization by sensitive and resistant cells—irrespective of the overall carriage prevalence of the species of interest—is needed to maintain widespread coexistence via within-host competition (Supplementary Note 4).

**Patterns of coexistence among pneumococcal serotypes.** So far, we have focused on a simplified scenario in which bacterial diversity is limited to sensitive versus resistant strains, but the mixed-carriage model can be extended in this respect. The nasopharyngeal colonizer *S. pneumoniae* exhibits extensive diversity in the expression of capsular proteins exposed to the host immune system, subdividing the species into nearly 100 distinct ‘serotypes’ that—like resistant versus sensitive strains—are known to stably coexist in host populations\(^7,36\). Understanding both the coexistence of these serotypes and the evolution of resistance within each host is vital for building a comprehensive picture of resistance evolution in pneumococci. We thus extended the two-strain mixed-carriage model by parameterizing it with the serotype-specific duration of carriage for 30 of the most common *S. pneumoniae* serotypes\(^7\), assuming a 10% transmission cost and a 20% growth cost of resistance, and introduced serotype-specific adaptive immunity to the model (that is, host immunity to colonization by previously cleared serotypes; see Supplementary Note 5 for details). The extended model captures much of the observed serotype diversity and patterns of resistance among serotypes (Fig. 5).

**General predictions of the mixed-carriage model.** Our extended serotype model illustrates that within-host competition can promote pathogen diversity more broadly than for resistance-associated phenotypes per se. For example, consider a host carrying cells of two different serotypes. If one serotype is cleared by the host immune system, the other serotype may benefit from competitive release. As long as clearance of one serotype does not result in clearance of all serotypes within a host, clearance will tend to promote rare serotypes, since the hosts they co-colonize are more likely to be carrying a different serotype, and hence they are more likely than common serotypes to be the beneficiaries of competitive release mediated by natural clearance. This effect can promote serotype diversity (Fig. 6a) even in the absence of any host acquired immunity\(^27,36\).

We conclude by considering the impact of carriage duration, transmission rate and growth rate on resistance evolution. In agreement with previous theoretical work\(^1\), we find that a longer duration of carriage promotes greater resistance when resistance carries a within-host growth-rate advantage (Fig. 6b). However, this association can be reversed when resistance instead carries a within-host growth-rate cost (Fig. 6c), because a longer duration of carriage affords sensitive cells a greater opportunity to outcompete resistant cells within hosts. Accordingly, the overall relationship between duration of carriage and resistance may depend on the balance of these two costs of resistance for a given species. Our model also predicts that a higher transmission rate promotes co-colonization. In co-colonized hosts, sensitive strains may be eliminated by treatment, while resistant strains may be eliminated by faster-growing...
existence, a high proportion of hosts must be colonized by both sensitive strains. The relative importance of these two forms of competition determines whether increased transmission promotes or inhibits resistance (Fig. 6b,c). This mechanism may elucidate an observed positive relationship between resistance prevalence and population density\(^5\). Finally, we find that resistance is promoted in serotypes with greater within-host growth, as they are less likely to be excluded by other serotypes before antibiotic treatment results in their competitive release. Each of these three trends appears stronger when serotypes circulate in the same population compared with when they circulate in different populations (Fig. 6b,c). Why various species exhibit different levels of resistance when faced with similar rates of antibiotic treatment appears to be common: genotyping studies have found up to 48% of the same species, but carriage of multiple strains more generally appears to be common: genotyping studies have found up to 48%

**Discussion**

Our model provides two advances over previous work: it harmonizes pathogen dynamics by mechanistically capturing both between-host and within-host processes, and it better captures empirical patterns of antibiotic resistance. We argue that frequency-dependent selection drives these patterns of resistance, and that explicitly tracking within-host dynamics helps to reproduce them.

For within-host competition to maintain substantial coexistence, a high proportion of hosts must be colonized by both resistant and sensitive bacteria. Co-colonizing strains must also compete with each other for transmission; models with co-colonization but no competitive release do not produce extensive coexistence\(^7\). Empirical estimates suggest that dual carriage may be widespread. A study of *Staphylococcus aureus* carriage in children found that 21% of carriers were colonized by both resistant and sensitive *S. aureus* strains\(^8\). Relatively few studies have measured simultaneous carriage of both sensitive and resistant strains of the same species, but carriage of multiple strains more generally appears to be common: genotyping studies have found up to 48%
multiple carriage of genetically distinct \textit{S. pneumoniae} strains\textsuperscript{13,14} and up to 86\% multiple carriage of \textit{E. coli} strains\textsuperscript{15,16}. Although we have focused on competition between conspecific strains, competition between different species could also promote coexistence, reducing the need for widespread carriage of multiple strains of the same species. There is ample opportunity for between-species competition: the nasopharynx typically hosts tens or hundreds of species\textsuperscript{45,46}, while the gut typically hosts thousands\textsuperscript{47,48}. The extent to which this extensive diversity may contribute to resistance evolution remains to be evaluated.

Alternative mechanisms that could explain coexistence between drug-sensitive and -resistant pathogens have been proposed\textsuperscript{9,10,28,30,38}. Some support only modest amounts of coexistence\textsuperscript{4,6}, while others may be less empirically generalizable, such as strongly age-assortative mixing\textsuperscript{49}, independent mappings of balancing selection or specific immune responses to resistance-associated phenotypes\textsuperscript{50,51}. We have focused on how within-host competition can promote substantial coexistence on its own. A more complex model incorporating additional drivers of coexistence would support similar amounts of coexistence while diminishing the relative importance of within-host competition.

The models we have contrasted here make a number of simplifying assumptions. We have assumed that observed resistance patterns represent the equilibrium state, following on from the lack of conclusive evidence for significant time lags in resistance prevalence\textsuperscript{52} (Supplementary Note 6). We have assumed that antibiotics kill all sensitive cells instantaneously rather than adopting a more mechanistically explicit model of treatment\textsuperscript{43}, and that host immunity completely prevents colonization by previously cleared serotypes rather than providing partial protection\textsuperscript{42}. We have ignored effects of population structure, such as age-assortative mixing\textsuperscript{53} and heterogeneity in antibiotic consumption\textsuperscript{4,6}, which may promote additional coexistence. We have assumed that co-colonization occurs through sequential transmission events, ignoring the alternative routes of de novo mutation (which may be especially important for long-lived chronic infections\textsuperscript{54,55}), acquisition or loss of resistance through horizontal gene transfer, and simultaneous transmission of multiple strains from co-colonized carriers. Finally, we have focused on modelling resistance to a single drug at a time rather than exploring multi-drug resistance\textsuperscript{43}. Elaborations of our simple mixed-carriage model incorporating these additional complexities may provide a means with which to explore the importance of these mechanisms.

Antibiotic resistance is one of the foremost threats to human health, and combating this threat will require the global deployment of coordinated interventions\textsuperscript{4,10}. Mathematical models of pathogen transmission will play a crucial role in this endeavour because they can explicitly integrate the mechanisms that drive resistance evolution in a population-level framework and allow us to quantify long-term trends as well as the probable impact and cost-effectiveness of any large-scale interventions for reducing resistance\textsuperscript{45}. Providing a framework in which to answer public health questions demands a balance between mathematical tractability and necessary complexity; building on the simple model proposed here will help to establish that balance. If mathematical models incorporate a truly mechanistic understanding of resistance evolution, they will be better able to explain empirical patterns of resistance and accurately predict the impact of interventions at a national and global level\textsuperscript{46}.

\textbf{Methods}

\textit{The problem of coexistence.} Data and sources. We use data from the European Centre for Disease Prevention and Control on the primary-care consumption of penicillins, fluoroquinolones and macrolides versus aminopenicillin resistance and fluoroquinolone resistance in \textit{E. coli}, and macrolide non-susceptibility and penicillin non-susceptibility in \textit{S. pneumoniae}, across up to 30 European countries.

All data are from 2015, except for \textit{S. pneumoniae} penicillin non-susceptibility versus penicillin consumption, which are from 2007 as the breakpoints for \textit{S. pneumoniae} penicillin non-susceptibility were changed in some countries after this year, yielding inconsistencies in the resistance data between countries\textsuperscript{46}.

Antibiotic use is classified into primary-care and hospital consumption, with the majority of consumption in primary care\textsuperscript{7}. We use primary-care data only, as we are focusing on community-acquired bacterial carriage. Resistance is measured in invasive isolates extracted from blood and cerebrospinal fluid\textsuperscript{8}. We assume that each isolate is an unbiased sample of commensally carried strains\textsuperscript{9}. See Supplementary Note 7 for full details.

\textbf{Trends in resistance prevalence.} In Fig. 1a, linear regressions are least-squares fits to maximum-likelihood estimates of the resistance prevalence in each country. In Fig. 1d, the average resistance prevalence in Europe is calculated as the population-weighted mean of resistance prevalence across countries that reported data for all years in 2007–2015. See Supplementary Note 6 for more details.

\textbf{Two models of within-host dynamics.} In the Results, we contrast two models of within-host dynamics: the existing knockout model\textsuperscript{4,28} and the novel mixed-carriage model. Here, we describe how the knockout and mixed-carriage models can be implemented for two strains in a stochastic individual-based framework, then we show how they can be approximated using systems of ODEs. The individual-based and ODE implementations are equivalent under certain limiting assumptions and produce similar results (Supplementary Note 1). We use the ODE implementations to illustrate coexistence between resistant and sensitive strains and for model fitting (Figs. 1, 3 and 4). The individual-based implementation of the mixed-carriage model can be extended to simulate an arbitrary number of strains (Supplementary Note 5) and is used to analyse serumotype dynamics (Figs. 3 and 4).

\textbf{Knockout model\textsuperscript{4,28}}. In a population of \textit{N} hosts indexed by \textit{i} \in \{1,\ldots,\textit{N}\}, there are \textit{N}_\text{S} non-carriers, \textit{N}_\text{SR} sensitive-strain carriers, \textit{N}_\text{R} resistant-strain carriers and \textit{N}_\text{SR} dual carriers; we note host \textit{i}’s state as \textit{h}_i \in \{X,S,R,SR\}. The following host-state transitions occur as inhomogeneous Poisson point processes at the specified per-host rates:

\begin{align*}
X & \xrightarrow{\lambda_x} S \quad \text{(sensitive-strain colonization)} \\
X & \xrightarrow{\lambda_s} R \quad \text{(resistant-strain colonization)} \\
S & \xrightarrow{\lambda_i} SR \quad \text{(resistant strain co- colonization)} \\
R & \xrightarrow{\lambda_i} SR \quad \text{(sensitive strain co- colonization)} \\
SR & \xrightarrow{\lambda_k} S \quad \text{(knockout of resistant strain)} \\
SR & \xrightarrow{\lambda_k} R \quad \text{(knockout of sensitive strain)} \\
SR & \xrightarrow{\lambda_s} X \quad \text{(resistant - strain carrier clearance or treatment)} \\
R & \xrightarrow{\mu} X \quad \text{(resistant - strain carrier clearance)} \\
SR & \xrightarrow{\mu} X \quad \text{(dual carrier clearance)} \\
SR & \xrightarrow{\mu} R \quad \text{(dual carrier treatment)}
\end{align*}

For example, non-carriers (\textit{X}) become sensitive-strain carriers (\textit{S}) at rate \textit{\lambda}_{\textit{x}} and so on. Above, \textit{\lambda}_i = \rho(\textit{N}_\text{S} + \frac{\textit{N}_\text{SR}}{\textit{N}})/\textit{N} is the sensitive strain’s force of infection, \textit{\lambda}_i = \rho(1-c)(\textit{N}_\text{R} + \frac{\textit{N}_\text{SR}}{\textit{N}})/\textit{N} is the resistant strain’s force of infection, \textit{\rho} is the transmission rate, \textit{c} is the transmission cost of resistance, \textit{k} is the relative efficiency of co-colonization, \textit{u} is the natural clearance rate and \textit{r} is the treatment rate. In this model, the resistance prevalence is \rho = \left(\textit{N}_\text{S} + \textit{N}_\text{SR}\right)/\left(\textit{N}_\text{S} + \textit{N}_\text{R} + \textit{N}_\text{SR}\right).

\textbf{Mixed-carriage model.} In a population of \textit{N} hosts indexed by \textit{i} \in \{1,\ldots,\textit{N}\} as above, host \textit{i}’s state is (\textit{s},\textit{r}), where \textit{s} \geq 0 is host \textit{i}’s carriage of the sensitive strain and \textit{r} \geq 0 is host \textit{i}’s carriage of the resistant strain. In a non-carrier, \textit{s} = \textit{r} = 0, while in a carrier, \textit{s} + \textit{r} = 1. We model transmission, clearance and treatment events as inhomogeneous Poisson point processes, while within-host strain growth is updated in each host at regular discrete time steps. The following host-state transitions occur at the specified per-host rates:

\begin{align*}
(s,0) & \xrightarrow{\omega_{\text{SS}}} (s+1,0) \quad \text{(sensitive-strain colonization)} \\
(s,0) & \xrightarrow{\omega_{\text{SR}}} (s+1,1) \quad \text{(resistant-strain colonization)} \\
(s,0) & \xrightarrow{\omega_{\text{SS}}} (0,0) \quad \text{(clearance)} \\
(s,0) & \xrightarrow{\omega_{\text{SS}}} (0,1) \quad \text{(treatment)}
\end{align*}
Finally, the mixed-carriage model with differential within-host growth can be approximated with ODEs by adding ‘intermediate’ compartments between $R_i$ and $S_i$:

$$\frac{dS_i}{dt} = \beta S_i N \left(1 - \frac{S_i}{N} \right) - k_i S_i \frac{R_i}{N}$$

$$\frac{dR_i}{dt} = k_i S_i \frac{R_i}{N} - \left(1 - \frac{R_i}{N} \right) R_i$$

Here, there are $Z$ ‘intermediate’ compartments between $R_i$ and $S_i$, labelled $D_1$ through $D_Z$ (we use $Z = 7$; see Supplementary Note 1 for a graphical illustration of the dynamics of these intermediate compartments). Here, $b$ determines the within-host rate of the sensitive strain relative to the resistant strain, setting the rate at which individuals move from the $R_i$ compartment through intermediate compartments and finally through to $S_i$ and the resistant strain is gradually outcompeted by the sensitive strain. A separate parameter $b_i$ sets the rate of the final transition from $S_i$ to $S_i$. In practice, we set $b_i = \frac{1}{\tau}$, which for $Z = 7$ and $\tau = 0.001$ corresponds to the resistant strain effectively becoming lost once its within-host frequency drops below $\frac{f_{\text{min}}}{Z} = 5 \times 10^{-6}$ (Supplementary Note 1). The parameters $b_i$ and $b_i$ replace the parameters $w_i$ and $w_i$ from the individual-based implementation of the mixed-carriage model above; all other parameters (that is, $b_i$, $c_i$, $u_i$, and $k_i$) correspond to those used in the individual-based implementation.

Notating the fraction of a host’s bacterial culture that is resistant as $r_i$, for a host with state $Y$, we assume that $r_i = \frac{1}{1 + e^{-y_i}}$, and that intermediate compartments are evenly spaced between these points on a logistic curve; that is, that $\frac{1}{1 + e^{-y_i}} = \frac{1}{1 + (\frac{x}{10})}$, where $y_i$ corresponds to the resistant strain effectively becoming lost once its within-host frequency drops below $f_{\text{min}} = 5 \times 10^{-6}$ (Supplementary Note 1). The parameters $b_i$ and $b_i$ replace the parameters $w_i$ and $w_i$ from the individual-based implementation of the mixed-carriage model above; all other parameters (that is, $b_i$, $c_i$, $u_i$, and $k_i$) correspond to those used in the individual-based implementation.

Initial conditions and solutions. For all individual-based model simulations, we use $r_i = 0$ as the initial condition, which is consistent with equation (2) and maintains structural consistency with equation (1). Accordingly, in the model above, $S_i = S_i + \sum_{D_i} \left(1 - r_i \right)$ and $R_i = R_i + \sum_{D_i} \left(1 - r_i \right)$. Note that the mixed-carriage model without differential within-host growth can be recovered from the above model by setting $b_i = b_i = 0$; in model fitting, when we allow differential growth (that is, $b_i > 0$) we assume this for the costs of resistance, and accordingly set $c = 0$. In this model, the overall resistance prevalence is $\rho = \sum_{i=1}^{N} r_i / N$.
For a given model fit with parameters $\theta$, suppose that country $m$ (where countries are numbered 1 to $M$) has antibiotic treatment rate $\tau_m$ and reports that $r_m$ out of $n_m$ isolates are resistant. Over all $M$ countries, these data are denoted $\tau=(\tau_1, \ldots, \tau_M)$ and $r=(r_1, \ldots, r_M)$ and $n=(n_1, \ldots, n_M)$, respectively. We also have $Y_{m\tau}$ and $Y_{m\tau}^1$, which are the lower and upper bounds for carriage prevalence in any country (see below). Together, $\tau, r, n, Y_{m\tau}$ and $Y_{m\tau}^1$ are the data to which the model is being fit, and the model parameters are $\theta=(\beta, \tau, \rho, \rho')$. (Note that, for certain datasets, not all of the parameters in $\theta$ are permitted to vary; specifically, we assume $\rho=1$ when fitting $S$. pneumoniae for consistency with previous studies, and we allow only one at a time to contrast these two alternative costs of resistance.) Suppose that, for a given treatment rate $\tau_m$, the model predicts a resistance prevalence of $\rho(r_m|\theta)$ and a prevalence of carriage $Y(r_m|\theta)$. Then, the likelihood of the model fit is:

$$L(\tau, r, n, Y_{m\tau}, Y_{m\tau}^1|\theta) = \prod_{m=1}^M L(\tau_m, r_m|\theta) \times Y_m^*(\theta) \times \exp(1-1,000)$$

where $L(\tau_m, r_m|\theta)$ is the likelihood of the model-predicted resistance prevalence $\rho(r_m|\theta)$ given that country holds that $r_m$ out of $n_m$ bacterial isolates are resistant. Above, $L(\tau, r, n, Y_{m\tau}, Y_{m\tau}^1|\theta)$ is the probability density function of a truncated normal distribution with bounds 0 and 1, where $\rho(x | \mu, \sigma) = \frac{1}{\sigma \sqrt{2 \pi}} \exp\left(-\frac{(x-\mu)^2}{2\sigma^2}\right)$ is the untruncated normal probability density function and $\phi(x | \mu, \sigma) = \frac{1}{\sigma \sqrt{2 \pi}} \exp\left(-\frac{(x-\mu)^2}{2\sigma^2}\right)$ is the untruncated normal cumulative distribution function; and $B(\tau, r, n, \rho) = \left(\frac{\beta}{\rho}ight)^r (1-\rho)^{n-r}$ is the binomial distribution probability mass function, such that the integral calculates a weighted likelihood over all possible ‘true’ resistance prevalences $s$. The parameter $\sigma(\theta)$ of the truncated normal distribution is fit as one of the parameters of the model so that between-country variation is estimated separately for each alternative model.

Prior used for model fitting, posterior distributions from model fitting, and further details of MCMC can be found in Supplementary Note 4. Note that since we are only fitting to the measured resistance prevalence in each European country and to a fixed range of carriage prevalence, the values of certain parameters are difficult to identify, particularly for the knockout model.

Model comparison. For each model fit, we calculate AIC = $2K - 2 \log L$, where $K$ is the number of free parameters and $L$ is the maximum likelihood for a given model fit.

Patterns of resistance and coexistence among bacterial subtypes. (For Figs. 5 and 6, we extend the individual-based mixed-cage model to accommodate an arbitrary number of strains [Supplementary Note 5]. For Fig. 5 only, we also introduce serotype-specific adaptive immunity. Hosts develop immunity to a serotype when they naturally clear that serotype, and immunity provides complete protection against future colonization by that serotype. We assume that hosts that are replaced by new, immunologically naive, uncolonized hosts at rate $\alpha = 1/60$ month$^{-1}$, reflecting the relative importance of hosts aged 55 years for pneumococcal transmission$^{13}$. Other parameters for Fig. 5 are $\beta = 3.2$ month$^{-1}$ for sensitive strains and $\beta = 2.88$ month$^{-1}$ for resistant strains (that is, a 10% transmission cost of resistance), $w$ ranging from 0.8–24 for resistant strains (that is, a 20% growth cost of resistance), $k = 1$, $\tau = 0.025$ and $N = 1 \times 10^6$. For Fig. 6, other parameters are $\beta = 2$ month$^{-1}$, $\mu = 1$ month$^{-1}$, $w = 1$ and $k = 1$ unless otherwise specified in the caption. The treatment rate is $r = 0.075$ for Fig. 6a for sensitive serotypes circulating both separately and in the same population. For Fig. 6c, the treatment rate is $r = 0.075$ when serotypes circulate together, but $r = 0.05$ when serotypes circulate separately. The reduced treatment rate when serotypes circulate separately is necessary to observe the trend in resistance prevalence among serotypes (with $r = 0.075$, all serotypes show 100% resistance prevalence, so trends are not apparent). We use a population size of $N = 1 \times 10^6$ for runs with serotypes circulating together, and $N = 2 \times 10^6$ for runs with serotypes circulating separately.

Received: 2 January 2018; Accepted: 11 December 2018; Published online: 11 February 2019

References
1. Goossens, H., Ferech, M., Vander Stichele, R. & Elseviers, M. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet 365, 579–587 (2005).
2. Antimicrobial Consumption Database (ESAC-Net) (European Centre for Disease Prevention and Control, 2018).
3. Data from the ECDC Surveillance Atlas—Antimicrobial Resistance (European Centre for Disease Prevention and Control, 2016).
4. Colijn, C. et al. What is the mechanism for persistent coexistence of drug-susceptible and drug-resistant strains of Streptococcus pneumoniae? J. R. Soc. Interface 7, 905–919 (2010).
5. Hardin, G. The competitive exclusion principle. Science 131, 1292–1297 (1960).
6. Cobey, S. et al. Host population structure and treatment frequency maintain balancing selection on drug resistance. J. R. Soc. Interface 14, 20170297 (2017).
7. Lehtinen, S. et al. Evolution of antibiotic resistance is linked to any genetic mechanism affecting bacterial duration of carriage. Proc. Natl Acad. Sci. USA 114, 1075–1080 (2017).
8. Austin, D. J., Kristinsson, K. G. & Anderson, R. M. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. Proc. Natl Acad. Sci. USA 96, 1152–1156 (1999).
9. United Nations High-Level Meeting on Antimicrobial Resistance (World Health Organization, 2016).
10. O’Neill, J. Tackling Drug-Resistant Infections Globally; Final Report and recommendations (https://amr-review.org (2016).
11. Kamgangna, A. W. et al. High multiple carriage and emergence of Streptococcus pneumoniae vaccine serotype variants in Malawian children. BMC Infect. Dis. 15, 234 (2015).
12. Turner, P. et al. Improved detection of nasopharyngeal colonization by multiple pneumococcal serotypes by use of lateral agglutination or molecular serotyping by microarray. J. Clin. Microbiol. 49, 1784–1789 (2011).
13. Martinez-Medina, M. et al. Molecular diversity of Escherichia coli in the human gut: new ecological evidence supporting the role of adherent-invasive E. coli (AIEC) in Crohn’s disease. Inflamm. Bowel Dis. 15, 872–882 (2009).
14. Mongkolrattanothai, K. et al. Simultaneous carriage of multiple genotypes of Staphylococcus aureus in children. J. Med. Microbiol. 60, 317–322 (2011).
15. Gordon, D. M., O’Brien, C. L. & Pavli, P. Escherichia coli diversity in the lower intestinal tract of humans. Environ. Microbiol. Rep. 7, 642–648 (2015).
16. Chaban, B. et al. Characterization of the upper respiratory tract microbiomes of patients with pandemic H1N1 influenza. PLoS ONE 8, e69539 (2013).
17. Ederven, T. H. A. et al. Haemophilus influenzae is overrepresented in the nasopharynx of infants hospitalized with RSV infection and associated with increased viral load and enhanced mucosal CXCL8 responses. Microbiome 6, 10 (2018).
18. Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K. & Knight, R. Diversity, stability and resilience of the human gut microbiota. Nature 489, 222–230 (2012).
19. Negri, M. C., Lipsitch, M., Blázquez, J., Levin, B. R. & Baquero, F. Concentration-dependent selection of small phenotypic differences in TEM beta-lactamase-mediated antibiotic resistance. Antimicrob. Agents Chemother. 44, 2485–2491 (2000).
20. Wipf, A. R., Hujhember, S., de Roode, J. C., Shepherd, J. & Read, A. F. Competitive release and facilitation of drug-resistant parasites after therapeutic chemotherapy in a rodent malaria model. Proc. Natl Acad. Sci. USA 104, 19914–19919 (2007).
21. Melnyk, A. H., Wong, A. & Kassen, R. The fitness costs of antibiotic resistance mutations. Evol. Appl. 8, 273–283 (2015).
22. Smanni, Y. et al. In vitro and in vivo reduced fitness and virulence in ciprofloxacin-resistant Acinetobacter baumannii. Clin. Microbiol. Infect. 18, 1–4 (2012).
23. Birch, L. C. The meanings of competition. *Am. Nat.* **91**, 5–18 (1957).
24. Hastings, I. M. Complex dynamics and stability of resistance to antimalarial drugs. *Parasitology* **132**, 615–624 (2006).
25. Ayala, F. J. Competition between species: frequency dependence. *Science* **171**, 820–824 (1971).
26. Ayala, F. J. & Campbell, C. A. Frequency-dependent selection. *Annu. Rev. Ecol. Syst.* **5**, 115–138 (1974).
27. Cobey, S. & Lipsitch, M. Niche and neutral effects of acquired immunity permit coexistence of pneumococcal serotypes. *Science* **335**, 1376–1380 (2012).
28. Lipsitch, M., Colijn, C., Cohen, T., Hanage, W. P. & Fraser, C. No coexistence for: neutral null models for multistrain pathogens. *Epidemics* **1**, 2–13 (2009).
29. Sinervo, B. & Lively, C. M. The rock–paper–scissors game and the evolution of alternative male strategies. *Nature* **380**, 240–243 (1996).
30. Gogord, L. D. B., Macnair, M. R. & Smithson, A. Negative frequency-dependent selection maintains a dramatic flower color polymorphism in the rewardless orchid *Dactylorhiza sambucina* (L.) Soó. *Proc. Natl Acad. Sci. USA* **98**, 6253–6255 (2001).
31. Rainey, P. B. & Travisano, M. Adaptive radiation in a heterogeneous environment. *Nature* **394**, 69–72 (1998).
32. Wale, N. et al. Resource limitation prevents the emergence of drug resistance by intensifying within-host competition. *Proc. Natl Acad. Sci. USA* **114**, 13774–13779 (2017).
33. Lewnard, J. A. et al. Impact of antimicrobial treatment for acute otitis media on carriage dynamics of penicillin-susceptible and penicillin–non-susceptible *Streptococcus pneumoniae*. *J. Infect. Dis.* **218**, 1356–1366 (2018).
34. Andersson, D. I. The biological cost of mutational antibiotic resistance: any practical conclusions? *Curr. Opin. Microbiol.* **9**, 461–465 (2006).
35. Andersson, D. I. & Hughes, D. Antibiotic resistance and its cost: is it possible to reverse resistance? *Nat. Rev. Microbiol.* **8**, 260–271 (2010).
36. Flasche, S. et al. The impact of specific and non-specific immunity on the ecology of *Streptococcus pneumoniae* and the implications for vaccination. *Proc. R. Soc. B* **280**, 20131939 (2013).
37. MacFadden, D. R., McGough, S. F., Fisman, D., Santillana, M. & Brownstein, J. S. Antibiotic resistance increases with local temperature. *Nat. Clim. Change* **8**, 510–514 (2018).
38. Dietz, K. Epidemiologic interference of virus populations. *J. Math. Biol.* **8**, 291–300 (1979).
39. Gupta, S., Swinton, J. & Anderson, R. M. Theoretical studies of the effects of heterogeneity in the parasite population on the transmission dynamics of malaria. *Proc. R. Soc. B** **256**, 231–238 (1994).
40. Lipsitch, M. Vaccination against colonizing bacteria with multiple serotypes. *Proc. Natl Acad. Sci. USA* **94**, 6571–6576 (1997).
41. Blanquart, F., Lehtinen, S. & Fraser, C. An evolutionary model to predict the frequency of antibiotic resistance under seasonal antibiotic use, and an application to *Streptococcus pneumoniae*. *Proc. R. Soc. B* **284**, 20170679 (2017).
42. Colijn, C. & Cohen, T. How competition governs whether moderate or aggressive treatment minimizes antibiotic resistance. *eLife* **4**, e10559 (2015).
43. Smith, E. E. et al. Genetic adaptation by *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients. *Proc. Natl Acad. Sci. USA* **103**, 8487–8492 (2006).
44. Yang, L. et al. Evolutionary dynamics of bacteria in a human host environment. *Proc. Natl Acad. Sci. USA* **108**, 7481–7486 (2011).
45. Lehtinen, S. et al. Mechanisms that maintain coexistence of antibiotic sensitivity and resistance also promote high frequencies of multidrug resistance. Preprint at https://arxiv.org/abs/1707.08351 (2017).
46. Atkins, K. E. et al. Use of mathematical modelling to assess the impact of vaccines on antibiotic resistance. *Lancet Infect. Dis.* **18**, e204–e213 (2018).
47. Goossens, M. C., Catry, B. & Verhaegen, J. Antibacterial resistance to benzylpenicillin in invasive pneumococcal disease in Belgium, 2003–2010: the effect of altering clinical breakpoints. *Epidemiol. Infect.* **141**, 490–495 (2013).
48. Berg, A. & Braak, C. A. Markov chain Monte Carlo version of the genetic algorithm Differential Evolution: easy Bayesian computing for real parameter spaces. *Stat. Comput.* **16**, 239–249 (2006).
49. Bogaert, D. et al. Colonisation by *Streptococcus pneumoniae* and *Staphylococcus aureus* in healthy children. *Lancet* **363**, 1871–1872 (2004).
50. Chewapreecha, C. et al. Dense genomic sampling identifies highways of pneumococcal recombination. *Nat. Genet.* **46**, 305–309 (2014).

Acknowledgements

We thank M. Davies and A. Levy for assistance and S. Lehtinen, C. Colijn and M. Lipsitch for discussion. N.G.D., M.J. and K.E.A. were funded by the National Institute for Health Research Health Protection Research Unit in Immunisation at the London School of Hygiene and Tropical Medicine in partnership with Public Health England. The views expressed are those of the authors and not necessarily those of the NHS, National Institute for Health Research, Department of Health or Public Health England. For part of this work, S.F. was supported by a Sir Henry Dale Fellowship jointly funded by the Wellcome Trust and Royal Society (grant number 208812/Z/17/Z).

Author contributions

N.G.D., S.F. and K.E.A. conceived the study. N.G.D. performed the analyses. N.G.D., M.J. and K.E.A. drafted the manuscript, which was revised by all authors.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41559-018-0786-x.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to N.G.D.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- [ ] The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- [ ] An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- [x] The statistical test(s) used AND whether they are one- or two-sided
- [ ] Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- [x] A description of all covariates tested
- [x] A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- [ ] A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- [ ] For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
- [ ] Give P values as exact values whenever suitable.
- [x] For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- [ ] For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- [x] Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated
- [x] Clearly defined error bars
- [x] State explicitly what error bars represent (e.g. SD, SE, CI)

Software and code

Policy information about availability of computer code

Data collection  No specialised software was used to collect data for this study.

Data analysis  R version 3.3.3 was used to analyse data.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data used in this analysis are publicly available from the European Centres for Disease Control and Prevention:
2. European Centre for Disease Prevention and Control. Antimicrobial consumption rates by country. (2018). Available at: http://ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/esac-net-database/Pages/Antimicrobial-consumption-rates-by-country.aspx.
Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences
☐ Behavioural & social sciences
☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | No new empirical data were collected. |
| Data exclusions | No new empirical data were collected. |
| Replication | No new empirical data were collected. |
| Randomization | No new empirical data were collected. |
| Blinding | No new empirical data were collected. |

Reporting for specific materials, systems and methods

Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ Unique biological materials |
| ☒ Antibodies |
| ☒ Eukaryotic cell lines |
| ☒ Palaeontology |
| ☒ Animals and other organisms |
| ☒ Human research participants |

Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ ChIP-seq |
| ☒ Flow cytometry |
| ☒ MRI-based neuroimaging |