Antibiotics as a selective driver for conjugation dynamics

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It is generally assumed that antibiotics can promote horizontal gene transfer. However, because of a variety of confounding factors that complicate the interpretation of previous studies, the mechanisms by which antibiotics modulate horizontal gene transfer remain poorly understood. In particular, it is unclear whether antibiotics directly regulate the efficiency of horizontal gene transfer, serve as a selection force to modulate population dynamics after such gene transfer has occurred, or both. Here, we address this question by quantifying conjugation dynamics in the presence and absence of antibiotic-mediated selection. Surprisingly, we find that sublethal concentrations of antibiotics from the most widely used classes do not significantly increase the conjugation efficiency. Instead, our modelling and experimental results demonstrate that conjugation dynamics are dictated by antibiotic-mediated selection, which can both promote and suppress conjugation dynamics. Our findings suggest that the contribution of antibiotics to the promotion of horizontal gene transfer may have been overestimated. These findings have implications for designing effective antibiotic treatment protocols and for assessing the risks of antibiotic use.

Horizontal gene transfer (HGT) is a main contributor to the spread of antibiotic resistance genes1–3. Conversely, it has been generally assumed that antibiotics promote HGT4–5. One of the most common mechanisms for HGT, particularly for the transfer of plasmids such as those carrying antibiotic resistance, is conjugation6–7. There are two broad ways in which antibiotics can promote HGT via conjugation. First, when dosed at sublethal concentrations, antibiotics can increase the conjugation rate by either activating the excision of transferrable genes from the host chromosome or by inducing the expression of conjugation machinery (or both)8–12. Second, it has been speculated, but not proven, that antibiotics can cause global cellular responses, such as changes in cell wall composition or upregulation of key survival genes13,14, which can indirectly increase the conjugation rate15–17.

The overall conjugation dynamics are determined by two components (Fig. 1a): the rate at which conjugation occurs (the conjugation efficiency) and the subsequent growth of transconjugants. An antibiotic can affect these dynamics by modulating the conjugation efficiency, serving as a selection force that acts on the population dynamics after conjugation, or both (Fig. 1b,c). In past studies, quantification of the effects of antibiotics on conjugation efficiency has been confounded by a lack of decoupling between these two components18–20. As a result, there are conflicting reports regarding whether or not antibiotics promote conjugation21. Typically, in vitro conjugation experiments have been performed in the presence of an antibiotic, followed by a period of growth, before quantification of transconjugants16,22. The conclusion that antibiotics promote conjugation has been based on the observed increase in transconjugants in the presence of an antibiotic as compared with the control without treatment23. Unfortunately, this experimental design cannot distinguish between the effects of the antibiotic on conjugation efficiency and its effect on selection dynamics, nor does it show how these effects might depend on antibiotic concentration.

In vivo and case studies have also yielded results indicating a link between conjugation-mediated transfer of resistance and antibiotic treatment, possibly as a result of antibiotic selection providing a favourable environment to enable transfer24–26. As with in vitro experiments, however, this speculation remains to be definitively proved due to experimental complexities.

Results

To determine how antibiotics affect the various aspects of conjugation dynamics, it is critical to (1) decouple antibiotic-mediated induction of conjugation machinery from the global effect an antibiotic might have on the conjugation efficiency, and (2) decouple the conjugation event from the ensuing growth dynamics. To this end, we used an engineered conjugation system27 derived from the F plasmid (Fig. 2a), in which the conjugation machinery is constitutively expressed28,29. In this system, each donor cell (G⁢⁺) carries two plasmids: an F helper plasmid (FHR) and a mobilization plasmid. FHR expresses the conjugation machinery and can mobilize any plasmid containing the oriT sequence, but is not self-transmissible. Thus, although the system is synthetic, the conjugation machinery on the F plasmid is non-engineered. The mobilization plasmid, which is transmissible by the helper plasmid, carries the oriT sequence, a gfp gene under the control of a P_LacZ Promoter, and a kanamycin (Kan) resistance gene (kanR)30. Each recipient cell (R⁢⁻) carries the FHR plasmid and a non-mobilizable plasmid, and the non-mobilizable plasmid contains an mCherry gene under the control of a P_LacZ Promoter32,33 and a chloramphenicol (Cm) resistance gene (cmR). When mixed, G⁢⁺ can transfer a copy of its mobilization plasmid to R⁢⁻, generating a transconjugant (Y), which expresses both green fluorescent protein (GFP) and mCherry (Supplementary Video 1; for control without oriT, see Supplementary Video 2). For a complete list of plasmids and strains see Supplementary Table 1. This system enables us to quantify conjugation dynamics by selective plating or fluorescence microscopy.
We first quantified the conjugation efficiency in the absence of antibiotic-mediated selection. In brief, we mixed high densities of R+ and G+ in an equal ratio (\( \sim 5 \times 10^8 \) cells ml\(^{-1} \) of each) with varying concentrations of an antibiotic. We then incubated the mixture at room temperature (25°C) for 1 h in M9 minimal medium before measuring the densities of R−, G− and Y cells by selective plating. The temperature of 25°C was used to ensure negligible growth over the incubation period. The R− and G− densities did not change significantly after mixing and incubation in the absence of an antibiotic (Supplementary Fig. 1a, \( P > 0.8 \), two-tailed \( t \)-test), indicating a lack of growth of either population.

These densities also did not change when cells were incubated with high concentrations of a range of different antibiotics (Supplementary Fig. 1b, \( P > 0.2 \), two-tailed \( t \)-test), indicating negligible cell death. Taken together, these measurements indicate that there was negligible antibiotic-mediated selection under these experimental conditions. For all conditions, a small population of transconjugants emerged, which had a negligible effect on the density of either parental population, as indicated by their approximately constant population sizes (Supplementary Fig. 1). We took this population to be the number of transconjugants produced directly via conjugation (that is, in the absence of selection dynamics).

Using the protocol above, we measured the effects of ten different antibiotics, namely Kan, Cm, gentamicin (Gen), streptomycin (Str), spectinomycin (Spc), penicillin-G (PC-G), carbenicillin (Carb), ceftriaxone (Ctx), erythromycin (Eryc) and norfloxacín (Nor). These cover six major classes, including aminoglycosides, β-lactams, cephalosporins, macrolides, amphenicols and quinolones (Supplementary Table 2). For each antibiotic we defined a rate constant for conjugation, or the conjugation efficiency, as \( \eta_c = Y/RG\Delta t \), where, \( R, G \) and \( Y \) are the colony forming unit (c.f.u.) counts of recipients, donors and transconjugants, respectively (we have omitted the transfer signs, ±, for generality), and \( \Delta t \) is the time of incubation (1 h). This metric avoids the potential bias in quantification that arises when only one parent is considered (that is, \( Y/R \) or \( Y/G \)).

When dosed up to values equal to \( 2 \times IC_{50} \) (where \( IC_{50} \) is the half-maximum inhibitory concentration; Supplementary Fig. 2), none of the antibiotics significantly increased the conjugation efficiency (Fig. 2b, Supplementary Table 3a, \( P > 0.15 \), one-tailed \( t \)-test). If an antibiotic did increase the efficiency, the effect was smaller than the error associated with our experimental measurements (mean \( < 27\% \)).

Although there was no effect of antibiotic on conjugation efficiency, basal conjugation efficiency did exhibit substantial day-to-day variations (up to 13-fold, Supplementary Fig. 3a). Past studies have also revealed such variability when reporting conjugation efficiencies\(^{16,34}\). This variability was probably due to differences in the physiological states of the two parental populations. These variations could result in differences in the expression of conjugation machinery\(^{35}\), which is greater in the exponential phase than in the stationary phase (Supplementary Fig. 3b). Indeed, the conjugation efficiency increased drastically (~300-fold) when donor cells were collected from the exponential phase (Fig. 2c, left). However, the conjugation efficiency decreased (approximately eightfold) when recipients were collected during the exponential phase (Fig. 2c, left), indicating a state-specific role for parental populations during conjugation\(^{16,37}\). Because the conjugation efficiency also increased significantly when both parents were collected during the exponential phase (~280-fold), this indicates that the increased metabolic activity of donors can compensate for the decreased receptivity of recipients.

We reasoned that the donor recognition of the recipient, which requires an efficient searching mechanism of the pilus tip in liquid culture, is more effective when the recipient is in the stationary phase, either due to decreased motility or modifications in the cell wall composition\(^{38,39}\). As both F-tip searching and DNA transfer require energy\(^{40,41}\), we suggested the hypothesis that energy availability would also impact conjugation efficiency. Consistent with this notion, we found that increasing the glucose concentration in the medium significantly increased the conjugation efficiency (approximately sevenfold, Fig. 2c, right, \( P < 0.01 \), left-sided
Figure 2 | Antibiotics did not significantly increase conjugation efficiency. a, Synthetic conjugation system (Supplementary Table 1). The recipient (R') carries an immobile plasmid expressing kanR and an mCherry reporter. When mixed, G+ transfers a copy of its plasmid to R', generating transconjugant Y. Transconjugants can be quantified by selective plating or by fluorescence imaging. b, Quantification of conjugation efficiency (ηC) for R- and G+ (Supplementary Table 2; see Methods for details) with ten antibiotics. The y axis represents the conjugation efficiency normalized by the efficiency in the absence of antibiotics, ηC. The x axis shows antibiotic concentration [A] normalized with each ηC measured in plate readers (Supplementary Fig. 2). There was no significant increase in ηC among all antibiotics and concentrations tested (P > 0.15; one-tailed t-test, Supplementary Table 3a). G+ was mated with R' as control. No transconjugants were detected (c.f.u. data not shown, but provided in Supplementary Video 2). c, Left: The physiological state of the cells significantly influenced the conjugation efficiency. G+ and R cells were grown into the exponential phase for 2 h (see Methods). All four combinations of R- and G+ from either the stationary (s) or exponential (e) phase were tested: from top to bottom, Ge and Rs (crosses), Ge and Re (pluses), Gs and Rs (circles) and Gs and Re (squares). Str was used here at concentrations of > 0.15, one-tailed P-test). Donors and recipients are labelled in each panel (Supplementary Table 1; see Methods). Error bars for all data in Fig. 2 indicate mean ± s.d. from four to six replicates.

To test the generality of the results shown in Fig. 2b, we measured the conjugation efficiency for five additional native self-transmissible conjugative systems, including the native F plasmid, RP4, R388, R6K and pESBL-283 (ref. 42; Fig. 2d,i–v, and Supplementary Fig. 3d) with five antibiotics, Eryc, Carb, Cm, Str and Nor. These systems cover five different incompatibility groups, namely IncF, IncP, IncW, IncX and IncL. We also measured the conjugation efficiency for four clinical isolate donors expressing extended spectrum β-lactamases (ESBL), given that genes encoding ESBLs are often spread by conjugation43 (Fig. 2d,vi–ix, and Supplementary Fig. 3e). Finally, we tested interspecies conjugation using FHR-bearing Escherichia coli donor R' cells with two ESBL clinical isolates of Klebsiella pneumoniae as recipients (Supplementary Fig. 3f). For all conditions tested, we found that antibiotics did not significantly increase the conjugation efficiency (P > 0.15, one-tailed t-test, see Supplementary Tables 1 and 3b for a list of donors and recipients as well as experimental conditions).

If antibiotics do not affect the conjugation efficiency, their effects on the emergence of transconjugants can be manifested through selection dynamics on the donor (g), recipient (r) and transconjugant (y) populations. To describe these dynamics, we constructed a kinetic model consisting of three non-dimensionalized ordinary differential equations (ODEs) (Supplementary equations (4) to (6)). Assuming that y ≪ g:

\[ \frac{dg}{dt} = \mu_g (1 - r - g - y) \]  

\[ \frac{dr}{dt} = \mu_r (1 - r - g - y) - \eta_r g \]  

\[ \frac{dy}{dt} = y (1 - r - g - y) + \eta_r g \]  

Briefly, we assume logistic growth for all three populations, where \( \mu_r \) and \( \mu_g \) are the dimensionless growth rates of r and g, respectively, and \( \eta_r \) is the dimensionless conjugation efficiency. Depending on how the antibiotic concentration a affects the growth of the parental populations, the model reveals four qualitatively different dynamics for the fraction of transconjugants, \( f_y = (y)(r + g + y) \) (Fig. 3b). Modulation of transconjugants can be due to two factors: the contribution through the conjugation term \( \eta_r g \), and whether the transconjugants have a selective advantage over the parental strains (that is, if \( \mu_r + \mu_g < \mu_y = 1 \), Fig. 3b, top row, grey region), the transconjugant
Figure 3 | Antibiotics can both promote and suppress conjugation dynamics. a, Conjugation dynamics of $r$ (recipient), $g$ (donor) and $y$ (transconjugant) in response to antibiotics, which can alter the growth rates of one or more of the three populations. Curved arrows represent cell proliferation, and blunt-ended arrows represent antibiotic inhibition. $r$ and $g$ conjugate to form $y$. b, Model (equations (1) to (3)) predictions of the dependence of conjugation dynamics on antibiotic concentration $a$ for all four trends. Top row: growth rates for the three populations. Middle row: total density $n$ of $r$, $g$ and $y$ after 16 arbitrary time units (a.u.). Bottom row: $f_y$ normalized by $f_y$ at $a = 0$ ($f_y^0$). Dashed lines indicate the contribution through conjugation ($fg$). Shaded boxes indicate regions of similar dynamics. (i) Antibiotic has no effect on either parent strain. (ii,iii) Antibiotic inhibits only one parent strain. (iv) Antibiotic inhibits both parent strains. c, Microfluidic platform to quantify conjugation. The microfluidic chip contains cell-trapping chambers, which allow for monolayer bacterial growth (Supplementary Fig. 5; see Methods for details of the device). Eight to ten chambers were measured for each growth condition for each experiment. Populations were determined by obtaining the total number of pixels of $R$ (mCherry), $G$ (GFP) and $Y$ (co-localization algorithm)44, normalized by the carrying capacity of the chamber ($N_{\text{max}} = 6 \times 10^5$ pixels per chamber), to obtain $r$, $g$ and $y$, respectively. Representative images (from ten replicates) show pure $G^+$ and $R^-$ populations, and mixed populations with or without oriT (see Methods). d, Experimental results confirm the model predictions in b. Panel numbers correspond to those in b. Standard deviation was from eight to ten chamber replicates, and those falling outside two standard deviations or having lost focus were removed. (i) $R^+$ and $G^+$ are both resistant to Kan (the x axis represents Kan). (ii) $R^+$ and $G^+$ are resistant to Cm and Kan, respectively (the x axis represents Kan). (iii) As in (ii), with 4 $\mu$g ml$^{-1}$ Cm to reduce the $G^+$ growth rate. (iv) $R^+$ and $G^+$ are resistant to Cm and Kan, respectively (the x axis represents both Cm and Kan). Top row: growth rates for the three populations quantified using plate reader measurements (see Methods). Middle row: total density $n$ of $r$, $g$ and $y$ after 12 h of growth. Bottom row: $f_y$ normalized by $f_y$ at $[A] = 0$, $f_y^0$ (Supplementary Table 5).

When transconjugants are at a selective advantage, $\mu_r + \mu_g < \mu_y$ (Fig. 3b,i,iv, blue region), the transconjugant population either decreases (Fig. 3b,iii, middle row) or increases then decreases (Fig. 3b,iii,iv, middle row), depending on whether the corresponding contribution through conjugation remains constant, increases or decreases (Fig. 3c, middle row; black lines indicate the contribution through conjugation).

To test the model predictions, we developed a microfluidic device (Fig. 3c and Supplementary Fig. 5a, see Methods) to analyse the population dynamics during conjugation. Bacteria form a monolayer in each growth chamber, which allows quantification of transconjugants by co-localizing pixels containing both GFP and mCherry44 (Supplementary Fig. 5). Furthermore, the platform enables precise control of antibiotic delivery by modulating the flow rate of the media. By varying the combinations of Kan and Cm to obtain the desired selection environments (Fig. 3d, top row), our experimental results validated the overall model predictions in terms of total density $n$ of each population ($r$, $g$ and $y$) normalized by the carrying capacity in the chamber (Fig. 3d, middle row), and the fraction of transconjugants ($f_y$) (Fig. 3d, bottom row). In each scenario, the predicted trends for the fraction of transconjugants were maintained when we switched the antibiotic or the direction of transfer (Supplementary Fig. 6a,b). These results confirmed that promotion of transconjugants by antibiotic-mediated selection occurred only under two conditions and over a particular antibiotic range (Fig. 3d,iii,iv, bottom row). For a summary of the microfluidic experiments, including the strains and concentrations used, see Supplementary Table 5.
In nature, conjugation typically occurs in the presence of buffer populations that may also participate in conjugation. To this end, we tested the effect of a buffer population (denoted B in the experiment and rb in the model), which can receive the mobile plasmid from donor cells and then pass this to recipient cells, generating transconjugants in the process. According to our simplified model (Supplementary Fig. 6c and Supplementary equations (14) to (17)), we predicted that the buffer population should maintain a monotonically decreasing transconjugant population. Our full model predicts the same outcome, as long as the above conditions are met (for example, the transconjugants never gain a selective advantage, and the contribution through conjugation decreases monotonically, Supplementary Fig. 6c). Using E. coli MG1655 carrying FHE, as the buffer population, our experimental results again validated the model predictions (Supplementary Fig. 6d).

As is evident in equation (3) and Fig. 3b, middle row, both r and g contribute critically to y dynamics. Consistent with this notion, it has been shown that pathogen-driven inflammation in mice generates blooms of both parents, resulting in a high conjugation frequency. Indeed, our modelling and experiments show an approximate power-law correlation between the transconjugant population y and the product of the donor and recipient populations, rg (Fig. 4), regardless of the selection environment or the time at which the data were collected (Supplementary Fig. 7). This intuitive correlation suggests a strategy that can be used to estimate the likelihood of conjugation, provided the two parental populations can be estimated in situ.

Discussion

It is well established that conjugation plays a major role in the worldwide spread of antibiotic resistance. However, the converse statement—that the use of antibiotics promotes conjugation—is not necessarily true. In general, conjugation must overcome mechanistic, physiological and selective barriers to be successful. Indeed, our results indicate that the contribution of antibiotics to the promotion of conjugation may be over-estimated, for two reasons. First, antibiotics do not significantly increase the efficiency of conjugation in systems where the conjugation machinery is constitutively expressed. Second, even considering selection dynamics, only a small subset of parameters promote an increase in the fraction of the transconjugants. This counterintuitive outcome arises from the opposing roles an antibiotic plays in conjugation. If an antibiotic does not affect either parent strain, there is no selective advantage for the transconjugant. If the antibiotic inhibits either or both parent strains, it does offer a selective advantage for the transconjugant. However, the antibiotic might still reduce the frequency of conjugation by reducing the population sizes of either or both parental populations, potentially negating the effect of positive selection for the transconjugant.

Although the presence of an antibiotic does not significantly increase the efficiency of conjugation per se, our results suggest that other factors, particularly the physiological state of cells before conjugation and energy availability during conjugation, have a drastic effect on the conjugation efficiency. When considering selection dynamics, population structure plays a dominant role in predicting transconjugant emergence (Fig. 4). Our findings underscore the importance of quantifying the growth dynamics of microbial populations (with or without antibiotic treatment), both to gauge the physiological states of cells and to estimate the effects of antibiotic-mediated selection. This information can also contribute to assessment of the risk associated with the dissemination of resistance. For example, a recent study demonstrated the feasibility of quantifying both abundances and growth rates of different microbial populations using sequencing. These measurements, when coupled with in vitro estimates of conjugation efficiency, can enable quantitative estimates of the extent of HGT in diverse natural environments.

Methods

Strains, growth conditions and plasmid construction. E. coli strain MG1655 carrying the FHE plasmid (F plasmid with a mutated oriT sequence, oriTm) was used as the background strain for all engineered experiments in this study. Donor cells (G+ ) contained mobilization plasmid pUA66, which carries the gfp gene under the control of the PUA66 promoter, oriT m for transfer, and Kan resistance (kanR), adapted from a plasmid used in previous work. Recipients (R) carried pRmCherry, an immobile plasmid containing a Tet-inducible mCherry gene and Cm resistance (cmyR) (Fig. 2a). Transconjugants (Y) were resistant to both Kan and Cm. For a complete list of strains and plasmids used in this study, see Supplementary Table 1.
For all experiments, single clones were grown separately for 16 h in 5 ml Luria-Bertani (LB) broth, supplemented with appropriate antibiotic concentrations (11-120 µg ml⁻¹), and transcribed into cDNA using the Applied Biosystems high-capacity cDNA Reverse Transcription kit (ThermoFisher Scientific, catalogue no. 4386814). For experiments using the Fhr conjugation system, G⁺ was used to quantify all dose responses, except for Kan, where R⁻ was used instead. Seven logarithmically spaced antibiotic concentrations were used, and 0 µg ml⁻¹ antibiotic was used as a control. The highest concentration of each antibiotic was selected to capture the appropriate range of both growth and death. Concentrations of 0, 0.95, 3.93, 16.6, 6.9, 28.8, 120 and 500 µg ml⁻¹ were used for Kan, Cm, Carb, Strept, Spc, Ctx and PC-G, concentrations of 0, 0.019, 0.08, 0.33, 1.38, 5.77, 24.01 and 100 µg ml⁻¹ for Nor and Eryc, and 0.047, 0.20, 0.83, 3.46, 14.4, 60.03 and 250 µg ml⁻¹ for Gen. A full description of all antibiotics (and suppliers) used in this study is provided in Supplementary Table 2. Three technical replicates per concentration were used to quantify the growth rate. Growth rates were quantified by log-transforming the growth curves, applying K-means clustering to non-arbitrarily locate the region of longest exponential growth, curve smoothing using MATLAB, and fitting of the linear portion. Dose–response curves were fit to a Hill function to determine the growth rate μ:

\[
\mu = \frac{\mu_{\text{max}} IC_{50}}{IC_{50} + A^n}
\]

where A is the antibiotic concentration, μ_{\text{max}} is the maximum rate, and n is the Hill coefficient. For subsequent c.f.u. measurement experiments using G⁺ and R⁻, five antibiotic concentrations, including 0 µg ml⁻¹ as control, were selected to span a range from twofold below to twofold above the IC_{50} value (Fig. 2b and Supplementary Table 3a). The conjugation efficiency, not normalized to the group in the absence of antibiotic, is shown in Supplementary Fig 3a.

We tested various additional plasmids and strains to demonstrate the generality of these findings. For these experiments, only three concentrations of antibiotics were chosen, namely 0, the IC_{50} value and twice the IC_{50} value. Because conjugation of the additional plasmids occurred between different strains, the IC_{50} of the recipient strain was used to choose the corresponding concentrations, unless the recipient was resistant to the tested antibiotic or the IC_{50} was so high that it caused a decrease in viability. In either of these cases the donor IC_{50} was used instead. The same five antibiotics were tested for each additional conjugation pair (Cam, Carb, Eryc, Strept and Nor). For all ESBL conjugation experiments, the IC_{50} values of the recipient, R⁻, were used for antibiotic scaling. All relevant IC_{50}, values, and donor/recipient pairs, are listed in Supplementary Tables 1 and 3b.

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR).

A single clone of G⁺ was grown overnight for 16 h at 37 °C. The exponential-phase overnight culture was obtained by diluting overnight cultures of G⁻ tenfold and growing these with shaking (250 r.p.m.) for 2 h at 37 °C before collection. RNA was extracted using the Qiagen RNeasy Protect Bacteria mini kit (Qiagen, catalogue no. 74524). On-column DNase digestion using the RNase-Free DNase set (Qiagen, catalogue no. 79234) was performed to remove any DNA present. RNA was then reverse-transcribed into cDNA using the Applied Biosystems high capacity cDNA Reverse Transcription kit (ThermoFisher Scientific, catalogue no. 4368814). qRT-PCR was

For experiments using the Fhr conjugation system, G⁺ was used to quantify all dose responses, except for Kan, and transcribed into cDNA using the Applied Biosystems high-capacity cDNA Reverse Transcription kit (ThermoFisher Scientific, catalogue no. 4368814). qRT-PCR was

The results of statistical testing using a left-sided one-tailed test to detect an increase are shown in Supplementary Table 3 for all conjugation experiments.
performed using Power SYBR Green Master Mix (ThermoFisher Scientific catalogue no. MW640) on a 7500 Real-Time PCR system. A gene specific for the transconjugant was used as the control gene. Data were collected from three replicates, and quantified using the standard curve method. The genes targeted were traD (forward: 5′-CCCCGAGTGGCGGTAATCT-3′ and reverse (RV) 5′-AGCGGGAGACGTC TTATTA-3′) and traD (FW 5′-GGCACTGGGCAATCATCT-3’ and RV 5′-GGG TTCGAGCCATGT-3′). The primary primer for traD was FW 5′-GGGAGGAAAGGATG C-3′ and RV 5′-GGGCAAATGCGGAAAAAGCAT-3′ (all primers from IDT). The results were normalized with respect to ffb first, and then compared for fold change between exponential- and stationary-phase cultures (Supplementary Fig. 3b). Error bars represent three technical replicates, and qPCR was performed twice on separate biological samples to ensure reproducibility.

Microfluidic device. The use of a microfluidic device is uniquely suited for antibiotic selection experiments, because the transconjugants are distinguishable from G+ and R+ as they express both GFP and mCherry. Trapping chambers from this device capture bacterial populations in a monolayer, which allows for highly accurate transconjugant quantification (Supplementary Fig. 5a).

The microfluidic device was fabricated with polydimethylsiloxane (PDMS, Dow Corning SYLGARD 184, lot no. 0007724118) based on soft lithography technology19. Each device consisted of six identical replicate units for different experimental conditions, and each unit consisted of a main channel and 24 culturing chambers (Fig. 3c and Supplementary 5a). The height of each chamber was 1.3 µm, which ensured that only a monolayer of bacteria was captured and monitored, and the height of the main channel was ~30 µm. Two different types of SU8 photoreist were used to fabricate a reusable mould for the device. SU8 2002 (MicroChem Corporation, lot no. 110533) was spun at 5,000 r.p.m. for 30 s to obtain the thin layer (1.3 µm) on a 3-inch silicone wafer. After being baked at 180 °C for 2 h, the second layer of SU8 3025 (MicroChem Corporation, lot no. 11050370) was spun on top of the first layer at 2,500 r.p.m. for 30 s to obtain the thick layer (30 µm). Following the protocol from the photoreist datasheet, the mould was baked at 180 °C for 2 h. This mould was subsequently used to fabricate individual devices. Specifically, 20 g 10:1 (weight ratio) of mixed PDMS (polymer and crosslinking agent) was poured on top of the mould, degassed, and then baked at 80 °C for 30 min to ensure complete crosslinking. A biopsy punch with a diameter of 0.75 mm (World Precision Instruments) was used to drill both the inputs and outputs. After being cleaned with scotch tape (3M Corporation) to remove the PDMS residue, the PDMS was left to dry for 75 min by 25 µm 3M polyester film treatment (30 W for 30 s). To maintain nutrient and antibiotic concentrations during the experiments, programmable syringe pumps (New Era model NE-1600) were used to control the medium flow rate at 120 µl h−1, with medium supplemented with different antibiotic concentrations.

Image calibration. R+ and G+ cells were grown overnight for 16 h shaking (250 r.p.m.) at 37 °C. Subcultures of R+ and G+ were developed 2 h before the start of each microfluidic experiment by inoculating a tenfold dilution of the overnight culture in 3 ml LB medium with appropriate antibiotic selection (100 µg ml−1 Cm for R+ and 50 µg ml−1 Kan with 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for G+ (for GFP induction), and 20 µg ml−1 Tet for both), and grown with shaking at 37 °C. After 2 h of subculture, each population was concentrated 60-fold for high-density loading. The c.f.u. counts for each population were then used to determine the relative cell density (Supplementary Fig. 5b). With this protocol, we found the c.f.u. values to be within standard deviations of one another, so we used the culture volume to determine the predefined ratios of the three populations in the mixtures. Predefined mixtures of R+, G+ and Y were loaded into individual channels, and ten chambers were imaged per channel as technical replicates, with each image containing 1,024 × 1,024 pixels. We adapted an image analysis algorithm from a previous publication18, which uses the co-localization of red and green pixels to determine the presence of transconjugants. Each bar shows the average and the standard deviation of measurements from ten chamber replicates (Supplementary Fig. 5c, error bars indicate standard deviation). Our detection limit was quantified from the calibration data of a mixture of pure R+ and G+ (Supplementary Fig. 5c). In the absence of yellow pixels, the detection algorithm will identify fewer than 3% of the pixels as containing both mCherry and GFP. The detection limit for transconjugants was thus set as anything greater than 500 pixels. All calibration and time-lapse images were obtained using a DeltaVision Elite imaging system; experiments used ×60 magnification with 80 ms exposure time and 5% transmission. The device was incubated at 37 °C for the entirety of the experiment, and images were obtained using a Coolscope HQ2 high-resolution charge-coupled device camera.

Requirement of oriT for transfer. To demonstrate that conjugation relies on the presence of oriT, we first grew R+ and G+ in the chip for 4 h before introducing both Kan and Cm to select for the resulting transconjugants (Fig. 3c, +oriT Supplementary Video 1). R+ and G+ (which carry pUA66 without oriT) showed no generation of transconjugants under the same conditions, thereby demonstrating the specificity of the oriT process (Fig. 3c, −oriT Supplementary Videos 1).

Specifically, R+ with G+ and R+ with G+ were mixed and loaded into the chamber. Kan (50 µg ml−1) and Cm (100 µg ml−1) were used for selection. Cells lacking oriT did not undergo conjugation, as evidenced by the lack of yellow pixels. This observation was also verified using c.f.u. counts.

Antibiotic selection in the microfluidic chip. Because the microfluidic experiments used different combinations of R and G + strains depending on the experiment (for example, R+ and G+ for Fig. 3d), for a full list of strains for each experiment see Supplementary Table 5a), we denote the parents as R and G without the transfer sign, to simplify explanations.

R+ and G+ were grown overnight for 16 h with shaking (250 r.p.m.) at 37 °C in LB medium supplemented with 100 µg ml−1 Cm and 20 µg ml−1 Tet. After subculturing, cells were immediately resuspended in M9 containing 20 µg ml−1 and IPTG (1 mM), condensed 40-fold, and mixed in an equal ratio. The chip was vacuumed for 20 min to facilitate bacterial entry into the chambers, by enclosing the chip within an air-tight desiccator connected to the vacuum pump (Rabinair Vacuumaster LS800). Between eight and ten chamber positions were marked for each antibiotic condition, each corresponding to one antibiotic condition. We quantified the percentage of transconjugants after 12 h of growth in the microfluidic chambers as the average of all ten chambers, with the appropriate antibiotic concentration administered at the start of each experiment, and maintained with continuous flow of medium throughout the duration of the experiment. Syringe pumps were used to achieve a continuous flow at a rate of 120 µl h−1. Those chambers falling outside two standard deviations, which lost focus, or had an air bubble, were removed before processing. All error bars represent standard deviations.

Image processing. Each video was imported into MATLAB and the time series was analysed. Data from the 12th hour were collected and analysed separately. The location of the chamber within the 1,024 × 1,024 image was detected using a Hough transform from the frame at the 12th hour. The total chamber size was estimated using this as 6 × 104 pixels per chamber. This value was used as N0 for density normalization.

Every experiment consisted of six independent antibiotic concentration, each administered in one channel of the device. Either eight or ten replicates per concentration were collected. A list of concentrations, total number of chambers collected, chamber number (1–10) removed in outlier processing and date of experiment are provided in Supplementary Table 5b. No more than three chambers were removed per experiment. At most, only one was removed from standard deviation outliers due to blurriness.

For processing, every channel was identified as ‘Date_KanXxCCmYY’, where XX and YY correspond to the appropriate concentration. Each concentration pair was investigated at least twice to ensure technical reproducibility (Supplementary Fig. 5d). Whenever possible, we used data sets collected on the same experimental day, that is, on the same chip. However, because of the technical reproducibility of our experiment, we could confidently combine concentrations from different experiments if one channel was not deemed usable, for example, if it had an air bubble. This was done for Fig. 3d, ii and iv, and for Supplementary Fig. 6b, right column.

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