Antibiotic-induced recombination in bacteria requires the formation of double-strand breaks

Arpita Nath[1], Dan Roizman[1], Nivetha Pachaimuthu[2,3], Jesús Blázquez[4], Jens Rolff[1] and Alexandro Rodríguez-Rojas[1, 2]*

[1]Evolutionary Biology, Institute for Biology, Freie Universität Berlin, Berlin, Germany[2] Department Internal Medicine, Vetmeduni Vienna, Austria

[3]Department Biocomparative Medicine and Food Science. Padova University. Italy

[4]Centro Nacional de Biotecnología-CSIC, Campus de la Universidad Autónoma de Madrid, Spain

*Correspondence: Alexandro.Rojas@vetmeduni.ac.at

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Abstract

Recombination is an essential process in bacterial drug resistance evolution. Fluoroquinolones, an important class of antibiotics, are known to stimulate recombination in *Escherichia coli.* On the other hand, when bacteria are exposed to sublethal concentrations of different antibiotic families, including strong inducers of the SOS system, which upregulates recombination pathways, there is no detectable change in the recombination rate. Here we explore why fluoroquinolones, but not any other tested antimicrobials, increase recombination rates. One feature that makes the fluoroquinolones different from other antibiotics is the generation of DNA double-strand breaks (DSBs). We tested whether other antimicrobials known for their ability to cause double-strand breaks, such as mitomycin C and bleomycin, are also affecting bacterial recombination rate. We found that both drugs indeed affected recombination rates in *E. coli*. Furthermore, there was a positive correlation between the number of DNA double-strand breaks and the recombination frequency. The manipulation of the level of DSBs directly impacted the recombination frequency. These results imply that recombination, at least in Gram-negative bacteria, is conservative, and even if multiple copies of a DNA sequence are available, the basal recombination rate is low. Our results highlight that homologous recombination is intended more for DNA damage repair than generating diversity, or at least that they occur at a very different scale. We also show that only antibiotics that cause DNA double-strand breaks can increase genetic diversity via recombination.

The stimulation of recombination by DSBs-causing antimicrobials is an additional factor leading to the risk of antibiotic resistance evolution.

Introduction

Antimicrobial failure to kill bacterial pathogens is becoming a global problem due to the very rapid emergence of resistant strains. According to a recent global report, antimicrobial resistance (AMR) led to an estimated 4.95 million deaths in 2019 (Baker, 2015). The number of deaths varied between regions, with the highest toll being reported in regions with lower resource settings. *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii*, and *Pseudomonas aeruginosa* were the six most significant pathogens leading to antimicrobial resistance (Baker, 2015). Resistance to fluoroquinolones and β-lactams, the first-line antibiotics in most infections, contributed to at least 70% of AMR deaths (Collaborators, 2022). Antibiotics are a selective pressure in themselves, but they also enhance evolvability by elevating mutation rates and account for increased recombination frequency and lateral gene transfer in bacteria (Rodríguez-Rojas et al., 2013).

An excellent example of an increasing issue with antibiotic resistance over the years is the quinolones, to which resistance emerges as soon as they are introduced (Witzany et al., 2020; Zhang et al., 2019). The mechanism of quinolone action is the inhibition of two essential bacterial type II topoisomerase enzymes, DNA gyrase and DNA topoisomerase IV (Hooper, 1998). Both of these enzymes are hetero-tetramers with two subunits. The activity of both enzymes causes the catalysis of a DNA double-strand break (DSB), passing another DNA strand through the break and then resealing it together to regulate the DNA supercoiling state. DNA strand passing occurs via domains localised in GyrA and ParC, whereas the ATPase activity is required for enzyme catalysis and is located in domains belonging to GyrB and ParE subunits (Hooper and Jacoby, 2016).

The SOS stress pathway is activated when several stressors including sub-lethal antibiotic treatments induce DNA damage. Consequently, error-prone alternative DNA polymerases repair the damage at the price of introducing mutations, a process called trans-lesion synthesis (Blázquez et al., 2012). The general stress response controlled by the sigma factor RpoS in Gram-negative bacteria also increases mutation rates under antibiotic stress by indirectly down-regulating MutS, a

key protein of the DNA mismatch-repair system (Gutierrez et al., 2013). Moreover, other classes of antibiotics such as sulphonamides increase mutagenesis directly due to the imbalance they produce in the nucleotide pool during replication (Nordman and Wright, 2008). All these direct or indirect mutagenic effects are common to many families of antimicrobials except for cationic antimicrobial peptides that do not increase the mutation rate or recombination probably because they do not induce mutagenic pathways such as the SOS system or RpoS general stress pathway (Rodríguez-Rojas et al., 2018, 2014).

Recombination is a fundamental driver of antimicrobial resistance by inducing, for example, gene duplication and amplification (Elliott et al., 2013; Sandegren and Andersson, 2009). It is known that fluoroquinolones cause an increase in the recombination rate in *E. coli* suggesting the potential for acquiring antibiotic resistance (Lopez et al., 2007). In pathogenic *E. coli* isolates, recombination rates can be significantly higher than in commensal strains (Rodríguez-Beltrán et al., 2015). However, the number of antibiotics that increase recombination in *E. coli* is limited to fluoroquinolones so far, suggesting this is a unique property of this family (López et al., 2009). Also, the human pathogen *Streptococcus pneumoniae* displays higher recombination rates in antibiotic-resistant strains when compared to sensitive ones adding another link between resistance and recombination (Mostowy et al., 2014).

Here we asked why fluoroquinolones, but not any other antimicrobials, increase bacterial recombination rate. Many antibiotics induce the SOS system, up-regulating several recombination proteins such as RecA. Nevertheless, this up-regulation is not sufficient to increase recombination. Previous data suggests that the induction of DSBs, a hallmark of fluoroquinolone-induced damage, could be responsible for boosting the recombination rate (Lopez et al., 2007). Therefore, we hypothesised that in order to stimulate recombination by antibiotics in Gram-negative bacteria such as *E. coli*, the induction of the SOS system alone is not sufficient and that the generation of DNA double-strand breaks is necessary. To test the generality of our findings, we compared ciprofloxacin to other non-fluoroquinolone drugs known for their capacity to generate DSBs: mitomycin C and bleomycin.

Results and Discussion

First, we measured the recombination frequency of several antimicrobials using a plasmidic system described elsewhere (Figure S1) (Radman, 1975; Radman et al., 1995). Since the plasmid is a multi-copy system, it has the advantage of being more sensitive (Radman, 1975; Radman et al., 1995) than other chromosomes integrated system used before. We used the minimal inhibitory concentration (Table S1) as a reference value for the experiments with antimicrobials that covered diverse antibiotic classes. This experiment confirmed that only ciprofloxacin increased recombination rates, which is in agreement with previous reports for ciprofloxacin (Lopez et al., 2007; López et al., 2009; Rodríguez-Rojas et al., 2018). What makes ciprofloxacin different from other antibiotics is its ability to induce DSBs, which we thought was the main cause of increase in recombination. As a result, we also tested other antimicrobials that can induce this type of DNA damage. In *E. coli* and enterobacteria in general, essential proteins of the recombination machinery are controlled by the SOS pathway (Radman, 1975; Radman et al., 1995). Besides fluoroquinolones, β-lactams and sulphonamides are also potent inducers of SOS response (Do Thi et al., 2011) but they failed to increase recombination. Interestingly, we found that mitomycin C and bleomycin significantly increased the rate of recombination by more than one order of magnitude compared with the controls' basal level and similar to ciprofloxacin (Figure 1). These results provide evidence that our hypothesis indicating that DSBs mediated the increase of recombination rate by fluoroquinolones is correct.

Ciprofloxacin and other antimicrobial used in this work can increase mutagenesis (Blázquez et al., 2018; Do Thi et al., 2011). One possible bias is the risk of spontaneous resistant mutants to tetracycline, which could be scored as recombination frequency increases. We treated *E. coli* MG1655 with the same concentration of antibiotics and experimental conditions, and we did not obtain any colony by plating the same inoculum and a ten-time higher one. These results ensure that the reconstitution of the tetracycline-resistant cassette generated all the colonies due to recombinational events and not due to spontaneous mutations.

Mitomycin C and bleomycin also induce the SOS response as they cause direct DNA damage (Xu et al., 2012). To understand the relevance of SOS system in the observed higher recombination phenotype, we used a RecA mutant unable to trigger the SOS response and lacking the most important protein for DSB repair in *E. coli*. We found that for ciprofloxacin, mitomycin C and bleomycin, the increase in recombination frequency was dependent on the recombination protein

RecA (Figure 2). A RecA deficient mutant suppressed the recombination increase to levels similar to the controls. This experiment along with our previous data suggests that the recombination stimulation requires SOS induction and DSBs indicating that the first step is DNA breakage, and the second step is damage repair. The activation of SOS is not always correlated with the presence of DSBs, but (Taddei et al., 1997) DSBs seem always to trigger the transcription of SOS genes in our experiments. In other words, the SOS system activation is necessary but not sufficient to increase the recombination rate.

To confirm that DSBs are significant determinant of the induction of recombination, we correlated the number of DSBs lesions and the recombination rate. For that purpose, we used ciprofloxacin at three different concentrations and determined the number of DSBs for ciprofloxacin, mitomycin C and bleomycin. We use a reporter system received as a kind gift from Susan M. Rosenberg that allows observing these lesions in real-time. The system consists of a green fluorescent protein (GFP) fusion containing bacteriophage GAM protein. GAM protein recognises the DNA ends of DSBs, while GFP allows the tagging and forms foci (Shee et al., 2013). We found a positive correlation between the number of DSB lesions and recombination frequency (Figure 3). We repeated this analysis to check if there was a positive correlation between antibiotic concentration and recombination frequency. For this, we used ciprofloxacin at three different concentrations and determined the number of DSBs at each dose. We found that as the ciprofloxacin concentration increased, the recombination frequency also increased (Figure 4). Microscopic pictures of cells treated with growing concentrations of DSB-inducing antibiotics show the induction of DSBs by ciprofloxacin, mitomycin C and bleomycin (Figure 5).

To determine whether there is a causal relationship between DSBs and increases in antibiotic-induced recombination, we devised an experiment to directly test whether DSBs caused by antibiotics are the trigger for antibiotic-induced recombination. One of the antibiotics that produce DSBs, bleomycin, requires iron as a cofactor to induce DSBs (Chen et al., 2008; Xu et al., 2012). In the experiments described so far, we used LB, an iron-rich medium (Rodríguez-Rojas et al., 2015). Therefore, we reduced the iron content of the medium by suppressing iron (by adding 2–2′ bipyridyl) during the bleomycin treatment. If DSBs cause changes in recombination, suppressing the iron content significantly decreases the recombination frequency (Figure 6). Complementarily, it is known that under low oxygen tension, the actual damage induced by bleomycin consists of forming oxidised abasic sites, while in aerobic environments, single and

double-strand breaks predominate (Povirk et al., 1977). The reason for the decrease of DSBs under anaerobic conditions is due to a decrease in Fenton reactions that do not require only iron, but also oxygen-stimulated hydrogen peroxide production during respiration (Jang and Imlay, 2007).

Our results entail an essential trait of recombination, at least in Gram-negative bacteria. The canonical recombination machinery of *E. coli* in the absence of stress is extremely conservative. Even in the presence of substrate for recombination, the frequency of recombination events is low, and it works more towards repair processes. One evidence of this notion is the need to use helper recombination systems (e.g., λ red recombinase) to increase the probability of DNA integration into the chromosome via homologous recombination (Chaveroche et al., 2000; Datsenko and Wanner, 2000). An additional implication of our result is that the induction of double-strand breaks and hence recombination by antibiotics could increase genetic diversity and enhance the risk for antimicrobial resistance. It is not a coincidence that two different soil bacteria produce bleomycin and mitomycin C (Kono et al., 1995) (Umezawa et al., 1966). Some other antibiotics, such as novobiocin and nitrofurantoin, damage DNA and are produced by soil microorganisms (May et al., 2017), pointing to the fact that this could be a ubiquitous phenomenon in environmental scenarios. Our study also reinforces the idea of controlling the pollution with other DNA damaging antibiotics such as fluoroquinolones that are incredibly stable and have been shown to accumulate in the environment (Riaz et al., 2018).

It does not escape our attention that in our methodology, some antibiotics could produce population imbalance or partial killing and thus, an uneven number of cell divisions could impact the recombination freqeuncy. For this reason, we did an additional experiment where we simulated this situation. We inoculated cultures of *E. coli* MG1655-pRHOMO, with inoculum sizes ranging from 10^4 to 10^8 bacteria (Figure S2). We did not find any detectable difference in recombination frequency due to an additional number of divisions.

Taken together, our results provide strong evidence that antibiotics that induce DSBs can increase the basal level of recombination in *E. coli*. This conclusion answers our central question on how ciprofloxacin could increase the recombination rate, at least in *E. coli*. Ciprofloxacin is a broad-spectrum antibiotic with conserved action mechanism across diverse bacterial species (Hooper and Jacoby, 2016; Millanao et al., 2021). Because the three antibiotics in the focus of this work are

broad-spectrum and produce similar damage in other bacterial species, we can foresee these results being potentially extendable to other microbes.

Our results might have some implications for cancer. Since bleomycin and mitomycin C are frequently used as antitumor drugs (Bolzán and Bianchi, 2018; Povirk et al., 1977), there is a chance that they would also increase gene shuffling in tumoral cells via recombination. Future studies could then uncover whether an increase in recombination rate is also possible in tumour cells, leading to chemotherapy resistance and carcinoma recidivation (Laehnemann et al., 2014).

Our results also have implications for bacterial evolution. It has been shown that recombination facilitates adaptive evolution, particularly in fluctuating environments that require complex genomic changes (Chu et al., 2017). For example, work in *E. coli* has shown that in the presence of doxycycline and erythromycin, costly adaptive evolution is facilitated by recombination (Laehnemann et al., 2014). Recent work in *pneumococci* demonstrated the importance of interspecies recombination in acquiring resistance against two antibiotics, tetracycline and macrolides (D'Aeth et al., 2021).

While fluoroquinolones are synthetic antibiotics, mitomycin C and bleomycin are naturally occurring antimicrobials produced by two soil bacteria: *Streptomyces caespitosus* produces mitomycin C (Kono et al., 1995), while bleomycin is produced by *Streptomyces verticillus* (Umezawa et al., 1966). This fact indicates that the increase of recombination induced by DSBs could be a ubiquitous mechanism operating widely on the soil, one of the most important reservoirs of microbial life and antimicrobial resistance genes.

Recombination in bacteria occurs via two principal mechanisms. On one hand, an allelic exchange can buffer genetic variability within bacterial populations as gene exchange mediates selective sweeps. On the other hand, incorporating new genes via horizontal gene transfer leads to an increase in the size of the genome (Lawrence and Retchless, 2009). Horizontal gene transfer, which frequently entails recombination events, is additionally a significant contributor to antibiotic resistance (Blázquez et al., 2012). Given the role of recombination in antibiotic resistance evolution, increasing recombination rates by administering drugs like those studied here will almost certainly accelerate resistance emergence.

225

226 **Material and methods**

227 **Bacteria and growth conditions.** The main bacterial strain in this study was the *Escherichia coli*
228 MG1655 transformed with the plasmid pRHOMO recombination system. We also used a *recA*
229 deficient derivative of MG1655 [7]. To determine the impact of antimicrobials on the generation of
230 DSBs, the *E. coli* strain SMR14354 was used [20]. The *E. coli* strain DH-5 alpha [*fhuA2 lac(del)U169*
231 *phoA glnV44 Φ80' lacZ(del)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*] was used to maintain the
232 plasmid of recombination system pRHOMO [11]. All strains were routinely cultured in Lysogeny
233 Broth (LB medium, Carl Roth, Germany), supplemented with antibiotics when appropriate. For
234 solid cultures, we added bacteriological agar to LB at a final concentration of 1.6 %.

235 **Minimal Inhibitory Concentration.** The minimal inhibitory concentration (MIC) was performed by
236 broth microdilution methods according to EUCAST guidelines, except that LB medium was used
237 instead of Mueller-Hinton. The antibiotics used were ciprofloxacin, mitomycin C, bleomycin,
238 trimethoprim, streptomycin, kanamycin, chloramphenicol, ceftazidime, cefotaxime, fosfomycin and
239 rifampicin (Supplementary Table S1).

240 **Recombination frequency.** A genetic assay was carried out as previously described to estimate the
241 impact of antimicrobials on recombination frequency [11]. The system consisted of a plasmid
242 harbouring two truncated *tetA* alleles separated by an antibiotic resistance cassette (*aacC1*,
243 conferring gentamycin resistance, Supplementary Figure S1). Recombination restores the
244 functional *tetA* gene, thereby conferring tetracycline resistance, which can be selected for [7,11].
245 Thus, this assay allows quantification of the recombination frequency with high sensitivity due to
246 the elevated copy number of the plasmid. We exposed 1 ml of culture containing approximately 1-
247 $2x10^7$ bacteria [dilution of 1/10 of mid-exponential culture containing $1x10^8$ colony-forming units
248 per millilitre (CFU/ml)] to different concentrations of different antimicrobials (to match for all of
249 them the MIC) for three hours, similarly to a previously described experiment [6]. After incubation
250 with gentle shaking, we added 9 ml of fresh LB to the cultures and centrifuged at 4000 x g for 10
251 minutes to reduce and remove the antimicrobials. The pellets were washed twice with 2 ml of LB,
252 and cells were allowed to recover for one hour before adding ampicillin (to maintain the plasmid)
253 to a final concentration of 50 µg/ml and incubated overnight with shaking at 37°C. The next day,
254 appropriate dilutions were plated in LB agar containing 50 µg/ml of ampicillin to estimate the
255 viability and LB agar containing 30 µg/ml of tetracycline to estimate the number of recombinants.

256 Each experiment consisted of five replicas and was repeated twice. Recombinant frequencies were
257 expressed as the ratio of means of recombinant number by the number of viable bacteria.

258 **Influence of antimicrobials in the generation of double-strand breaks.** The strain SMR14354 was
259 used to measure the number of double-strand breaks when the strain is incubated with different
260 concentrations of tested antimicrobials. This strain carries a reporter fusion of the protein Gam,
261 able to detect double DNA ends and a green fluorescent protein (GFP). The strain also harbours a
262 unique I-*SceI* restriction site in the chromosome and carries the gene for the restriction enzyme
263 *SceI* whose expression is regulated by an arabinose inducible promoter [20]. Mid exponential
264 cultures containing $0.5x10^8$ CFU/ml were exposed to different concentrations of selected
265 antibiotics for four hours. Then, 5 µl drops of the cultures were added to LB agar blocks containing
266 1 µM Propidium Iodide (PI, Sigma, Germany) and the covered bacterial area of the blocks were
267 placed on a circular bottom glass open cultivation system II (PeCon, Germany). This device consists
268 of a metallic holder with a 0.17 mm round-bottom glass and a glass lid that limits evaporation. The
269 device was placed on a robotic inverted microscope Nikon Eclipse T2 (Nikon, Japan). Several fields
270 of each sample were taken automatically using the red and green channels to capture the signal of
271 PI (as viability criteria) and the signal of Gam-GFP fusion to detect DSBs, respectively. Three
272 independent overnight cultures were analysed per antimicrobial per concentration.

273 **Influence of iron on the level of double-strand break and recombination frequency of bleomycin**
274 **treated cells.** Double-strand breaks induced by bleomycin depends on intracellular iron
275 concentration [19]. To understand the relationship between double-strand breaks and
276 recombination frequency, bleomycin treated cells were subjected to iron depletion. The *E. coli*
277 MG1655 harbouring the pRHOMO construct cultures treated with 1X MIC of bleomycin alone and
278 bleomycin plus 2-2′ bipyridyl (50 µM) were examined for changes in recombination rate and the
279 number of double-strand breaks determined as described elsewhere in this section.

280 **Statistical analysis**. To compare experimental groups with their control, a Welch test was
281 performed. To avoid biases introduced by multiple corrections due to comparison against control,
282 each antimicrobial experiment had its non-treated control. In addition, covariance analysis was
283 carried out to estimate the co-occurrence of double-strand breaks and increase in recombination
284 rate induced by different antibiotics. P-values less than or equal to 0.05 were considered
285 statistically significant. Statistical tests and graph plotting were performed with R (R Core Team,
286 2017) and GraphPad Prism version 9.00 (GraphPad Software, Inc., San Diego, CA).

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Figure legends

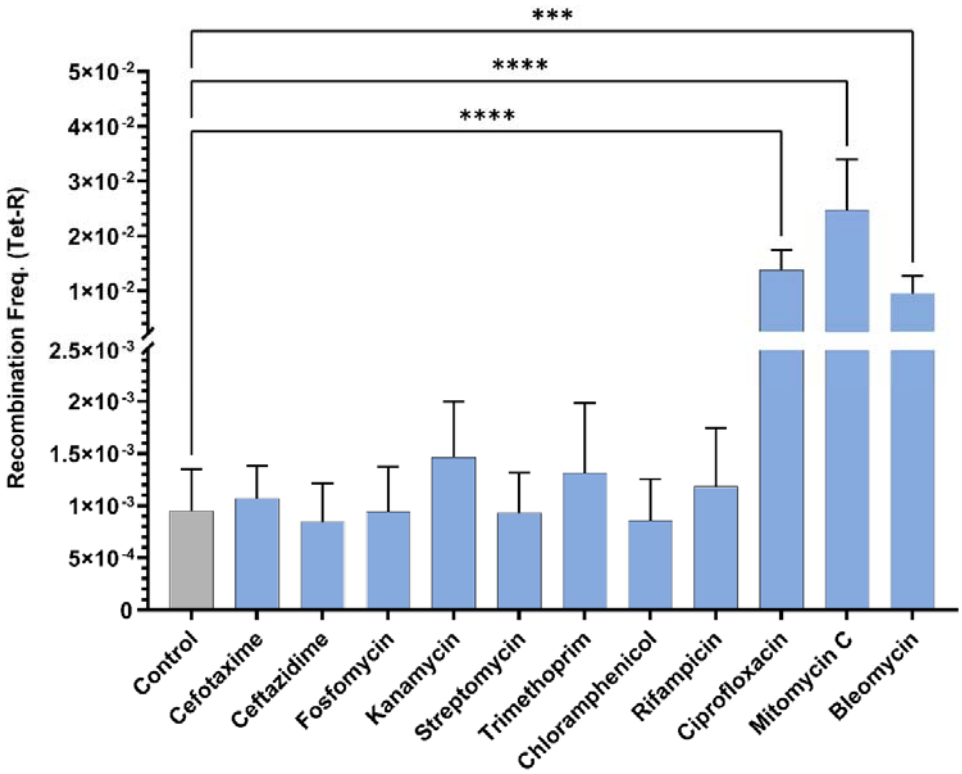


Figure 1. **Recombination frequencies of *E. coli* MG1655 harbouring the plasmid pRHOMO (recombination construct) treated with antibiotics at their 1xMIC for three hours**. Bars represent the respective means of five biological replicates and their standard deviation (n=5 + SD) values of recombination frequency of the bacteria. Differences in recombination frequencies between treatments were analysed by an ordinary one-way ANOVA followed by Dunnett's multiple comparisons tests with a single pooled variance, performed using GraphPad Prism 9 (*p*-values denote: *** - 0.0005, **** - <0.0001). Ciprofloxacin, mitomycin C and bleomycin had a significantly higher recombination frequency compared to the control group.

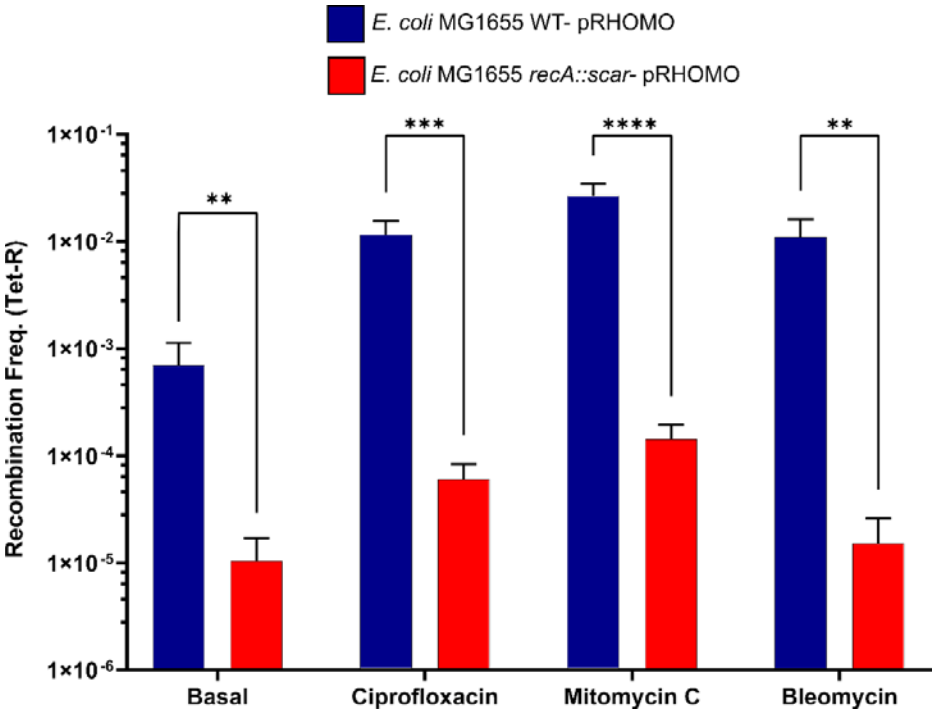


Figure 2. **Effect of RecA inactivation on recombination frequency induced by antibiotics that promotes double-strand breaks.** Antibiotic-stimulated recombination for mitomycin C, bleomycin, and ciprofloxacin (1xMIC during three hours) is primarily dependent on RecA protein repair activity since *E. coli* MG1655 *recA*::scar harbouring the plasmid pRHOMO suppressed the change in recombination rate observed in the wild-type strain when treated with DSB-inducer antimicrobials. Differences in recombination rates between strains were analysed by unpaired two-tailed student t-tests, considering similar SD and 95% Confidence Intervals. Asterisks represent statistical differences (Basal: ** - p=0.0076, Ciprofloxacin: *** - p=0.0002, Mitomycin C: **** - p<0.0001, Bleomycin: ** - p=0.0015, respectively). Each experiment consisted of five replicates, including non-treated controls (basal recombination rate value of the non-treated bacteria). The bars represent the means while the error bars represent the standard deviations (n=5 + SD).

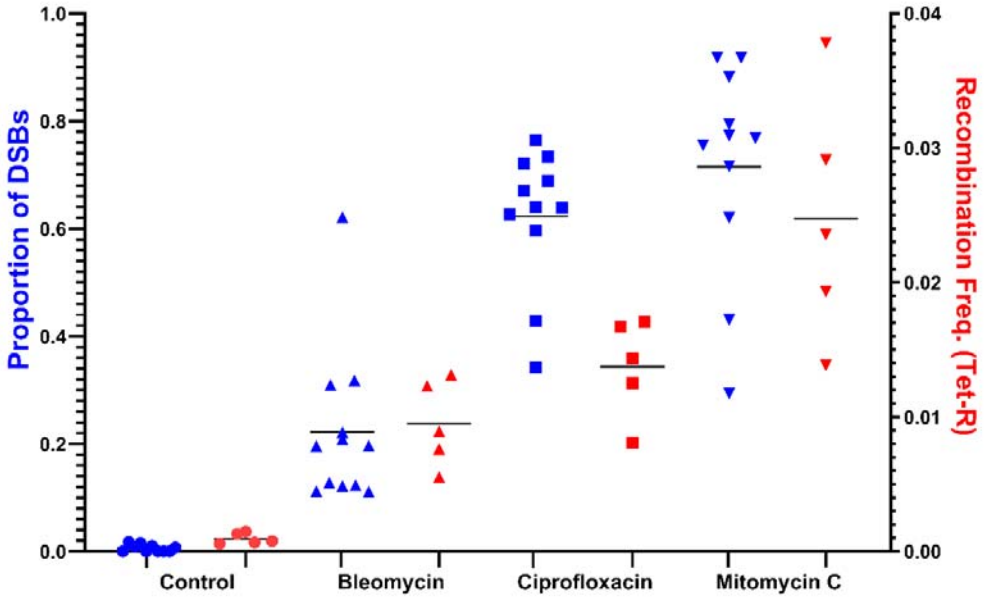


Figure 3. Co-occurrence of DSB and increase of recombination rate for bleomycin, ciprofloxacin and mitomycin C. The graph shows a significant positive linear trend (Spearman correlation coefficient r= 0.819, p<0.0001, Slope=0.253 +/- 0.0193) between the number of DSBs lesions and increased recombination frequency when bacteria are treated with antibiotics that induce DSBs. Each experiment consisted of five replicates and had its non-treated control (basal recombination rate value of the non-treated bacteria, n=5). The positive control for DSBs (I-*SceI*) consisted of a unique restriction site in the bacterial chromosome that can be cut by inducing the expression of the restriction enzyme *SceI*.

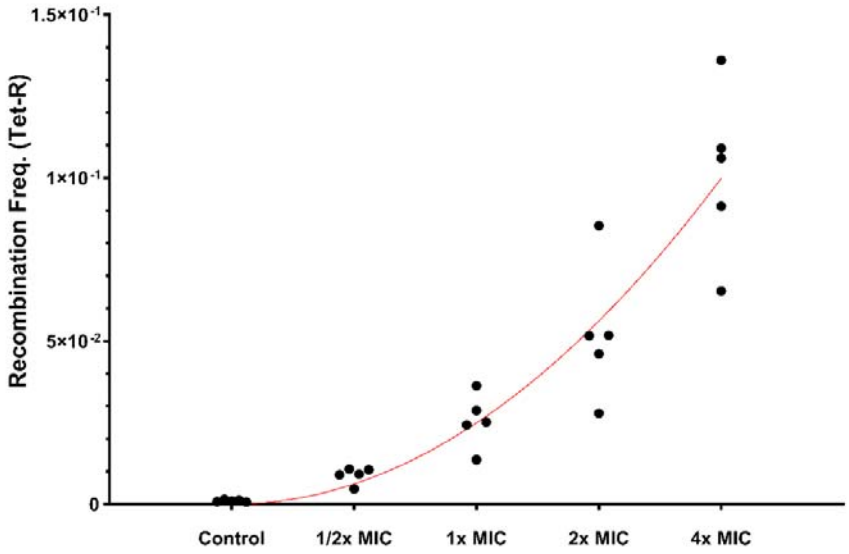


Figure 4. **Dose-response of recombination frequency of *E. coli* MG1655-pRHOMO treated with growing ciprofloxacin.** The graph shows a positive trend in the recombination frequency and increasing ciprofloxacin dose (Fit based on simple quadratic equation, Spearman correlation coefficient r=0.873, p-value<0.0001). Each experiment consisted of five replications and had its non-treated control (basal recombination rate value of the non-treated bacteria, n=5).

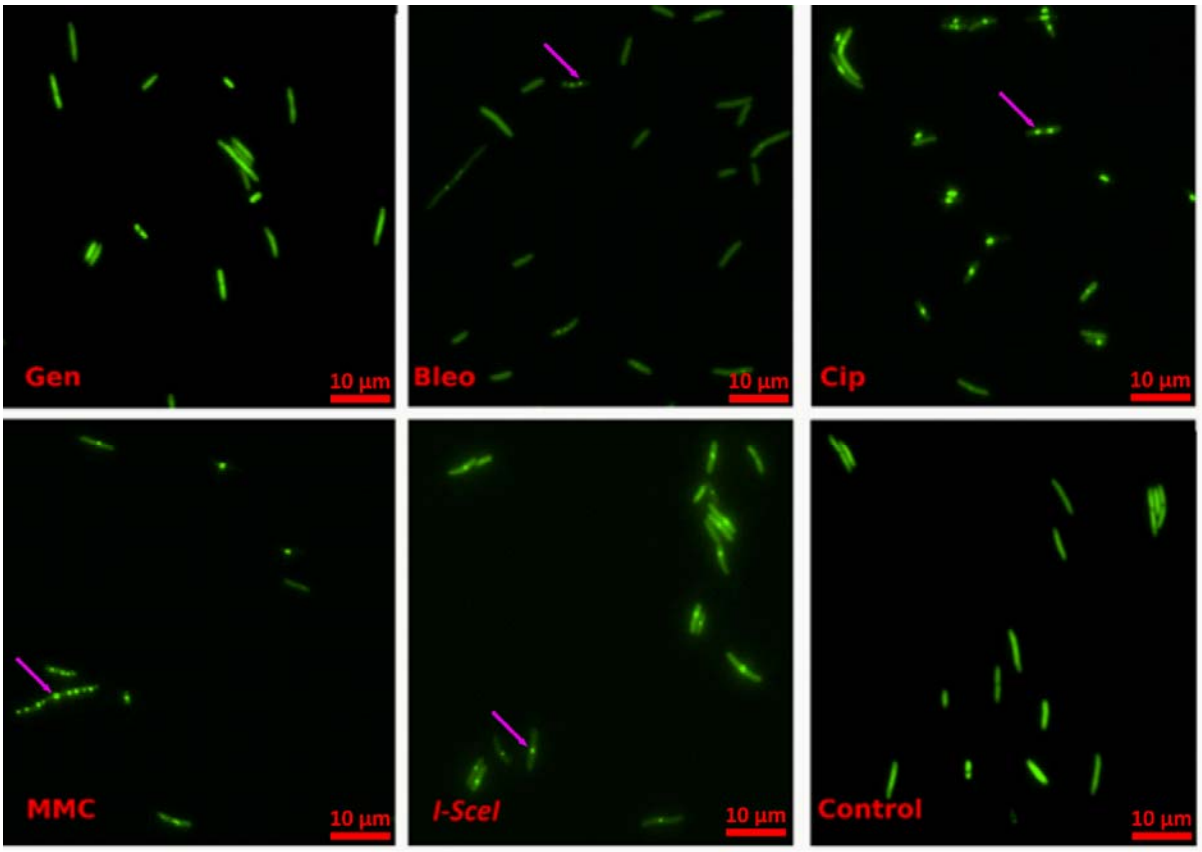


Figure 5. Fluorescent microscope images of antibiotic-generated double-strand breaks after treating the *E. coli* strain SMR14354 with gentamycin (Gen), bleomycin (Bleo) and ciprofloxacin (CIP), MIC of mitomycin C (MMC) with the minimal inhibitory concentration for 3 h. The green foci (indicated by magenta arrows) indicate the binding of the GFP-GAM fusion protein to free DNA ends and are only found at high frequency in treated bacteria with bleomycin, ciprofloxacin and mitomycin C. The foci formation (indicating the presence of DSBs) can also be observed in the positive control (I-*SceI*), which consists of a unique restriction site in the bacterial chromosome that can be cut by inducing the expression of the restriction enzyme *SceI*. Gentamycin is used as a control antibiotic, which does not increase recombination frequency or the number of DSBs.

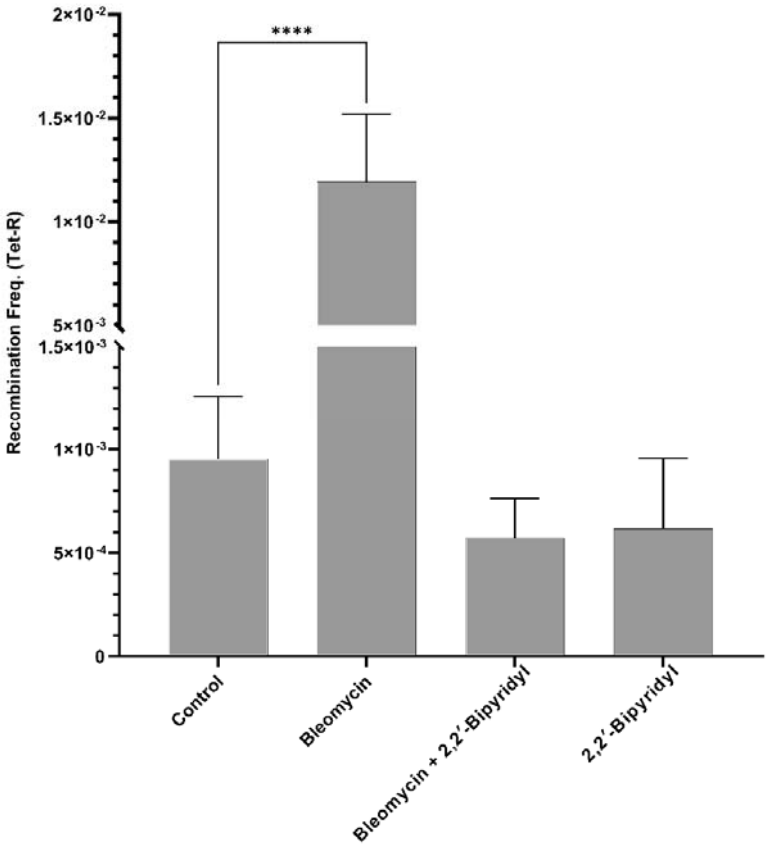
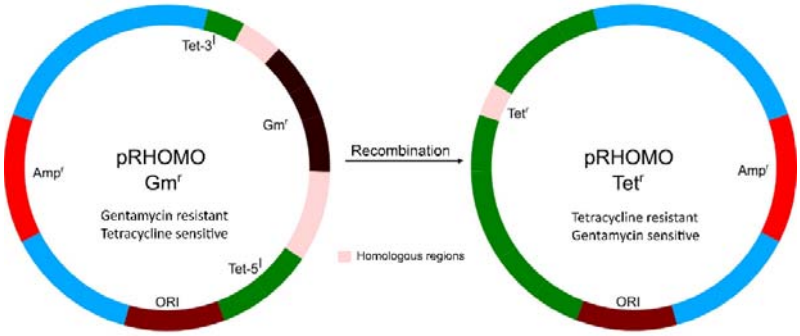


Figure 6. **Impact of iron chelation by 2–2′ bipyridyl on recombination frequency of *E. coli* MG1655-pRHOMO treated with 1xMIC of bleomycin.** LB medium contains between 15 to 20 µM of iron (Rodríguez-Rojas et al., 2015), which potentiates the capacity of bleomycin to induce DSBs (Xu et al., 2012) (Analysed by an ordinary one-way ANOVA followed by Dunnett's multiple comparisons tests with a single pooled variance, performed using GraphPad Prism 9, p-value denotes: **** - <0.0001). The experiment consisted of five independent replicates (n=5), bars representing the mean and standard deviation. A significant decrease in recombination rate was observed when an iron-titrating level of 2–2′ bipyridyl (final concentration of 50µM) was added together with bleomycin, leading to its return to basal level.

Supplementary material

Figure S1. Representation of the plasmid system to measure the recombination frequency (modified from reference (Rodríguez-Beltrán et al., 2015). The system consists of a plasmid harbouring two truncated *tetA* alleles separated by an antibiotic resistance cassette (*aacC1*, conferring resistance to gentamycin). Recombination restores the functional *tetA* gene, thereby restoring a functional tetracycline-resistant cassette, which can be selected on an agar plate containing tetracycline.



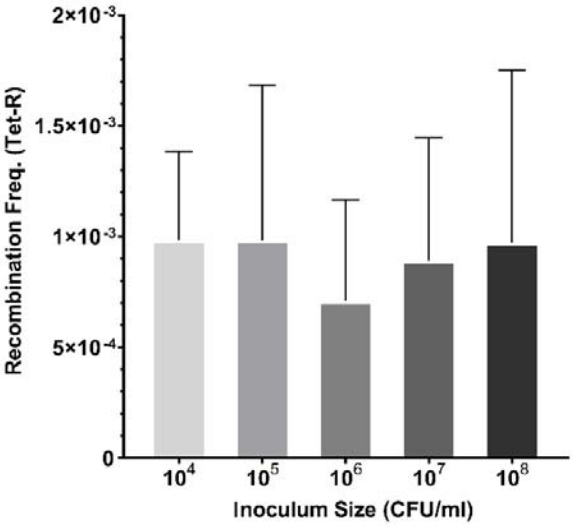


Figure S2. Effect of population size on recombination frequency. Cultures *E. coli* MG1655 harbouring the plasmid pRHOMO were inoculated with growing bacterial numbers ranging from 10^4 to 10^8 bacteria (Figure S2). There are not statistical difference difference in recombination

frequency due to an additional number of divisions. Each experiment consisted of five replicates. The bars represent the means while the error bars represent the standard deviations (n=5 + SD).

Table S1. Minimal inhibitory concentrations (MIC) values for *E. coli* MG1655 and its derivative mutant *E. coli* MG1655 *recA*::scar for different antimicrobials used in this work.

		MIC (µg/ml)	
Antibiotics	**Class**	*E. coli* MG1655 WT	*E. coli* MG1655 *recA*::scar
bleomycin	glycopeptide	4	0.5
cefotaxime	cephalosporins	0.125	0.062
ceftazidime	cephalosporins	0.25	0.125
chloramphenicol	amphenicols	2	2
ciprofloxacin	fluoroquinolones	0.125	0
fosfomycin	fosfomycin	2	1
gentamycin	aminoglycosides	0.5	0.5
kanamycin	aminoglycoside	1	1
mitomycin C	mitomycins	2	0.012
rifampicin	rifampicins	4	4
streptomycin	aminoglycosides	4	4
trimethoprim	sulfonamides	1	0.125
tetracycline	tetracyclines	2	2