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TisB protein protects *Escherichia coli* cells suffering massive DNA damages from environmental toxic compounds

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Toxin-antitoxin systems are genetic elements that are widespread in prokaryotes. Although molecular mode of action of many of these toxins has been identified, their biological functions are mostly unknown. We investigated the functional integration of the TisB/IstR toxin-antitoxin system in the Escherichia coli SOS genotoxic stress response network. We showed that the tisB gene is induced in cells exposed to high doses of the genotoxic antibiotic trimethoprim. However, we also found that TisB contributes to the trimethoprim-induced lethality. This is a consequence of the TisB-induced drop in the proton motive force (PMF), which results in blocking the thymine import and therefore the functioning of the pyrimidine salvage pathway. Conversely, TisB-induced PMF drop protects cells by preventing the import of some other toxic compounds, like the aminoglycoside antibiotic gentamicin and colicin M, in the SOS-induced cells. Colicins are cytotoxic molecules produced by Enterobacterales when they are exposed to strong genotoxic stresses in order to compete other microbiota members. We indeed found that TisB contributes to E. coli’s fitness during mouse gut colonization. Based on the obtained results, we propose that the primary biological role of the TisB toxin is to increase the probability of survival and maintenance in the mammalian gut of their bacterial hosts when they have to simultaneously deal with massive DNA damages and a fierce chemical warfare with other microbiota members.

Contribution of toxin-antitoxin systems to the persistence of bacteria to antibiotics has been intensively studied. This is also the case with the E. coli TisB/IstR toxin-antitoxin system, but the contribution of TisB to the persistence to antibiotics turned out to be not as straightforward as anticipated. In this study we show that TisB can decrease, but also increase, cytotoxicity of different antibiotics. Such inconsistency has a common origin, i.e., TisB-induced collapse of the PMF, which impacts the import and the action of different antibiotics. By taking into account the natural habitat of TisB bacterial hosts, the fact that this toxin-antitoxin system is integrated into the genotoxic stress response regulon SOS, that both SOS regulon and TisB are required for E. coli to colonize the host intestine, and the phenotypic consequences of the collapse of the PMF, we propose that TisB protects its hosts from cytotoxic molecules produced by competing intestinal bacteria.
INTRODUCTION

Toxin-antitoxin (TA) systems are genetic entities composed of two genes, one coding for a toxin and another for its cognate antitoxin (1). Toxins typically inhibit essential cellular processes and components, such as translation, DNA replication, membranes, and cytoskeleton. TAs are classified in different types according to their genetic structure and the mode of toxin neutralization. TAs are widely distributed on plasmids, bacteriophages and bacterial chromosomes. While the molecular mode of action of many TAs is extensively studied, their biological roles are still mostly unknown (1). Identification of TAs’ mode of action responds to the mechanistic “how”, but not necessarily to the evolutionary/ecological “why” question. Notable exceptions are some plasmid-borne TAs, which assure plasmid stabilization, such as the F plasmid-encoded CcdA/CcdB TA system (2). Identification of TAs’ biological functions is impeded by the fact that the inactivation of genes coding for most of the TAs has no phenotype under laboratory conditions (2). For many toxins, phenotypes were observed only when they were produced over the physiological levels (2), which is often artifact-prone.

Important information about TAs’ biological roles may come from the understanding of how the control of their expression is integrated in cellular signaling pathways. Particularly convenient for this purpose is when regulatory mechanisms are well characterized. One such example is the SOS regulon in Escherichia coli, which is composed of about 50 genes including six TAs (3). In unstressed cells, the SOS regulon genes are repressed by the LexA protein. The LexA-controlled SOS regulon has an ancient origin, and is present in a large number of widely diverged bacterial phyla (4). This regulon can be induced by a wide variety of stressors, all of which cause the accumulation of persistent single-strand DNA (ssDNA). The RecA protein polymerizes on the ssDNA, which activates its co-protease activity that promotes the proteolytic auto-cleavage of the LexA protein, thus activating the transcription
of the SOS regulon genes. When the DNA damage is repaired, persistent ssDNA disappears and the SOS regulon is repressed again. Because the SOS regulon was the first identified as a response to genotoxic stress, and because many LexA-regulated genes code for DNA damage processing functions, roles of other genes that are not obviously involved in the DNA repair were largely ignored. This is the case for six LexA-regulated chromosomal TAs: DinQ/AgrB, HokE/SokE, SymE/SymR, TisB/IstR, YafQ/DinJ, and YafN/YafO (3). An unanswered question is: why are these TAs integrated in such a complex, strictly regulated genotoxic stress response?

The best-studied LexA-regulated TA is TisB/IstR (5) (Fig. S1A). This type I TA is composed of the TisB protein toxin and the IstR RNA antitoxin. The tisB gene is repressed by LexA, while istR is constitutively expressed (5). In unstressed cells, the toxin is not synthetized because the 5’ UTR secondary structure of tisB primary transcript prevents its translation. Processing of the tisB primary transcript generates translationally active mRNA, but the binding of the antitoxin istR RNA triggers RNase III cleavage and tisB mRNA degradation (5). Under SOS regulon-inducing conditions, massively synthetized tisB mRNA out-titrates istR RNA and the TisB protein is produced. TisB is a small hydrophobic protein that targets the cytoplasmic membrane and disrupts the proton motive force (PMF) (6), which is necessary for many membrane transactions including nutrient uptake and ATP production (Fig. S1A). While the overproduction of the plasmid-borne tisB gene, with or without concomitant SOS induction, provokes a severe decrease in transcription, translation, DNA replication and results in rapid cell death, the induction of the chromosomal tisB gene by SOS-inducing treatments does not kill the cells (5).

It was hypothesized that upon SOS induction, TisB renders cells transiently metabolically inactive, and consequently tolerant to antibiotics (7). However there is no unambiguous evidence for the involvement of TisB in the drug-induced persistence to antibiotics. For
example, it was reported that ciprofloxacin (CIP), a fluoroquinolone antibiotic that poisons DNA-gyrase, induces SOS response and increases TisB-dependent persistence to CIP (7). However, another study reported that TisB-dependent increased persistence to CIP is not due to the SOS regulon induction by CIP but due to the presence spontaneously induced persisters that were present in the bacterial population prior to CIP treatment (8). It was also shown that TisB does not impact persistence of growing and stationary phase *E. coli* cells to another SOS-inducing fluoroquinolone antibiotic, ofloxacin (9, 10). These data indicate that drug-induced TisB production does not prevent generation of DNA damages by fluoroquinolones and that it does not contribute to the repair of these damages. So the question why TisB/IstR toxin-antitoxin system is integrated in the SOS genotoxic stress response network remains open.

In order to answer this question, we investigated the impact of TisB on the survival of *E. coli* cells treated with the antibiotic trimethoprim (TMP).TMP is a competitive inhibitor of bacterial dihydrofolate reductase, which prevents the synthesis of methionine, glycine, purines and deoxythymidine triphosphate (dTTP) (11) (Fig. S1B). In rich growth medium, TMP-bactericidal effect resides entirely in the intracellular dTTP depletion, which affects DNA synthesis, leads to DNA damage, SOS induction and cell death. The advantage of using TMP instead of fluoroquinolones is that TMP-bactericidal effect can be reversed by the addition of thymine or thymidine, which can be used for dTTP synthesis via a pyrimidine salvage pathway (12) (Fig. S1C). Importantly, the import of thymine or thymidine is PMF-dependent, which is affected by TisB. We also tested if TMP-induced TisB impacts the PMF-dependent import of some other compounds: lactose, gentamicin (GM), and colicin M (colM). Finally, we investigated if TisB may play a role in the colonization of mouse intestine by *E. coli*. Based on the obtained results, we propose that the primary role of TisB is to transiently block the import of host and/or microbiota-produced toxic compounds into cells suffering
massive DNA damages and thus to increase the probability of survival and maintenance in the mammalian gut.

RESULTS

Impact of TisB on SOS induction and the survival of TMP-treated cells. For this study, we used *E. coli* K-12 MG1655 strain, referred to as Wild Type (WT) in the text, and its ΔtisB derivative (Table S1). To investigate which of the TisB-dependent phenotypic alterations contribute to the survival of stressed cells, we treated cultures of WT and ΔtisB strains growing in LB medium. We first tested the susceptibility of WT cells from different growth phases to different TMP concentrations (Fig. S2AB), and decided that the treatment of exponentially growing cultures having around $2 \times 10^8$ colony-forming units (CFU)/ml ($OD_{600nm} = 0.6$) and the TMP concentration of 10 µg/ml, i.e., $20 \times$ MIC (Table S2), are most suitable for our study. This concentration of TMP was chosen because further increase of the TMP concentration did not further decrease survival, i.e., the dose-response relationship displayed the Eagle effect (13, 14). This cell concentration was chosen because cells at this growth phase were more susceptible to TMP. The stationary phase cells ($OD_{600nm} = 2$) were barely impacted.

We first confirmed that TMP causes dTTP depletion which blocks DNA replication and induces SOS response. This conclusion is based on the observation that addition of thymidine, which can be used for the dTTP synthesis via a pyrimidine salvage pathway, completely abolished the TMP cytotoxic effect (Fig. S1BC and S2C). We further validated this conclusion by modulating the flow of thymine and thymidine through the pyrimidine salvage pathway (Fig. S2C), by deleting *deoC* and *deoR* genes. The deletion of the *deoC* gene, which results in decreased utilization of thymine and thymidine for energy production thus
allowing cells to use it for the nucleotide synthesis, increased the survival. Deletion of the
deoR gene, which results in an increased thymine catabolism by favoring its flux towards the
TCA cycle, decreased the survival. Finally, we showed that delaying dTTP depletion, by
introducing the dhaA(Sx) allele that reduces DNA replication initiation frequency, increased
the survival 20-fold (Fig. S2D).

We investigated the induction of the tisB gene by TMP treatment in our experimental
conditions. We also compared the kinetics of the tisB gene induction with the induction of
other LexA-controlled genes. We used a collection of low-copy plasmids, each carrying a
promoter region of a SOS gene inserted upstream of a gene coding for a fast-folding gfpmut2
(Fig. 1A; see Fig. S3A for all 12 promoters). PrecA was the first to be induced after ~20 min,
followed by PlexA, PsulA, and PrecN, while PtisB was induced after ~1 h of treatment. Other
promoters were induced later and at a much lower rate. We performed the same experiment
using CIP as a SOS inducing agent and found that the pattern of induction of the tested SOS
genes was very similar to the one obtained with TMP (Fig. S3B). Finally, we showed that the
induction of the PrecA-GFP and PtisB-GFP reporters by TMP treatment is due to a bone fide
SOS induction regulation, i.e., it is LexA dependent (Fig. 1BC). Importantly, although PrecA,
PlexA, and Pruva were induced in cells treated with 0.1 × MIC of TMP, PtisB was not (Fig.
S3D).

Next, we investigated the involvement of the SOS regulon induction in the modulation
of the capacity of cells to survive 20 h of 20 × MIC of TMP treatment. We tested the survival
of a lexA1 (Ind−) mutant, which cannot induce the SOS regulon, and of a ΔsulA mutant that
cannot prevent cell division, i.e., cells do not filament (Fig. 1D). These two mutants were
chosen because we previously found that lexA1 (Ind−) and ΔsulA mutants have respectively
decreased and increased susceptibility to sub-MIC of TMP (15), which correlates with the
MICs of these strains (Table S2). The survival of lexA1 (Ind−) to the lethal TMP treatment was
10-fold higher than the survival of the WT strain, suggesting that the induction of one or more of the SOS functions contributes to the death of TMP-treated cells. Inactivation of the sulA gene had no significant impact on the survival of TMP-treated cells. However, the survival of the ΔtisB mutant was 20-fold higher than that of the WT strain (Fig. 1D), although the MICs of these two strains are not different (Fig. 1E and Table S2). Such a discrepancy between MIC and survival to lethal antibiotic treatment is a hallmark of tolerance to antibiotics (16). Importantly, by using PrecA-GFP reporter, we showed that the deletion of the tisB gene did not affect the induction of the SOS regulon as indicated by the induction of the recA gene promoter (Fig. 1B), which could impact cell DNA repair capacity and survival to TMP.

Because the tisB gene is upstream of the emrD gene that codes for a multidrug efflux pump (Fig. S1A), we verified if the deletion of the tisB gene has a polar effect on the emrD gene and consequently increases cell capacity to deal with TMP (Fig. 1D). However, this was not the case because ΔtisB and ΔtisB-emrD mutants had the same susceptibility. TisB’s antitoxin IstR alone has no impact on TMP cytotoxicity because the survival of the ΔistR ΔtisB strain is not significantly different from the one of the istR+ ΔtisB strain (Fig. 1D). Higher production of the TisB protein in the ΔistR Δl-41 strain (5)which lacks both IstR and the small DNA region coding for the 5’ UTR structure in tisB mRNA that delays TisB translation, resulted in reduced survival compared to the WT strain (Fig. 1D). This result confirms that strictly regulated transcription of tisB gene is required for appropriate TisB functioning.

We also used a pBAD plasmid carrying the tisB gene under an arabinose inducible promoter to evaluate the impact of the modulation of TisB production on cell viability. Strong tisB’s overexpression upon induction by L-arabinose, blocks E. coli’s growth even in the absence of any treatment (17). Therefore, we did not add the inducer, and observed that the leakiness of the promoter assures a TisB production sufficient to reduce the survival of the
ΔtisB mutant treated with lethal TMP dose, while it had not a significant impact on the survival of the WT strain (Fig. S2E). This result confirms that the absence of the TisB protein alone, and not a secondary consequence of the tisB gene deletion, is responsible for most, if not all, of the killing resulting from the SOS regulon induction with TMP.

**Impact of TisB on the morphology of TMP-treated cells.** The comparison of the kinetics of TMP-induced killing of the WT and ΔtisB mutant strains, measured by colony forming units (CFU) counts, showed that their survival was not significantly impacted during the first 3 h of treatment (Fig. 2A). After 3 h, the survival of both strains started decreasing, albeit at different rates, *i.e.*, CFUs of the WT were reduced faster than CFUs of the ΔtisB mutant. After 4 h of treatment, the fraction of surviving WT and ΔtisB mutant cells decreased 22- and 4-fold, respectively. After 20 h of treatment, the fraction of surviving WT and ΔtisB mutant cells decreased 1780- and 37-fold, respectively.

We compared the size of the WT and ΔtisB cells during TMP treatment. After 3 h of treatment, the WT and ΔtisB cells had similar size distributions (Fig. 2BC). However, the two strains displayed different cell size distributions after 20 h of treatment, *i.e.*, ΔtisB cells were longer than WT cells. Because dormant stationary phase cells are usually smaller than growing cells, it is unclear if surviving ΔtisB cells are dormant or metabolically active growing cells. In order to address this question, we took advantage of the observation that the combination of TMP and vancomycin (VAN) displays a synergistic effect on *E. coli* (18) VAN inhibits peptidoglycan synthesis but it is ineffective on *E. coli* because it cannot penetrate Gram-negative bacteria outer-membrane due to its large size. However, VAN can affect Gram-negative bacteria when their outer membrane is damaged. The fact that TMP and VAN have a synergistic effect, suggests that TMP treatment damages the outer membrane thus allowing VAN to interact with its target. Cells that are not affected by TMP, like non-growing
stationary phase cells, should not be affected by VAN either. In addition, peptidoglycan synthesis is active in growing cells and slows down in stationary phase cells (19).

So, we used VAN as a probe to investigate if peptidoglycan synthesis is active in TMP-treated cells. WT and ΔtisB cells were treated with TMP and/or with 0.25 × MIC of VAN (Table S2) during 20 h. We showed that the TMP treatment sensitized WT cells to VAN, which accelerated the killing of the cells. However, the fraction of the WT cells surviving 20 h of TMP+VAN treatment was the same as with TMP treatment alone (Fig. 2D). The WT cells treated with TMP+VAN were also significantly smaller than cells treated with TMP alone (Fig. 2B). These cells had the same size as stationary phase cells, which further confirmed that WT survivors are small non-growing cells. However, the treatment of ΔtisB with TMP+VAN accelerated the killing and diminished 725-fold the fraction of cells surviving 20 h of treatment compared to that of TMP treatment alone, to a level 23-fold lower than that of the WT cells (Fig. 2E). In addition, the ΔtisB cells were smaller after TMP+VAN than after TMP treatment alone (Fig. 2C), which indicates that most of the ΔtisB cells surviving TMP treatment are bigger, metabolically active cells.

Impact of TisB on the membrane potential and ATP pool in TMP-treated cells. It was reported that TisB overproduction affects cytoplasmic membrane integrity and disrupts the PMF (6), which is necessary for many membrane transactions including ATP production (17) (Fig. S1A). Because TisB-dependent decrease of ATP levels was proposed to induce persistent state to CIP (7) we examined how the TMP treatment impacts cell membrane and ATP level. First, we tested membrane potential using bis-(1,3-dibutylbarbituric acid)-trimethine oxonol (DIBAC₄(3)), which enters cells with depolarized membranes and flow cytometry (20). We found that the TMP-treated WT cells started to be stained after 3 h of treatment, whereas the
The \( \Delta tisB \) strain was not stained at this time point (Fig. 3A). After 20 h of TMP treatment, 80% of WT and 35% of \( \Delta tisB \) cells were stained with DIBAC\(_4\) (3).

Second, we investigated the impact of TMP treatment on the ATP pool in WT and \( \Delta tisB \) cells and in the growth medium. We found that the intracellular ATP amount increased in both strains, which corroborates reports showing that treatments with UV radiation, bleomycin and DNA gyrase inhibitors increase intracellular ATP concentration in \( E. coli \) (21, 22). After 2 h of TMP treatment, the amount of ATP stopped increasing in WT but continued in the \( \Delta tisB \) mutant. After 20 h of treatment, ATP amount was still higher in \( \Delta tisB \) than in WT cells (Fig. 3B). Observed difference between the two strains is probably due to the TisB-mediated drop in ATP production in WT cells. Measurement of the extracellular ATP amounts showed that they stayed constant during the first 3 h in the medium with \( \Delta tisB \) cells, but that it increased in the medium with WT cells (Fig. 3C). Such increase of the ATP in the medium with treated WT cells most probably results from the leakage from dead cells and/or cell lysis.

Therefore, because WT cells have less ATP, and reduced survival compared to \( \Delta tisB \) cells, it can be concluded that, unlike what was proposed for the persisters to CIP, the intracellular ATP levels were not anti-correlated with the survival to TMP treatment.

**Impact of TisB on death of cells after TMP treatment.** The survival measured by CFU counts is the sum of cells’ ability to survive during the exposure to a drug and to recover and start growing after the drug removal. This may explain why the frequency of the DIBAC\(_4\)(3)-stained cells detected by flow cytometry after 20 h of TMP treatment did not correspond to the CFU-based survival frequency (Fig. 1D and 3A). However, it should be noted that DIBAC\(_4\)(3) stains dead cells (20), but that it also stains live cells with depolarized membrane. For this reason, we decided to investigate the kinetics of the post-TMP treatment staining with DIBAC\(_4\)(3) but also with Alexa Fluor™ 633 Hydrazide (AFH633), which detects
carbonylated macromolecules and is a reliable marker of cell death (20, 23). As a positive
control, we showed that both dyes stain heat-killed cells.

We loaded the cells immediately after the removal of TMP, into the channels of a
“mother machine” microfluidic device, and incubated them during 16 hours in LB medium
supplemented with DIBAC₄(3) and AFH633 (Fig. 4A). The vast majority of the initially non-
stained cells become stained over several hours, which indicates that many cells die during
the recovery phase. None of the stained cells was able to grow again. Mortality curves based
on the kinetics of appearance of the stained cells showed that DIBAC₄(3) stained cells before
AFH633 and that ΔtisB cells were stained 1.76 and 1.59 fold faster with DIBAC₄(3) and
AFH633, respectively, than WT cells (Fig. S4). Comparison of the CFU-based survival
frequencies and frequencies of staining with DIBAC₄(3) after 20 h of TMP treatment, i.e., t₀
after drug removal, indicates that around 75% and 31% of the ΔtisB and WT cells,
respectively, die during the recovery phase after treatment. Therefore, the absence of TisB is
advantageous during TMP treatment, but ΔtisB cells die faster during recovery phase after the
drug removal.

**TisB modulates membrane trafficking during TMP treatment.** Many membrane
transporters require energy and/or PMF and are expected to be less active in the WT cells in
which TisB is induced. Because the import of thymine and thymidine depends also on such
transporters (24), we hypothesized that TisB expression during TMP treatment may accelerate
intracellular thymidylate depletion by blocking its import, and consequently dTTP production
via the pyrimidine salvage pathway (Fig. S1C). On the contrary, the inactivation of the tisB
gene is expected to allow continuation of thymine import and dTTP production. Our
hypothesis is supported by the observation that the inactivation of deoR gene, which results in
an increased thymine catabolism (Fig. S1C), decreased the survival of the WT (Fig. 4B and S2C), but not of the ΔtisB strain.

To further confirm that TisB impacts the activity of PMF- and energy-requiring transporters, we investigated if TisB affects lactose import during TMP treatment because lactose import depends on the PMF-dependent LacY transporter (25). We added lactose to the medium after 1 h of TMP treatment and measured the induction of the PlacZ-GFP reporter in WT and ΔtisB mutant (Fig. 4C). As expected, the PlacZ-GFP reporter was induced significantly faster and stronger in the ΔtisB mutant than in the WT strain. Slopes of the linear regressions for WT and ΔtisB mutant were significantly different (linear regression slope comparison test, p-value < 0.0001).

However, besides being important for uptake of nutrients, PMF may also facilitate the import of some toxic molecules, like aminoglycoside antibiotics. Therefore, we examined the impact of gentamicin (GM), whose uptake requires PMF (26), on the survival of TMP-treated cells. We first visualized the import of GM in WT and ΔtisB cells after 2 h of TMP treatment, by using a gentamicin-Texas Red (GTx) conjugate and microscopy imaging (Fig. 4D). We observed that GTx penetrated in ΔtisB 3.4-fold more than in WT cells (Mann-Whitney test, p < 0.0001). There was practically no difference in the fluorescence between the two strains when they were not treated (WT/ΔtisB = 0.9) or when they were treated only with GTx (WT/ΔtisB = 1.1). We also measured the survival of WT and ΔtisB cells when GM was added at the MIC (Table S2) after 3 h of TMP treatment. GM reduced the survival of TMP-treated ΔtisB mutant 161-fold more (Mann-Whitney test, p < 0.02) than the survival of the TMP-treated WT (Fig. 4E).

Finally, we tested if TisB can protect TMP-treated cells against colicin M (ColM). Colicins are toxic molecules produced by Enterobacterales to reduce competition from other bacteria in the intestine (27). Importantly, colicin-coding genes are controlled by the LexA
repressor. Uptake of ColM requires its binding to the FhuA outer membrane receptor followed by an energy-dependent translocation into the periplasm through the TonB system and the inner membrane PMF (28). Once in the periplasm, ColM hydrolyses peptidoglycan lipid precursors and causes cell lysis. We found that ColM added after 3 h of TMP treatment, reduced the survival of TMP-treated ΔtisB mutant 12.4-fold (Mann-Whitney test, p < 0.0079) than the survival of the TMP-treated WT cells (Fig. 4F). Therefore, we showed that TisB activity could be advantageous to the SOS-induced cells when toxic molecules are present in the environment.

Impact of TisB on *E. coli*’s fitness in ecologically relevant environments. Because *Enterobacterales* use colicins to fight competitors in the mammalian gut, which is *E. coli*’s primary natural habitat, we investigated if TisB may have an impact on the colonization of mouse gut by *E. coli*. For this, we performed competitions between WT and ΔtisB strains during intestinal colonization. We used a modified streptomycin-treated mouse model, where the streptomycin treatment was stopped 5 days before the inoculation of bacteria (29, 30). The two strains were inoculated with a 1-to-1 ratio and their relative ability to colonize the mouse intestine was determined from the fecal bacterial load at days 1, 3, and 7 post-inoculation (Fig. 5). We observed small but significant reduction of the competitive ability of ΔtisB mutant relative to WT already after one day. After seven days, competitive ability of the ΔtisB mutant has further decreased. The CmR cassette used for the *tisB* gene deletion has negligible effect on the competitive index in these assays. These data suggest that TisB may contribute to *E. coli*’s fitness in the mammalian intestine.

Phylogenetic analysis of the TisB/IstR TA system. Because natural selection eliminates deleterious functions, it is plausible that observed TisB-associated deleterious effects, like
increased susceptibility to TMP due to the blocking of nutrients uptake, are largely
counterbalanced by TisB-associated selective advantages in natural *E. coli* habitats. However,
TisB may also be selected for in some ecological niches and counter-selected in others. In
order to answer this question, we performed phylogenetic analysis of the TisB/IstR TA system
in bacteria. We found that the *tisB/istR* genes are present only in the *Enterobacter-Escherichia*
clade of the *Enterobacterales* order (31), i.e., they were found in *Citrobacter, Salmonella* and
*Escherichia/Shigella* genera strains, as well as in the *Enterobacter cancerogenus* (formerly *E.
taylorae*) *MiY-F* strain (Table S3). The phylogeny of the 586 bp region flanking *tisB* (90bp)
and *istR* (140bp) genes follows the strain phylogeny (Fig. S5A), which was established with
the multilocus sequence analysis (MLSA) using the core genome *atpD, gyrB, infB* and *rpoB*
gene sequences (31) (Fig. S5B). These observations suggest unique, relatively recent arrival
of this TA during diversification of the *Enterobacter-Escherichia* clade within the
*Enterobacterales* order.

Next, we examined the distribution of the *tisB/istR* genes within the *Escherichia* genus
using a collection of 72 strains, which were chosen to represent the *Escherichia* phylogenetic
diversity (32). The analysis showed that the *tisB/istR* genes are present in most of the strains
(Fig. 6), always at the same chromosomal location between *ilvB* and *emrD* genes (Fig. S5C),
and that the *tisB/istR* region phylogeny (Fig. S5D) follows the phylogeny of the examined
strains based on the core genome genes (Fig. 6). However, the *tisB/istR* genes were absent in
*Escherichia albertii, Escherichia* clade V, and *E. coli sensu stricto* phylogroup B2 strains.
Interestingly, one B2 strain, FN-B26, has an empty canonical chromosomal site of the
*tisB/istR* gene integration (Fig. S5E), but we detected presence of the *tisB* gene at an unknown
location in the sequenced genome. Precise analysis of the *ilvB-emrD* region in *E. albertii, E.
clade V and E. coli* B2 strains showed three species-specific complex deletion/insertion (Fig.
S5E).
DISCUSSION

In this study, we investigated the functional integration of the TisB/IstR TA system in the *E. coli* SOS genotoxic stress response regulon. This regulon, which is highly conserved in eubacteria, has a large variability of regulon members as a function of adaptation of different bacterial species to diverse ecological niches. So, we investigated the presence of the *tisB/*istR locus in bacteria, and found that it is present only in the recently emerged genera of the *Enterobacterales* order, probably due to a unique ancestral arrival event. The *tisB/*istR locus is present in most *Escherichia* genus clades, except in *E. albertii*, *E. clade V* and *E. coli* phylogenetic group B2, each of which have lost it by an independent event. The primary natural habitat of *E. coli* is the warm-blooded animals’ gut. It was previously reported that the capacity to induce the SOS response is important for *E. coli* during intestinal colonization in order to compete with other members of the intestinal microbiota (33). Our study shows that TisB may be one of LexA-controlled functions that contribute to *E. coli*’s fitness during intestinal colonization. We investigated which of the different TisB-induced phenotypes may provide competitive advantage to its host.

The SOS regulon is induced by and deals with DNA damages. So, does TisB participate in DNA repair or contributes to the cell survival in another way? We found that the deletion of the *tisB* gene increases the survival to lethal TMP treatment. This indicates that TisB does not participate in DNA repair. However, we showed that wild-type strain is more affected than ∆*tisB* by the TMP-treatment because TisB inhibits the activity of the PMF-dependent thymine transporters and thus prevents dTTP production via a pyrimidine salvage pathway (15). We also showed that TisB modulates the activity of other PMF-dependent transporters, by showing that it impacts lactose import. Therefore, TisB prevents import of
nutrients, like sugars. However, this is not expected to increase DNA repair capacity of the WT cells because maintaining access to nutrients is essential for stressed cells to simultaneously deal with the cellular damages and to sustain functioning metabolism (33).

However, we also showed that TisB-dependent disruption of the PMF reduces the uptake of toxic compounds, such as the aminoglycoside antibiotic gentamicin and colicin M, into the SOS-induced cells. We propose that the primary biological role of TisB toxin is to prevent the uptake of colicins (and other toxic compounds) in strongly stressed cells. This hypothesis is based on the following facts: (i) SOS induction is important for intestinal colonization because it provides a competitive edge over other microbiota members (32), (ii) colicin-coding genes are controlled by the LexA repressor, (iii) colicins are toxic molecules produced by Enterobacteriales to reduce competition with other bacteria in the intestine (26, 34), and (iv) TisB protects stressed cells against colicin M.

Using low-copy plasmid-borne transcription reporters for twelve LexA-controlled genes, we observed that tisB gene expression was induced by TMP treatment with 20 × MIC but not with 0.1 × MIC of TMP. If these plasmid-borne transcriptional reporters faithfully reproduce the kinetics of the SOS regulon induction at high TMP doses, then it can be concluded that tisB gene induction was later compared to other LexA-controlled genes, such as recA and sulA. This is consistent with the fact that the sequence of tisB gene’s LexA box has the lowest heterology index among the LexA-controlled genes, which means that it binds to LexA with a very high affinity (36). In addition, it was shown that LexA dissociates much slower from the tisB gene operator than from other LexA-controlled gene operators (37).

A similar delay was observed for the appearance of TisB-dependent phenotypes, such as membrane depolarization or cell death upon TMP treatment. TisB-dependent membrane depolarization was also observed only after prolonged CIP treatment (38). Importantly, the colicin-coding genes are also induced only with high doses of SOS-inducing agents and with
a pronounced delay (39). So, the timing of the tisB gene induction should give time to the DNA repair machinery to deal with DNA damages before TisB transiently blocks cell functioning in order to protect cells against colicins and other environmental toxic compounds.

The conservation of the order of induction of the LexA-controlled genes by different stressors most likely reflects the frequency and the importance by which different genes were required for dealing with recurrent genotoxic challenges E. coli experienced in its natural habitats during its evolutionary history. Such capacity for predictive behavior of transcription networks based on déjá vu challenges is exemplified by coordinated induction of distinct transcription responses of E. coli to temperature up-shift and oxygen downshift, both of which happen upon transition from the external environment to the mammalian gastrointestinal tract (40). Similarly, Saccharomyces cerevisiae responses to a succession of stresses occurring during the process of alcoholic fermentation reflect their natural temporal order of appearance in such way that the early stresses are used as predictive signals for the arrival of later stresses (41).

What would be the ecological situation that selected for the timing of tisB gene induction and for TisB functions? One such situation may be when bacterial pathogen-induced inflammation of the intestine induces microbiota dysbiosis. For example, it was reported that invading Salmonella enterica serovar Typhimurium induces inflammation to disrupt colonization resistance. Host innate immunity defense, such as reactive oxygen species, induces SOS and colicin production by Salmonella, which eliminate commensal E. coli that are major Salmonella competitors under inflammatory conditions (42). However, our study indicates that E. coli, which possesses TisB are not disarmed against attack by colicins and other bacteriocins. Therefore, TisB toxin may provide a competitive edge to its hosts when they are simultaneously exposed to genotoxic stresses and fierce chemical warfare with other bacteria in the intestine.
MATERIALS AND METHODS

Bacterial strains

Bacterial strains and plasmids used in this study are described in Table S1. All strains are derived from the MG1655 strain.

Growth conditions

Growth was performed in LB medium at 37°C with 150 rpm shaking. The medium is prepared fresh by filter-sterilization. If needed, antibiotics were added at the following concentrations: 30 µg/ml chloramphenicol, 100 µg/ml ampicillin, and 50 µg/ml kanamycin. For supplementation experiment, thymidine was added at the final concentration of 0.3 mM. The chemicals were purchased from Sigma Aldrich unless otherwise indicated.

Determination of the Minimum Inhibitory Concentration (MIC)

The MIC of antibiotic of used bacterial strains (Table S2) was defined as the antibiotic concentration that inhibited growth after 24 h of incubation in LB liquid medium at 37°C. Cells from fresh overnight cultures were diluted 1/10,000 (v/v) and inoculated into 96-well plates (Greiner Bio-One 96 rounded) containing LB medium and different concentrations of antibiotics. Microtiter plates were incubated for 24 h at 37°C in a microplate reader and incubator (Infinite M200 PRO, Tecan). The optical density (OD) at 600 nm was measured every 10 min.

Measuring Expression of the GFP-Based Transcription Reporters
Fresh overnight (o.n.) cultures were diluted (1/10,000) and inoculated in 96-well microtiter plates containing LB. For sub-MIC experiments, TMP was added to the medium at the final concentration of 0.05µg/ml (0.1 × MIC), 50 µl of mineral oil were added into each well to avoid evaporation and the plates were incubated at 37°C in a microplate reader incubator (Spark® from TECAN). For lethal conditions, the plates were first incubated in a microplate incubator at 37°C with orbital 800 rpm shaking during 4 h to reach exponential growth phase. TMP or CIP was then added to the cultures at the final concentration of 10 µg/ml (20 × MIC) and 0.1 µg/ml (10 × MIC), respectively. Control cultures were incubated without treatment.

Optical density at 600 nm (OD$_{600\text{nm}}$) and GFP fluorescence (480/510 nm excitation/emission) were read every 10 min for 4 h.

Survival to bactericidal treatment

O.n. cultures were diluted 1/1,000 in LB medium and incubated at 37°C and 150 rpm, until early exponential phase OD$_{600\text{nm}}$ ≈ 0.2. Cultures were subsequently diluted 1/1,000 to prevent the carryover of cells of stationary phase and grown until mid-exponential phase OD$_{600\text{nm}}$ ≈ 0.6. Cells were then treated with lethal concentrations of antibiotics, either TMP at 10 µg/ml or CIP at 0.1 µg/ml. VAN was used at the sub-MIC of 0.125 mg/ml (0.25 × MIC). At given times, cells were collected by centrifugation and washed with 0.01 M MgSO$_4$. The survival was quantified by plating serial dilutions of cells on LB plates before and after antibiotic treatment and by counting the number of CFU after an o.n. incubation at 37°C.

TDtest

TDtest allows the detection of bacterial tolerance to lethal antibiotic concentrations (43). To evaluate the impact of TisB on the survival to TMP treatment, we modified the TDtest as follows. E. coli WT cells were grown in LB medium until OD$_{600\text{nm}}$ ≈ 0.6 as for testing the survival to bactericidal treatments. Growth medium was sterilized by filtration. Conditioned
sterilized medium was used for the preparation of plates upon which bacterial suspensions, with approximately $10^5$ bacteria/ml, were streaked using a sterile cotton swab. Absorbent paper discs were placed in the middle of the plates. 50 µl of 300 µg/ml of TMP were added on each disc. Plates were incubated 24 h at 37°C. Bacteria growing outside of the inhibition zone exhaust nutrients from the whole plate (even from the inhibition zone). To allow growth of surviving bacteria, 10 µl of 30% glucose that provide energy for growth, and 20 µl of 100 µg/ml thymidine that counteracts the effects of TMP, were spotted on the discs and plates were incubated 24 h at 37°C. Colonies of surviving bacteria can be visualized in the inhibition zone.

Membrane potential assay

500 µl samples of bacterial cultures were collected and centrifuged 2 min at 20,000 g. After the removal of the supernatant, the cell pellet is resuspended in 500 µl of DIBAC$_4$(3) at 2.5 µg/ml in 0.01 M MgSO$_4$. After 15 min of incubation at room temperature in the dark, samples are washed in 0.01 M MgsO$_4$ then analyzed using Gallios Flow Cytometer (Beckman Coulter). Single-cell fluorescence was measured using a 488 nm excitation laser and 525 nm emission filter. Cells exposed to a temperature of 90°C during 5 min were used as a positive control. Minimally 50,000 cells were analyzed per experiment.

ATP measurement

The ATP measurement was done using the ATP Determination Kit (Molecular Probes). All the sample preparation steps were carried out on ice or at 4°C. At given times of the TMP treatment, 10 ml of culture were collected and centrifuged 10 min at 4000 rpm. The supernatant was filtered and stored at 4°C until the assay. The cell pellet was precipitated with 120 µl of ice-cold 6% perchloric acid and lysed for 10 min using Lysing Matrix B (MP
Biomedicals). The samples are then centrifuged for 5 min at 12,000 rpm, the supernatant is collected and the pH is adjusted to ~ 7 with 2M K$_2$CO$_3$. A 20 µl-volume of samples was mixed with 180 µl of luciferin-luciferase solution using 96-well white microtiter plates. After 30 min of incubation at room temperature, the luminescence was measured using a CLARIOstar® Plus plate reader (BMG Labtech). The luminescence of ATP solutions ranging from 0.1 to 1 µM was measured to obtain a standard curve ($r^2 = 0.9991$). The ATP concentrations were calculated using the standard curve. Obtained results were corrected for sample biomass, approximated by the cell pellet weight.

**Determining post-antibiotic treatment mortality rates**

After TMP lethal treatment, cells are collected, washed and loaded in a microfluidic device as described before (34). The microfluidic device we used consist of a series of growth channels through which the LB medium with DIBAC$_6$(3) 2.5 µg/ml and AFH633 4 µg/ml is passed at a constant rate. The samples were monitored using a Nikon Ti-E inverted microscope coupled to MetaMorph® software and images were acquired every 20 min during 16 h. Mortality curves were analysed using the R package ‘growthrates’ (44) by applying the logistic growth model.

**PMF-dependent transporters’ activity**

WT and ∆tisB cells are grown in LB and treated with 20 × MIC of TMP. For cells carrying the PlacZ-GFP reporter, 0.2 % lactose was added to the cultures after 1 h of TMP treatment and a 150 µl-volume was transferred to a 96-well microtiter plates. 50 µl of mineral oil were added into each well. The plates were incubated at 37°C in a microplate reader incubator (Spark® from TECAN), control cultures were incubated without lactose. OD$_{600nm}$ and GFP fluorescence (480/510 nm excitation/emission) were read every 15 min for 8 h.
To observe the import of gentamicin (GM), we used a gentamicin-Texas Red (GTx) conjugate which was obtained by mixing 4.4 ml of GM 50 mg/ml with 0.6 ml of Texas Red (Molecular Probes) 2 mg/ml and agitated at 4°C during 48 h (45). After 2h of TMP treatment, GTx was added to the cultures at the MIC (2.5 µg/ml) during 15min. For control cultures, GTx 2.5 µg/ml was added to the cultures at OD$_{600nm}$=0.6 during 15min. Cells are then collected and washed in MgSO$_4$ 0.01M. 1 µl of sample is spotted on 1.5% agarose pads prepared with MgSO$_4$ 0.01M for microscopy imaging. For fluorescence detection, we used a 561nm excitation laser and a 630/60nm filter. For each observation field, we acquired 16 images by varying the Z parameter with a 0.1 µm step in order to obtain images at different focal planes and a depth of field adapted to the bacteria’s width. Using ImageJ Z-projection tool, the images were summed into one image for analysis. The mean fluorescence of each cell was obtained after analysis using the MicrobeJ plugin (46) on Fiji (ImageJ).

Susceptibility of TMP-treated cells to gentamicin and colicin M treatments

To test the susceptibility of TMP-treated cells to GM, 2 µg/ml (= MIC) of GM was added to the cell cultures after 3 h of TMP treatment. The survival was quantified by plating serial dilutions of cells on LB plates before and after the addition of GM and by counting the number of CFU after an o.n. incubation at 37°C.

Colicin M (ColM) was prepared using the *E. coli* strain C43(DE3) carrying the pMLD238 plasmid as previously described (47). Briefly, cells were grown at 37°C in 500 ml of LB medium containing kanamycin. When the OD$_{600nm}$ reached 0.6, isopropyl 1-thio-β-D-galactopyranoside (IPTG) was added at the final concentration of 1 mM, and growth was continued for 3 h at 37°C. Cells were collected and washed with 100 ml of cold 20 mM Tris-HCl buffer (pH 7.4) containing 10 mM 2-mercaptoethanol, 200 mM NaCl and 10% glycerol. The cell pellet was suspended in 10 ml of the same buffer and lysed for 10 min using Lysing
Matrix B (MP Biomedicals). The resulting suspension was centrifuged at 4 °C for 15 min at 15,000 g and the supernatant stored at 20 °C before purification of the His₆-tagged protein. The purification of the His₆-tagged proteins was performed using a HisTrap™ purification column. The column was equilibrated in the previously described buffer supplemented with 10mM imidazole, the sample was applied using a pump at 1 ml/min flow rate. The proteins of interest were eluted with 200 mM imidazole and dialyzed over night against 100 volumes of the buffer. The final preparation was stored at -20°C. Protein concentrations were determined by using a colorimetric assay based on the Bradford method (Dye Reagent Concentrate, Bio-Rad Protein Assay). 3 µg/ml ColM (= MIC) was added to the cultures after 3 h of TMP treatment. The survival was quantified by plating serial dilutions of cells on LB plates before and after the addition of ColM and by counting the number of CFU after an o.n. incubation at 37°C.

**Intestinal colonization**

Six-week-old female mice (Charles River CD-1) treated with streptomycin were used to monitor the ability of the WT and ΔtisB strains to colonize the intestine of mammalian host as previously described (48). Five days before inoculation, mice were isolated and streptomycin was added to the sterile drinking water at a final concentration of 5 g/l. Streptomycin was maintained until the day before inoculation. The antibiotic treatment efficacy against the coliform intestinal population was controlled by plating a pure suspension of feces in physiological water on Drigalski selective agar medium. Mice free of coliform flora were inoculated *per os* with 10⁹ bacteria in 200 µL of physiological water. Mice were inoculated with a 1-to-1 ratio of WT and ΔtisB::Cm cells or a 1-to-1 ratio of ΔtisB::Cm and ΔtisB::FRT as a control for the competitive cost of the Cm<sup>R</sup> cassette (29). At day 1, 3 and 7 postinoculation, the intestinal population of *E. coli* was estimated by plating dilutions of
weighted fresh feces on LB agar with or without chloramphenicol 30 \(\mu\)g/ml and colony forming units (CFU) counts after an o.n. incubation at 37\(^\circ\)C. The competitive index (CI) was calculated as the ratio of chloramphenicol-resistant CFU to sensitive CFU at given times, divided by the same ratio present in the inoculum for each competition.

All animal experiments were approved by the French Ministry of Research and by the Ethical Committee for Animal Experiments, CEEA-121, Comité d’éthique Paris-Nord (APAFIS#4951-2016020515004032 v2, 2016021216251548 v4).

Phylogenetic analyses

tisB gene sequences of the *Escherichia* strains representative of the genus (32) were translated. We obtained 4 protein sequences that were searched in the ‘nr’ NCBI database using a BLASTP to identify TisB bearing strains from other genus in the database of identity and coverage of 90%.

tisB and istR genes as well as the atpD, gyrB, infB and rpoB genes of the MLSA (31) were used as gene database in Abricate for identification of these genes in the genomes (https://github.com/tseemann/abricate) with a minimum of identity and coverage of 80%.

Sequences were aligned with MAFFT (49, 50). Phylogenetic trees were reconstructed using the maximum likelihood method with PhyML (51) and the GTR model.

The phylogenetic tree of the 72 *Escherichia* strains representative of the genus diversity was reconstructed from the SNPs (n=374,678) of core genome genes (n=1,302) using ROARY and RAxML as in reference (32).

Statistical analysis

All analyses were done using GraphPad Prism 8 (GraphPad Software, Inc., La Jolla, CA).
SUPPLEMENTAL MATERIEL

Supplemental material is available online only.

FIG S1, PDF file, 639 Ko.

Regulation of the tisB gene expression, folate cycle and dTTP synthesis.

FIG S2, PDF file, 278 Ko MB.

TMP killing activity depends on the cell culture growth phase, thymidine, the pyrimidine salvage pathway and DNA replication activity.

FIG S3, PDF file, 255 Ko.

Kinetics of the induction of the SOS genes by antibiotic treatments.

FIG S4, PDF file, 405 Ko.

Post-antibiotic treatment mortality rates.

FIG S5, PDF file, 847 Ko.

Phylogenetic analysis of the TisB/IstR TA system.

TABLE S1, PDF file, 1.6 Mo.

Bacterial strains and plasmids used in this study.

TABLE S2, PDF file, 981 Ko.

MIC of the studied strains for different antibiotics.

TABLE S3, PDF file, 94 Ko.

Strain ID and genome accession numbers of the Enterobacter-Escherichia clade taxa possessing the TisB/IstR TA system presented in the Figure S5AB.

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Author contributions were as follows: W.-L.S, A.G., E.D. and I.M designed the research; E.D. and I.M. secured funding; W.-L.S, M.-F.B., S.D., J.D. and B.C. performed the experiments and analyzed data; and W.-L.S, E.D. and I.M. prepared and edited the manuscript.

We declare no conflicts of interest.
REFERENCES

1. Harms A, Brodersen DE, Mitarai N, Gerdes K. 2018. Toxins, targets, and triggers: an overview of toxin-antitoxin biology. Molecular Cell 70:768–784.

2. Fraikin N, Goormaghtigh F, Van Melderen L. 2020. Type II toxin-antitoxin systems: evolution and revolutions. J Bacteriol 202:e00763-19, /jb/202/7/JB.00763-19.atom.

3. Simmons LA, Foti JJ, Cohen SE, Walker GC. 2008. The SOS regulatory network. Ecosal Plus 2008.

4. Erill I, Campoy S, Barbé J. 2007. Aeons of distress: an evolutionary perspective on the bacterial SOS response. FEMS Microbiology Reviews 31:637–656.

5. Wagner EGH, Unoson C. 2012. The toxin-antitoxin system \textit{tisB-istR1}: expression, regulation, and biological role in persister phenotypes. RNA Biology 9:1513–1519.

6. Gurnev PA, Ortenberg R, Dörr T, Lewis K, Bezrukov SM. 2012. Persister-promoting bacterial toxin TisB produces anion-selective pores in planar lipid bilayers. FEBS Letters 586:2529–2534.

7. Dörr T, Vulić M, Lewis K. 2010. Ciprofloxacin causes persister formation by inducing the TisB toxin in \textit{Escherichia coli}. PLOS Biology 8:e1000317.

8. Edelmann D, Oberpaul M, Schäberle TF, Berghoff BA. 2021. Post-transcriptional deregulation of the \textit{tisB/istR-1} toxin–antitoxin system promotes SOS-independent persister formation in \textit{Escherichia coli}. Environmental Microbiology Reports 13:159–168.

9. Völzing KG, Brynildsen MP. 2015. Stationary-Phase Persisters to Ofloxacin Sustain DNA Damage and Require Repair Systems Only during Recovery. mBio 6.

10. Goormaghtigh F, Van Melderen L. 2019. Single-cell imaging and characterization of \textit{Escherichia coli} persister cells to ofloxacin in exponential cultures. Sci Adv 5:eaav9462.
11. Miovic M, Pizer LI. 1971. Effect of trimethoprim on macromolecular synthesis in *Escherichia coli*. Journal of Bacteriology 106:856–862.

12. Sangurdekar DP, Zhang Z, Khodursky AB. 2011. The association of DNA damage response and nucleotide level modulation with the antibacterial mechanism of the antifolate drug Trimethoprim. BMC Genomics 12:583.

13. Eagle H, Musselman AD. 1948. The rate of bactericidal action of penicillin in vitro as a function of its concentration, and its paradoxically reduced activity at high concentrations against certain organisms. J Exp Med 88:99–131.

14. Lewin CS, Amyes SGB. 1991. The role of the SOS response in bacteria exposed to zidovudine or trimethoprim. Journal of Medical Microbiology, 34:329–332.

15. Giroux X, Su W-L, Bredeche M-F, Matic I. 2017. Maladaptive DNA repair is the ultimate contributor to the death of trimethoprim-treated cells under aerobic and anaerobic conditions. PNAS 114:11512–11517.

16. Balaban NQ, Helaine S, Lewis K, Ackermann M, Aldridge B, Andersson DI, Brynildsen MP, Bumann D, Camilli A, Collins JJ, Dehio C, Fortune S, Ghigo J-M, Hardt W-D, Harms A, Heinemann M, Hung DT, Jenal U, Levin BR, Michiels J, Storz G, Tan M-W, Tenson T, Van Melderen L, Zinkernagel A. 2019. Definitions and guidelines for research on antibiotic persistence. Nat Rev Microbiol 17:441–448.

17. Unoson C, Wagner EGH. 2008. A small SOS-induced toxin is targeted against the inner membrane in *Escherichia coli*. Molecular Microbiology 70:258–270.

18. Liu A, Tran L, Becket E, Lee K, Chinn L, Park E, Tran K, Miller JH. 2010. Antibiotic sensitivity profiles determined with an *Escherichia coli* gene knockout collection: generating an antibiotic bar code. Antimicrob Agents Chemother 54:1393–1403.

19. Yamaguchi Y, Inouye M. 2011. Regulation of growth and death in *Escherichia coli* by toxin–antitoxin systems. 11. Nat Rev Microbiol 9:779–790.
20. Saint-Ruf C, Crussard S, Franceschi C, Orenga S, Ouattara J, Ramjeet M, Surre J, Matic I. 2016. Antibiotic susceptibility testing of the Gram-negative bacteria based on flow cytometry. Front Microbiol 7.

21. Barbé J, Villaverde A, Guerrero R. 1983. Evolution of cellular ATP concentration after UV-mediated induction of SOS system in Escherichia coli. Biochem Biophys Res Commun 117:556–561.

22. Guerrero R, Llagostera M, Villaverde A, Barbe J. 1984. Changes in ATP concentration in Escherichia coli during induction of the SOS system by mitomycin C and bleomycin. Microbiology 130:2247–2251.

23. Saint-Ruf C, Cordier C, Mégret J, Matic I. 2010. Reliable detection of dead microbial cells by using fluorescent hydrazides. Applied and Environmental Microbiology 76:1674–1678.

24. Botou M, Lazou P, Papakostas K, Lambrinidis G, Evangelidis T, Mikros E, Frillingos S. 2018. Insight on specificity of uracil permeases of the NAT/NCS2 family from analysis of the transporter encoded in the pyrimidine utilization operon of Escherichia coli. Molecular Microbiology 108:204–219.

25. Busch W, Saier MH. 2002. The Transporter Classification (TC) System, 2002. Critical Reviews in Biochemistry and Molecular Biology 37:287–337.

26. Mingeot-Leclercq M-P, Glupczynski Y, Tulkens PM. 1999. Aminoglycosides: Activity and Resistance. Antimicrob Agents Chemother 43:727–737.

27. Cascales E, Buchanan SK, Duché D, Kleanthous C, Lloubès R, Postle K, Riley M, Slatin S, Cavard D. 2007. Colicin biology. Microbiol Mol Biol Rev 71:158–229.

28. Jakes KS, Cramer WA. 2012. Border crossings: colicins and transporters. Annu Rev Genet 46:209–231.
29. Diard M, Garry L, Selva M, Mosser T, Denamur E, Matic I. 2010. Pathogenicity-associated islands in extraintestinal pathogenic *Escherichia coli* are fitness elements involved in intestinal colonization. Journal of Bacteriology 192:4885–4893.

30. Duprilot M, Baron A, Blanquart F, Dion S, Pouget C, Lettéron P, Flament-Simon S-C, Clermont O, Denamur E, Nicolas-Chanoine M-H. 2020. Success of *Escherichia coli* O25b:H4 sequence type 131 clade C associated with a decrease in virulence. Infect Immun 88:e00576-20.

31. Adeolu M, Alnajar S, Naushad S, Gupta R. 2016. Genome-based phylogeny and taxonomy of the ‘Enterobacteriales’: proposal for *Enterobacterales* ord. nov. divided into the families *Enterobacteriaceae*, *Erwiniaeae* fam. nov., *Pectobacteriaceae* fam. nov., *Yersiniaceae* fam. nov., *Hafniaceae* fam. nov., *Morganellaceae* fam. nov., and *Budviciaceae* fam. nov. Int J Syst Evol Microbiol 66:5575–5599.

32. Denamur E, Clermont O, Bonacorsi S, Gordon D. 2021. The population genetics of pathogenic *Escherichia coli*. Nat Rev Microbiol 19:37–54.

33. Samuels AN, Roggiani M, Zhu J, Goulian M, Kohli RM. 2019. The SOS response mediates sustained colonization of the mammalian gut. Infection and Immunity 87.

34. Mathieu A, Fleurier S, Frénoy A, Dairou J, Bredeche M-F, Sanchez-Vizuete P, Song X, Matic I. 2016. Discovery and function of a general core hormetic stress response in *E. coli* induced by sublethal concentrations of antibiotics. Cell Reports 17:46–57.

35. Riley MA, Gordon DM, Riley MA, Gordon DM. 1999. The ecological role of bacteriocins in bacterial competition. Trends in Microbiology 7:129–133.

36. Fernández De Henestrosa AR, Ogi T, Aoyagi S, Chafin D, Hayes JJ, Ohmori H, Woodgate R. 2000. Identification of additional genes belonging to the LexA regulon in *Escherichia coli*. Mol Microbiol 35:1560–1572.
37. Culyba MJ, Kubiak JM, Mo CY, Goulian M, Kohli RM. 2018. Non-equilibrium repressor binding kinetics link DNA damage dose to transcriptional timing within the SOS gene network. PLoS Genet 14:e1007405.

38. Berghoff BA, Hoekzema M, Aulbach L, Wagner EGH. 2017. Two regulatory RNA elements affect TisB-dependent depolarization and persister formation. Molecular Microbiology 103:1020–1033.

39. Žgur-Bertok D. 2012. Regulating colicin synthesis to cope with stress and lethality of colicin production. Biochem Soc Trans 40:1507–1511.

40. Tagkopoulos I, Liu Y-C, Tavazoie S. 2008. Predictive behavior within microbial genetic networks. Science 320:1313–1317.

41. Mitchell A, Romano GH, Groisman B, Yona A, Dekel E, Kupiec M, Dahan O, Pilpel Y. 2009. Adaptive prediction of environmental changes by microorganisms. 7252. Nature 460:220–224.

42. Nedialkova LP, Denzler R, Koeppel MB, Diehl M, Ring D, Wille T, Gerlach RG, Stecher B. 2014. Inflammation fuels colicin Ib-dependent competition of Salmonella serovar Typhimurium and E. coli in enterobacterial blooms. PLoS Pathog 10:e1003844.

43. Gefen O, Chekol B, Strahilevitz J, Balaban NQ. 2017. TDtest: easy detection of bacterial tolerance and persistence in clinical isolates by a modified disk-diffusion assay. Sci Rep 7:41284.

44. Petzoldt T. 2021. growthrates: estimate growth rates from experimental data. R package version 0.8.2. https://github.com/tpetzoldt/growthrates. Retrieved 7 July 2021.

45. Dai CF, Mangiardi D, Cotanche DA, Steyger PS. 2006. Uptake of fluorescent gentamicin by vertebrate sensory cells in vivo. Hear Res 213:64–78.
46. Ducret A, Quardokus EM, Brun YV. 2016. MicrobeJ, a tool for high throughput bacterial cell detection and quantitative analysis. Nat Microbiol 1:1–7.

47. Barreteau H, Bouhss A, Fourgeaud M, Mainardi J-L, Touzé T, Gérard F, Blanot D, Arthur M, Mengin-Lecreulx D. 2009. Human- and plant-pathogenic Pseudomonas species produce bacteriocins exhibiting colicin M-like hydrolase activity towards peptidoglycan precursors. J Bacteriol 191:3657–3664.

48. Vimont S, Boyd A, Bleibtreu A, Bens M, Goujon J-M, Garry L, Clermont O, Denamur E, Arlet G, Vandewalle A. 2012. The CTX-M-15-producing Escherichia coli clone O25b: H4-ST131 has high intestine colonization and urinary tract infection abilities. PLoS One 7:e46547.

49. Katoh K, Rozewicki J, Yamada KD. 2019. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Brief Bioinform 20:1160–1166.

50. Kuraku S, Zmasek CM, Nishimura O, Katoh K. 2013. aLeaves facilitates on-demand exploration of metazoan gene family trees on MAFFT sequence alignment server with enhanced interactivity. Nucleic Acids Res 41:W22–W28.

51. Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307–321.
FIGURE LEGENDS

**FIG 1** The induction and involvement of different SOS genes in the survival of cells treated with TMP. (A) Kinetics of the induction of five SOS genes by 20 × MIC of TMP measured by following the GFP fluorescence intensity. The promoter-less \( P_{\text{less}} \) plasmid was used as a negative control. Each dot represents the mean value (± SEM) of results obtained from 3 independent experiments. (B) The induction of the SOS response in cells treated with 20 × MIC of TMP measured using a PrecA-GFP reporter. (C) The induction of \( \text{tisB} \) in cells treated with 20 × MIC of TMP measured using a \( P_{\text{tisB}} \)-GFP reporter. (B and C) \( \text{lexA1} \) (Ind') strain and untreated condition (NT) are used as negative controls for SOS induction. The presented results are representative of 3 independent experiments. (D) The survival of mutants of several SOS genes to 20 h of 20 × MIC of TMP treatment. Each bar represents the mean value (± SEM) of results obtained from at least 4 independent experiments (Mann-Whitney test vs WT unless otherwise indicated, * p-value < 0.05, *** p-value < 0.001, **** p-value < 0.0001, NS p-value > 0.05). (E) TDtest. Left column: susceptibility to TMP, which was spotted on the discs, after 24 h of incubation. Right column: colonies of surviving bacteria within the growth inhibition zone visualized after additional 24 h incubation with glucose (Glu) that provide energy for growth, and thymidine (Thy) that counteracts the effects of TMP, which were spotted on the discs.

**FIG 2** Morphology and survival of the antibiotic-treated cells. WT and Δ\( \text{tisB} \) cells were treated with (A) 20 × MIC of TMP, (D and E) 0.25 × MIC of vancomycin (VAN) or the combination of TMP + VAN and the killing kinetics are represented as the survival over time. Each dot represents the mean value (± SEM) of results obtained from 3 independent experiments (Mann-Whitney test, * p-value < 0.05, **** p-value < 0.0001). (B) The WT and
(C) \( \Delta tisB \) cell size (FSC-area) distribution after 3 h and 20 h of antibiotic treatment determined by flow cytometry (data from (D) and (E) respectively). 50,000 cells are analyzed for each condition. Stationary phase untreated cells were used as a control for cell size. The presented histograms are representative of 3 independent experiments. The tables show the percentage of cells with a size higher than that of the control population of stationary phase untreated cells. The values represented are mean values (± SEM) of 3 independent experiments.

**FIG 3.** Membrane permeability and ATP pool in cells treated with 20 × MIC of TMP. (A) The cell permeability assayed by DIBAC\(_4\)(3) staining after 3 h and 20 h of TMP treatment is determined by flow cytometry. Each histogram represents the distribution for 50,000 cells analyzed. Stationary phase untreated cells were used as a negative control. Heat-killed cells were used as a positive control. The presented results are representative of at least 3 independent experiments. The tables show the percentage of cells with a size higher than that of the control population of stationary phase untreated cells. The values represented are mean values (± SEM) of 3 independent experiments. (B) Intracellular and (C) extracellular ATP concentrations during TMP treatment. The results shown are the mean values (± SEM) of results obtained from 3 independent experiments (Student t-test, ** p-value < 0.01, *** p-value < 0.001, **** p-value < 0.0001).

**FIG 4** Impact of TisB on cell death kinetics after TMP treatment and on the import of different extracellular compounds during TMP treatment. (A) The kinetics of AFH633 and DIBAC\(_4\)(3) cell staining after 20 × MIC of TMP treatment is plotted as the frequency of stained cells over time. For this experiment, cells were loaded into the channels of a “mother machine” microfluidic device, incubated during 16 hours in LB medium supplemented with
DIBAC₄(3) and AFH633, and monitored using fluorescent microscope. The results shown are obtained from 4 independent experiments, a total of 329 and 657 cells for WT and ΔtisB respectively, were analyzed. (B) The impact of TisB on the pyrimidine salvage pathway is measured by the survival of the WT, ΔtisB, ΔdeoR and ΔtisB ΔdeoR strains treated with 20 × MIC of TMP. Each bar represents the mean value (± SEM) of data obtained from at least 3 independent experiments (Mann-Whitney test, * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001, NS p-value > 0.05). (C) The import of lactose was measured using the PlacZ-GFP reporter. Lactose was added after 1 h of treatment with 20 × MIC of TMP. The obtained values were corrected by subtracting values from the control condition without lactose. Each dot represents the mean value (± SEM) of 5 independent experiments. The lines represent the linear regression (slope mean value (± SEM): WT = 146 (4.2), ΔtisB = 374.7 (7.9); r²: WT = 0.9619, ΔtisB = 0.9791). Prism Linear regression slope comparison test, **** p-value < 0.0001. (D) The import of the gentamicin-Texas Red (GTx) conjugate in WT and ΔtisB cells monitored using fluorescent microscope. Each dot represents the value of summed fluorescence corrected by the area for a single cell. The results shown are obtained from 3 independent experiments and a total of 112, 85, 529, 430, 517 and 597 cells were analyzed for WT NT, ΔtisB NT, WT GTx, ΔtisB GTx, WT TMP GTx, and ΔtisB TMP GTx condition respectively. For this experiment, TMP and GTx were used at 20 × MIC and MIC, respectively. (E) Gentamicin (GM) at the MIC was added to the cultures after 3 h of treatment with 20 × MIC of TMP, the survival after 20 h is determined by CFU counts. (F) Colicin M (ColM) at the MIC was added to the cultures after 3 h of 20 × MIC of TMP treatment, the survival after 20 h is determined by CFU counts. (E and F) Paired results from 4 and 5 independent experiments are presented (Mann-Whitney test, * p-value < 0.05, ** p-value < 0.01). Each bar represents the median of obtained data.
FIG 5 Impact of TisB on the intestinal colonization. Competition between WT and ΔtisB mutant during mouse intestinal colonization. Competitive indexes (CI) are given for day 1, day 3 and day 7 after bacterial inoculation. Mice were inoculated with a 1-to-1 ratio of WT and ΔtisB::Cm cells or a 1-to-1 ratio of ΔtisB::Cm and ΔtisB::FRT. The first competition (COMP) is for measuring the CI. The second competition (CTR) is to evaluate the impact of the CmR cassette on the CI. Each symbol represents the CI for one mouse. Horizontal bars represent median values. Statistical differences were calculated using Mann-Whitney test (* p-value < 0.05, ** p-value < 0.01).

FIG 6 Presence of the TisB/IstR TA system in the genomes of Escherichia genus strains. Phylogenetic history of 72 Escherichia strains representative of the genus diversity with the presence/absence of the TisB/IstR TA system. The tree was reconstructed from the SNPs (n=374,678) of core genome genes (n=1,302) using ROARY and RAxML and rooted on the E. albertii species. The strains are identified by their ID followed by the ST number according to the Achtman (Warwick University) scheme. The external circle corresponds to the phylogenetic groups according to the given color code. The presence of the TisB/IstR TA system is indicated by a star. Of note, the B2 phylogroup E. coli FN-B26 strain possesses only the tisB gene, but at an unknown location in the sequenced genome.
**Figure A**

- **t<sub>3h</sub>** and **t<sub>20h</sub>**
- Cell count
- DIBAC<sub>4</sub>(3) fluorescence (A.U.)
- % DIBAC<sub>4</sub>(3) +
  - WT **t<sub>3h</sub>** 39.9 ± 16.5
  - ΔtisB **t<sub>3h</sub>** 3.0 ± 1.2
  - WT **t<sub>20h</sub>** 81.7 ± 4.2
  - ΔtisB **t<sub>20h</sub>** 35.1 ± 5.7

**Figure B**

- WT vs ΔtisB
- [ATP]<sub>intracellular</sub> (µM)
- Time (h)
- WT: 0, 1, 2, 3, 20
- ΔtisB: 0, 1, 2, 3, 20
- **p < 0.01**

**Figure C**

- WT vs ΔtisB
- [ATP]<sub>extracellular</sub> (µM)
- Time (h)
- WT: 0, 1, 2, 3, 20
- ΔtisB: 0, 1, 2, 3, 20
- **p < 0.001**
- **p < 0.0001**
Intestinal colonization

Day 1 | Day 3 | Day 7

COMP: ΔtisB::Cm/WT | CTR: ΔtisB::Cm/ΔtisB::FRT

Competitive index

** | * | *
A. **LexA**

- LexA box
- SOS response

B. **IstR**

- +1 (inactive)
- +42 (active)
- IstR binding
- RNase III cleavage
- IstR cleaved

C. **TisB**

- Translation of active +42 mRNA
- IM
- OM

**De novo synthesis**

- dUTP
- dUMP
- ATP
- GTP
- THF
- DHF
- 5,10 methylene THF
- gly
- hcy
- met
- ser

**Salvage**

- deoxycytidine
- deoxyuridine
- deoxyribose-1-phosphate
- P_i
- thymine
- thymidine
- dTMP
- dUTP
- dTMP

**Trimethoprim**

- Trimethoprim
- DeoC
- TCA cycle
- DeoR
- H^+
- H^+
- IM
- OM
FIG S1 Regulation of the *tisB* gene expression, folate cycle and dTTP synthesis. (A) Regulation of the *tisB* gene expression. Under normal growth conditions, SOS repressor LexA binds to the *tisB* gene LexA box and prevents its transcription, while *istR* gene is constitutively transcribed. The primary *tisB* transcript +1 is translationally inactive due to the secondary structures that prevent ribosome binding. This transcript is processed into translationally active +42 mRNA, but IstR RNA binding prevents its translation and induces its cleavage by RNase III. The cleaved *tisB* mRNA (+106) is inactive for translation. When the SOS response is induced, resulting strong *tisB* transcription overcomes IstR RNA capacity of inhibition. Consequently, TisB toxin can be synthesized. TisB protein affects the inner membrane, which results in the disruption of the proton motive force and the inhibition of the ATP synthesis. (B) Folate cycle and dTTP synthesis. TMP inhibits the reduction of dihydrofolate (DHF) to tetrahydrofolate (THF) by blocking the DHF reductase, thus blocking the folate cycle which involves the synthesis of amino-acids: methionine (met), glycine (gly); the synthesis of purines: ATP and GTP; and the neo-synthesis of deoxythymidine triphosphate (dTTP). (C) dTTP can also be synthesized through a pyrimidine salvage pathway that involves either the successive conversions of deoxycytidine into dTTP or the import of thymine and thymidine - when present in the medium - that is converted into dTTP. The salvage pathway is under the control of the DeoR repressor. DeoC, one of the salvage pathway enzymes, is responsible for the utilization of deoxyribose as a carbon and energy source.
FIG S2 TMP killing activity depends on the cell culture growth phase, thymidine, the pyrimidine salvage pathway and DNA replication activity. (A) We first tested susceptibility of WT cells from different growth phases to different TMP concentrations, and decided that treatment of the exponentially growing cultures having around $2 \times 10^8$ colony-forming units (CFU)/ml ($OD_{600nm} = 0.6$) and TMP concentration of 10 µg/ml, i.e., $20 \times$ MIC (Table 2), are most suitable for our study. This concentration of TMP was chosen because further increase of the TMP concentration did not further decrease survival, i.e., dose-response relationship displayed the Eagle effect. This cell concentration was chosen because cells at this growth phase were most susceptible to TMP. The stationary phase cells ($OD_{600nm} = 2$) were barely impacted. The presented results are mean values (± SEM) obtained from at least 3 independent experiments. (B) The concentrations of cells in the untreated cultures at different time points are plotted on the left axis. The dashed line indicates the density of the culture at $OD_{600nm} = 0.6$. At each time point, cell samples were collected and treated with TMP. Survival frequencies of treated cultures are plotted on the right axis. Mean values (± SD) of data obtained from 3 independent experiments are presented. (C) The survival of the TMP-treated WT strain in LB medium supplemented or not with 0.3 mM of thymidine, and the survival of TMP-treated ΔdeoC and ΔdeoR mutants. (D) The survival of the TMP-treated dnaA(Sx) strain, whose DNA replication initiation frequency is diminished. (E) The survival of WT and ΔtisB strains carrying either the P_{BAD} or the P_{BAD}-tisB\textsuperscript{+} plasmids, to 20h of TMP treatment. Each bar represents the mean value (± SEM) of data obtained from at least 3 independent experiments. Mann-Whitney test (vs WT unless indicated), * p-value < 0.05, ** p-value < 0.01, *** p-value < 0.001, **** p-value < 0.0001, NS p-value > 0.05.
**FIG S3** Kinetics of the induction of the SOS genes by antibiotic treatments. Each strain carries a plasmid with a transcriptional fusion of the *gfp* gene to the promoter of a LexA-controlled gene. Cultures of exponentially growing cells of each strain were treated with (A) 20 × MIC of TMP, (B) 10 × MIC of CIP, and (D) 0.1 × MIC of TMP. (C and E) Untreated cultures and the promoter-less (*P*<sub>less</sub>) plasmid were used as negative controls. Each dot represents the mean value (± SD) of the results obtained from 3 independent experiments.
FIG S4. Post-antibiotic treatment mortality rates. (A) The kinetic of AFH633 and DIBAC$_4$(3) cell staining after TMP treatment is plotted as the frequency of stained cells over time. The blue line represents the fitting curve of the obtained data. The curves were analyzed using the ‘growth rates’ package on R and by applying the logistic growth model. (B) Three parameters obtained from the fitting: Y0, the initial death frequency, $\mu_{\text{max}}$ interpreted as the death rate, and K as the maximum death frequency.
FIG S5. Phylogenetic analysis of the TisB/IstR TA system. (A and B) Maximum likelihood phylogenetic trees reconstructed from (A) the 586 bp region flanking tisB and istR and (B) the atpD, gyrB, infB and rpoB concatenated genes (2504 bp) of the Enterobacter-Escherichia clade taxa possessing the TisB/IstR TA system. The trees were rooted on Enterobacter cancerogenus and reconstructed using PhyML and the GTR model. The tree of the four concatenated genes corresponds to the strain phylogeny. See Table S1 for the strain ID and genome accession numbers. (C) Chromosomal map of the region surrounding the tisB and istR genes. An example of a tisB/istR negative (E. coli phylogroup B2 CFT073) and positive (E. coli phylogroup B1 NILS24) strains is given. The numbers below the CFT073 map correspond to the CFT073 chromosome coordinates. (D) Maximum likelihood phylogenetic tree reconstructed from the 586 bp region flanking tisB and istR of the Escherichia strains carrying these genes (Fig. 6). The tree was rooted on Escherichia clade III and IV strains and reconstructed using PhyML and the GTR model. The external circle corresponds to the phylogenetic groups according to the given color code. (E) Alignments of the nucleotides of the ilvB/emrD region in genomes of the E. coli phylogroup B2, E. albertii and Escherichia clade V strains lacking the TisB/IstR TA system. Grey arrows indicate positions corresponding to 5’ and 3’ of the tisB/istR region in the chromosomes of strains that possess entire tisB/istR TA system.
### TABLE S1 Bacterial strains and plasmids used in this study

| Strain               | Relevant genotype          | Source                                           |
|----------------------|---------------------------|-------------------------------------------------|
| WT                   | MG1655 wild-type (parental strain) | Laboratory strain collection                    |
| ∆deoC                | ∆deoC::kan                | MG1655 P1 (KEIO ∆deoC::kan, Suppl. ref. (1))    |
| ∆deoR                | ∆deoR::kan                | MG1655 P1 (KEIO ∆deoR::kan, Suppl. ref. (1))    |
| ΔaulA                | ΔaulA::kan                | Ref. (15)                                       |
| ΔaulA (Sx)           | ΔaulA(Sx)721 Δzib::Tn10   | Ref. (15)                                       |
| lexA1 (Ind)          | Δins::FRT lexA1           | Ref. (15)                                       |
| ΔtisB                | ΔtisB::cm                 | MG1655 × FRTcatFRT from pKD3, Suppl. ref. (3)   |
| ΔtisB-emrD           | ΔtisB-emrD::cm            | Forward primer ATTACTGTTTATTATACAGTAAAC TTCTATAATATCAGTGAGCTGGAGCTGCTTC Revers primer GTAGCATGATCAGCATACACCC CGCAGCTAAAATACATAGAATATCCTCTTTAG |
| ΔistR Δ1-41          | Δ1-41 ΔistR::kan          | B. Berghoff laboratory strain collection        |
| ΔistR ΔtisB          | ΔtisB::cm                 | L. Van Melderen’s laboratory strain collection  |
| ΔtisB ∆deoR          | ΔtisB::cm ∆deoR::kan      | ΔtisB P1 (KEIO ∆deoR::kan, Suppl. ref. (1))     |

| Plasmids             | Description                                      | Source                                           |
|----------------------|--------------------------------------------------|-------------------------------------------------|
| P<sub>BAD</sub>      | Derived from pBAD-TOPO, amp<sup>R</sup>           | B. Berghoff laboratory strain collection        |
| P<sub>BAD-tisB</sub>| P<sub>BAD</sub> with ΔtisB<sup>+</sup> from +42 to +354 | B. Berghoff laboratory strain collection        |
| SOS genes’ and lac promoters carried by pUA66 | pSC101 origin, promoter region cloned upstream of gfpmut2 gene, kan<sup>R</sup> | Suppl. ref. (4) |
| P<sub>cat</sub>      | isitR-tisB promoter region cloned in pUA66       | forward primer CTCGAGACAAAAAACCCCGGCCAGAGC reverse primer GGATCCACCGTCTCTGCTGTCG |
| pMLD238colM<sup>+</sup>| colM gene under IPTG inducible promoter cloned in the pET plasmid | D. Duché laboratory strain collection |

Supplementary references

1. Baba T, Ara T, Hasegawa M, Takai Y, Okumura Y, Baba M, Datsenko KA, Tomita M, Wanner BL, Mori H. 2006. Construction of Escherichia coli K12 in frame single gene knockout mutants: the Keio collection. Mol Syst Biol 2.
2. Dapa T, Fleurier S, Bredeche M-F, Matic I. 2017. The SOS and RpoS regulons contribute to bacterial cell robustness to genotoxic stress by synergistically regulating DNA polymerase Pol II. Genetics 206:1349–1360.
3. Datsenko KA, Wanner BL. 2000. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products.
**TABLE S2** MIC of the studied strains for different antibiotics

| Strain               | MIC of TMP (µg/ml) | MIC of VAN (mg/ml) | MIC of CIP (µg/ml) | MIC of AMP (µg/ml) | MIC of GM (µg/ml) | MIC of ColM (µg/ml) |
|----------------------|--------------------|--------------------|--------------------|--------------------|-------------------|---------------------|
| WT                   | 0.5                | 0.5                | 0.01               | 2                  | 2                 | 3                   |
| dnaA(Sx)             | 1                  | ND                 | ND                 | ND                 | ND                | ND                  |
| ΔdeoC                | 0.5                | ND                 | ND                 | ND                 | ND                | ND                  |
| ΔdeoR                | 0.38               | ND                 | ND                 | ND                 | ND                | ND                  |
| lexA1 (Ind⁻)         | 0.38               | ND                 | ND                 | ND                 | ND                | ND                  |
| ΔsulA                | 0.75               | ND                 | ND                 | ND                 | ND                | ND                  |
| ΔtisB                | 0.5                | 0.5                | 0.01               | 2                  | 2                 | 2                   |
| ΔistR Δ1-41          | 0.5                | ND                 | ND                 | ND                 | ND                | ND                  |
| ΔistR ΔtisB          | 0.5                | ND                 | ND                 | ND                 | ND                | ND                  |
| ΔtisB-emrD           | 0.5                | ND                 | ND                 | ND                 | ND                | ND                  |
| ΔtisB ΔdeoR          | ND                 | ND                 | ND                 | ND                 | ND                | ND                  |

ND: not determined
**TABLE S3** Strain ID and genome accession numbers of the *Enterobacter-Escherichia* clade taxa possessing the TisB/IstR TA system presented in the Fig. S5AB.

| Taxa                        | Strain ID   | GenBank access | NCBI Reference Sequence | Assembly accession |
|-----------------------------|-------------|----------------|-------------------------|--------------------|
| *Citrobacter freundii*     | CFNIH1      | CP007557.1     | NZ_CP007557.1           | GCA_000648515.1    |
| *Citrobacter koseri*       | ATCC BAA-895| CP000822.1     | NC_009792.1             | GCA_000018045.1    |
| *Citrobacter portucalensis*| Effluent 1  | CP039327.1     | NZ_CP039327.1           | GCA_004801555.1    |
| *Citrobacter werkmani*     | BF-6        | -              | NZ_CP019986.1           | GCA_002025225.1    |
| *Enterobacter cancerogenus*| MiY-F       | CP045769.1     | NZ_CP045769.1           | GCA_009648915.1    |
| *Escherichia* clade I      | H442        | -              | -                       | -                  |
| *Escherichia* clade II     | ROAR19      | -              | -                       | -                  |
| *Escherichia* clade IV     | H605        | -              | -                       | -                  |
| *Escherichia coli* phylogroup A | 101-1  | -              | -                       | GCA_000168095.1    |
| *Escherichia coli* phylogroup F | DAEC14 | -              | -                       | -                  |
| *Escherichia fergusonii*   | B253        | -              | -                       | GCA_000190495.1    |
| *Salmonella bongori*       | NCTC 12419  | FR877557.1     | NC_015761.1             | GCA_000252995.1    |
| *Salmonella enterica*      | CT18        | AL513382.1     | NC_003198.1             | GCA_000195995.1    |
| *Shigella boydii*          | ATCC 8700   | CP026731.1     | NZ_CP026731.1           | GCA_002946735.1    |
| *Shigella dysenteriae*     | WRSd3       | -              | -                       | GCA_000499065.1    |
| *Shigella flexneri*        | FDAARGOS_535| CP034060.1     | NZ_CP034060.1           | GCA_003855135.1    |