Vibrio cholerae triggers SOS and mutagenesis in response to a wide range of antibiotics: a route towards multiresistance.
Zeynep Baharoglu, Didier Mazel

To cite this version:
**Vibrio cholerae** Triggers SOS and Mutagenesis in Response to a Wide Range of Antibiotics: a Route towards Multiresistance\(^7\)\(^\dagger\)

Zeynep Baharoglu\(^1,2\) and Didier Mazel\(^1,2\)*

Institut Pasteur, Unité Plasticité du Génome Bactérien, Département Génomes et Génétique, F-75015 Paris, France,\(^1\) and CNRS, URA2171, F-75015 Paris, France\(^2\)

Received 9 November 2010/Returned for modification 3 December 2010/Accepted 27 January 2011

Antibiotic resistance development has been linked to the bacterial SOS stress response. In *Escherichia coli*, fluoroquinolones are known to induce SOS, whereas other antibiotics, such as aminoglycosides, tetracycline, and chloramphenicol, do not. Here we address whether various antibiotics induce SOS in *Vibrio cholerae*. Reporter green fluorescent protein (GFP) fusions were used to measure the response of SOS-regulated promoters to subinhibitory concentrations of antibiotics. We show that unlike the situation with *E. coli*, all these antibiotics induce SOS in *V. cholerae*.

The emergence of multiply resistant bacteria has been correlated with widespread antibiotic usage. It has been noted that sub-MICs of antibiotics induce several changes in gene expression (8). Fluoroquinolones (FQs), beta-lactams, and trimethoprim (TMP) induce the SOS stress response in *Escherichia coli* (19, 23, 25), resulting in increased mutation frequency (17, 25), whereas other antibiotics, such as aminoglycosides (AGs), chloramphenicol (CAM), rifampin (RIF), or tetracycline (TCN), do not (23). However, TCN induces mutagenesis requiring SOS-regulated DNA polymerases (7), suggesting a link between tetracycline and SOS. Interestingly, AGs, as well as FQs and mitomycin C (MMC), induce the competence regulon (*com*) in *Streptococcus* (21). *Streptococcus* does not have any homologue for the SOS repressor LexA; however, most of the DNA repair genes, including recA, belong to the *com* regulon, considered a parallel of SOS in *Streptococcus*.

A recent study demonstrated how beta-lactams, FQs, and AGs stimulate production of reactive oxygen species (ROS) in bacteria (12). ROS can damage DNA and induce mutagenesis, leading to multiple resistances. Damaged DNA is a potent SOS inducer, suggesting that all antibiotics have the potential to induce the bacterial stress response, which is a fundamental mechanism of adaptation and resistance development.

*Vibrio cholerae* is a Gram-negative human pathogen which can be deadly if not treated appropriately with antibiotics (e.g., TCN, TMP, FQs, and sometimes CAM, according to WHO recommendations). AGs are also generally used against Gram-negative bacteria, although not specifically against cholera infections. All strains of *V. cholerae* carry a chromosomal superintegron (SI), composed of an array of promoterless adaptive gene cassettes, that can be recombined and summoned when necessary through the action of the integrase IntIA (3), which is regulated by SOS (5, 11).

The *V. cholerae* response to sub-MICs of antibiotics has not been thoroughly studied. In this study we addressed whether antibiotics that do not induce SOS in *E. coli* might act differently in *V. cholerae*.

**Results and discussion.** We constructed *V. cholerae* and *E. coli* reporter strains carrying the green fluorescent protein gene (*gfp*) fused to the *recN* promoter. RecN expression is upregulated during SOS induction in both bacteria (1). Figure 1 shows the percentage of GFP-induced cells after treatment with sub-MICs of specified antibiotics. Sub-MICs were determined as concentrations 100-fold lower than the MIC for each bacterium (Fig. 1). Bacteria were cultured overnight with specified antibiotics on Mueller-Hinton (MH) medium, and the percentage of fluorescence-induced cells was determined by flow cytometry as described previously (1).

Our data confirm that FQs and TMP are strong SOS inducers in *E. coli* (Fig. 1, black bars). The isogenic *lexA* strain, which is not inducible for SOS (due to an unclavable LexA repressor) corroborated that the increase in GFP expression was indeed due to SOS induction (Fig. 1, gray bars). Conversely, AGs, TCN, and CAM do not induce SOS in *E. coli*. Supporting this fact, abrogation of SOS (*lexA* strain) does not impact fluorescence values obtained with TCN and AGs. In order to examine how these antibiotics act on *V. cholerae* SOS, we used the quorum-sensing-proficient N16961 *hapR* strain (4, 16), because the overall SOS level (even without antibiotics) was three to five times higher (and thus easier to detect) in the *hapR* context than in the *hapR*-deficient mutant (Fig. 1, red versus pink bars for MH medium; see also Fig. S1 in the supplemental material). Besides, many other natural strains of *V. cholerae*, such as the classical O1 strain responsible for the 6th pandemic (O395), the O1 strain El Tor MJ1236, and strains A1552, O37, HK1, CA401, and SG21, are *hapR*+. As expected, ciprofloxacin (CIP) (an FQ) and TMP strongly induced SOS.

\*Corresponding author. Mailing address: Institut Pasteur, Unité Plasticité du Génome Bactérien, Département Génomes et Génétique, 25 rue du Dr. Roux, F-75015 Paris, France. Phone: 33 1 40 61 32 84. Fax: 33 1 45 68 88 34. E-mail: mazel@pasteur.fr.

\†Supplemental material for this article may be found at http://aac.asm.org/.

\‡Published ahead of print on 7 February 2011.
The mutation frequency in TMP), ampicillin (AMP), TCN, and CAM strongly increased as other pathways exist (2, 20). Note here that mutagenesis may not exclusively be due to SOS, which was undetectable by flow cytometry. It is important to frequency, suggesting a low effect of these antibiotics on SOS, RIF, and spectinomycin (SPC) also slightly induced mutation frequencies in wild-type \( V. \) cholerae, although to a lower degree than FQs and TMP. GFP expression was dramatically reduced in the \( lexA \) inducer strain MG1655 (Fig. 2A). TCN, CAM, RIF, and TCN also induced SOS in \( V. \) cholerae, whereas no effect is observed in \( E. \) coli MG1655 (Fig. 2A). Unlike \( E. \) coli, \( V. \) cholerae is thus prone to SOS response activation by a wide set of antibiotics at sub-MICs. We conclude that \( E. \) coli should not be taken as a paradigm regarding the effects of antibiotics on bacteria.

SOS induction by antibiotics has to be taken seriously, as it can result in undesired changes in the behavior of bacteria and their adaptation to hostile environments. Examples are numerous: SOS can induce the expression of the cholera toxin (22), and can trigger SI cassette rearrangements conferring resistance to FQs and TMP. GFP expression was dramatically reduced in the \( lexA \) inducer strain MG1655 (Fig. 2A). Unlike \( E. \) coli, \( V. \) cholerae is thus prone to SOS response activation by a wide set of antibiotics at sub-MICs. We conclude that \( E. \) coli should not be taken as a paradigm regarding the effects of antibiotics on bacteria.

In conclusion, activation of SOS appears to be a major threat to the efficiency of antibiotic treatments. An engineered bacteriophage that suppresses the SOS network has been reported to enhance killing by antibiotics in \( E. \) coli and to increase survival of infected mice (14). Another example in treatment failure due to SOS is the resistance to FQs mediated by translesional DNA-polymerase-dependent mutations appearing upon SOS induction (6). Consequently, the inhibition of
the SOS response in pathogens by the use of “anti-SOS” components could be promising for prevention of resistance development.

We thank David Bikard for his help with statistical analysis of the flow cytometry data.

This work was supported by the Institut Pasteur, the Centre National de la Recherche Scientifique (CNRS-URA 2171), the French National Research Agency (ANR-08-MIE-016), the EU (NoE EuroPathoGenomics, LSHB-CT-2005-512061), and the Fondation pour la Recherche Médicale (équipe FRM 2007). Z.B. is supported by a postdoctoral fellowship from the Roux Foundation and a DIM Malinf fellowship (Conseil régional d’Île-de-France).

REFERENCES