Differential Roles of Antimicrobials in the Acquisition of Drug Resistance through Activation of the SOS Response in *Acinetobacter baumannii*

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The effect of antimicrobials on SOS-mediated mutagenesis induction depends on the bacterial species and the antimicrobial group. In this work, we studied the effect of different families of antimicrobial agents used in clinical therapy against *Acinetobacter baumannii* in the induction of mutagenesis in this multiresistant Gram-negative pathogen. The data showed that ciprofloxacin and tetracycline induce SOS-mediated mutagenesis, whereas colistin and meropenem, which are extensively used in clinical therapy, do not.

Acinetobacter baumannii is one of the main infectious nosocomial agents that lead to sporadic outbreaks and multidrug-resistant strains worldwide (1). The factors contributing to high-level antimicrobial resistance include the acquisition of mutations and gain of genetic determinants (2). The SOS system mediates a transitory response to DNA damage, during which bacteria can introduce changes in their genomes, acquiring phenotypic characteristics that potentiate adaptation to changing environments (3–5).

Antimicrobials may cause genetic changes mediated by the SOS response. Previous studies showed that the UmuDAB regulon, encoding several error-prone DNA polymerase V subunits, is an important component of the SOS response and mutagenesis in *A. baumannii* (6–8). Point mutations in the bacterial genome caused by these error-prone DNA polymerases can result in the acquisition of drug resistance. For instance, in *A. baumannii*, point mutations in the genes *rpoB* and *pmrB* confer resistance to rifampin and colistin, respectively (6, 9). Activation of the SOS response and the induction of mutagenesis by subinhibitory doses of antimicrobials have been demonstrated in several bacterial species, such as *Escherichia coli*, *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus* species (10). Given the clinical importance of multidrug-resistant strains of *A. baumannii* in nosocomial infections, especially in intensive care units, the aim of this study was to evaluate the relationships between different groups of common therapeutic antimicrobials and their ability to induce both the SOS response and mutagenesis in this pathogen.

As cited above, subinhibitory concentrations of antibiotics promote genetic variation by increasing the rates of mutations through activation of the SOS system. Some of these antimicrobials, such as quinolones, trigger the SOS response as a consequence of their direct action on DNA (11). Moreover, the induction of SOS can be also caused by antimicrobials that do not directly lead to DNA damage, i.e., β -lactams; instead, induction is attributed to the generation of reactive oxygen species (12, 13). In addition, the mutagenic response to a given antimicrobial may be different depending on both the antimicrobial group and the bacterial species. For instance, in *E. coli*, quinolones and β -lactams (excluding carbapenems) are able to increase both SOS gene expression and

mutagenesis, whereas other antibiotics, such as tetracycline, do not (11, 13, 14). However, tetracycline induces SOS genes and mutagenesis in *V. cholerae*, although to a lower degree than that by quinolones (14).

A. baumannii contains multiple *umuDC* operons and unlinked *umuC* and *umuD* genes, all of which encode components of error-prone DNA polymerase V (6). In *A. baumannii* strain ATCC 17978, the induction of these components after DNA damage leads to the introduction of point mutations in the bacterial genome, including those able to generate antibiotic resistance (7, 8). Previous studies showed that the *umuD* and *umuC* genes of *A. baumannii* are induced by ciprofloxacin, which is also a strong inducer of the SOS response (6). To assess the effect of the main antimicrobial therapies used in the treatment of infections by this multidrug-resistant pathogen, and, specifically, the role of these antimicrobials in the generation of antibiotic resistance, we studied the effects of meropenem and colistin with respect to their ability to induce genes encoding components of the error-prone DNA polymerase V in *A. baumannii*, comparing these drugs with ciprofloxacin, a known SOS inducer, and with tetracycline, which induces both SOS genes and mutagenesis in *V. cholerae* but not in *E. coli* (11, 14).

In accordance with the methodology used in most studies of chemical compound-induced mutagenesis (15, 16), the effect of temporary exposure (2 h) of bacteria to the selected antibiotics (meropenem, colistin, ciprofloxacin, and tetracycline) was analyzed. It has been established that in this kind of study, the most

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FIG 1 (A) MC2h of the antimicrobials used in this study, tested on *A. baumannii* ATCC 17978. (B) Expression of the genes *A1S_0636*, *A1S_1174*, *A1S_1389*, *A1S_2008*, and *A1S_2015* (encoding components of error-prone DNA polymerase V [8]) in cultures of *A. baumannii* ATCC 17978 treated with MC2h of the indicated antimicrobial (COL, colistin; MP, meropenem; TET, tetracycline; CIP, ciprofloxacin) compared to expression in nontreated cultures. The mRNA level of each gene was determined by comparison with a standard curve generated by the amplification of an internal fragment of the *A. baumannii gyrB* gene. The error bars represent the standard deviations of the mean values from at least two independent experiments, each carried out in duplicate. A value of ≥ 2 (indicated by the black arrow) was considered induction. See Aranda et al. for the primer sequences (8).

efficient method is to obtain a high frequency of mutants with low lethality (17). For this reason, the maximal antimicrobial concentration producing no more than a 50% reduction in *A. baumannii* viability after 2 h of treatment (MC2h) was determined for each antibiotic (Fig. 1A).

The effect of the MC2h of the selected antimicrobials on the induction of *umuDC* gene expression in *A. baumannii* ATCC 17978 was analyzed. To do so, a saturated culture of the bacterium was diluted 1:20 in Luria-Bertani (LB) medium (100 ml) and incubated at 37°C until the mid-exponential phase of growth was reached (optical density at 600 nm, ~ 0.6 ; $\sim 10^8$ CFU/ml). At this time, 10-ml aliquots were removed from the culture and treated with the MC2h of the antimicrobial (with the exception of the negative control). After an additional 2 h of incubation at 37°C, the cultures were harvested for RNA extraction by centrifugation. The cells were then resuspended in Tris-EDTA (TE) buffer and treated with 50 mg/ml lysozyme for 10 min at 37°C. Total RNA was subsequently extracted using an RNeasy minikit (Qiagen). DNA contaminants were removed from the RNA by digestion with DNase Turbo (Ambion). Gene expression was determined by reverse transcription-quantitative real-time PCR (RT-qPCR) using the LightCycler RNA master SYBR green I (Roche) on a LightCycler 480 instrument (LC480) (Roche), according to the manufacturer's instructions. Specific oligonucleotides (Roche) were used to quantify the genes encoding error-prone DNA polymerase components belonging to the UmuDAB regulon of *A. baumannii*: *A1S_0636*, *A1S_1174*, *A1S_1389*, *A1S_2008*, and *A1S_2015* (8). The relative mRNA concentration obtained from the genes studied was determined according to a standard curve generated by amplifying an internal fragment of the *gyrB* gene, which does not belong to the SOS regulon (8). The expression factor was calculated as the ratio between the mRNA concentration of the genes expressed in the treated cultures versus that from the untreated culture (8). The results showed that genes encoding

components of the error-prone DNA polymerase V of *A. baumannii* were induced by either ciprofloxacin or tetracycline but not by meropenem or colistin (Fig. 1B). The *dinB* gene, which is not induced in *A. baumannii* after DNA damage (6), was used as the negative control in all antimicrobial treatments. As expected, there was no increase in the expression of this gene in response to any of the treatments (data not shown).

Rifampin resistance can be acquired through genomic point mutations, most commonly following DNA base pair substitutions in the *rpoB* gene, which encodes the β -subunit of RNA polymerase. We recently demonstrated that inactivation of any of the *umuD* genes present in the genome of *A. baumannii* ATCC 17978 significantly decreases its ability to generate DNA damage-induced mutants (7). To measure the capacity of the antimicrobials tested in this study to induce mutagenesis in *A. baumannii*, we used an established rifampin resistance assay for this bacterial species (6). Briefly, a saturated culture of *A. baumannii* ATCC 17978 was diluted 1:20 in LB and incubated under the above-described conditions. At the mid-exponential phase of growth, 10 ml of culture was supplemented (with the exception of the negative control) with the MC2h of ciprofloxacin, colistin, meropenem, or tetracycline. After 2 h of incubation, the cultures were washed twice with 0.9% (wt/vol) saline, followed by centrifugation at $13,000 \times g$. The pellets were resuspended with fresh LB broth and incubated for 24 h at 37°C with shaking at 150 rpm. Appropriate cell dilutions were plated on LB agar with and without 50 μ g/ml rifampin to assess the number of resistant mutants and the total number of CFU, respectively. The plates were incubated at 37°C for 24 to 48 h. The mutation frequency was calculated by dividing the number of resistant mutants by the total number of CFU. As shown in Fig. 2, there was a clear correlation between the increased expression of several *umuDC* genes and the increase in mutagenesis, which might be explained by the direct action of the error-prone DNA polymerase V (encoded by the *umuDC* genes) in the

FIG 2 Frequency of the occurrence of rifampin-resistant mutants in *A. baumannii* ATCC 17978 cultures treated with MC2h of the indicated drug (COL, colistin; MP, meropenem; TET, tetracycline; CIP, ciprofloxacin). C–, negative control. The error bars represent the standard deviations of the mean of the results from 10 independently tested cultures. The data were analyzed by a two-tailed one-way analysis of variance (ANOVA), followed by the Tukey test for *post hoc* multiple group comparisons. *, $P < 0.01$ compared to the non-treated *A. baumannii* strain (ATCC 17978).

generation of antibiotic resistance, including to rifampin, as we recently demonstrated (7). Specifically, mutagenesis increased significantly after the treatment of *A. baumannii* cultures with the MC2h of either ciprofloxacin or tetracycline ($P < 0.01$), whereas the induction of mutagenesis was not augmented in response to the MC2h of either meropenem or colistin (Fig. 2). In conclusion, the results reported in this work provide additional support for the use of meropenem and colistin in the treatment of *A. baumannii* infections, since neither drug triggers SOS-mediated mutagenesis in this multiresistant nosocomial pathogen.

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We declare no conflicts of interest.

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