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Antibiotic Resistance Acquired through a DNA Damage-Inducible Response in *Acinetobacter baumannii*

Matthew D. Norton, Allison J. Spilkia, Veronica G. Godoy

Department of Biology, Northeastern University, Boston, Massachusetts, USA

***Acinetobacter baumannii* is an emerging nosocomial, opportunistic pathogen that survives desiccation and quickly acquires resistance to multiple antibiotics. *Escherichia coli* gains antibiotic resistances by expressing genes involved in a global response to DNA damage. Therefore, we asked whether *A. baumannii* does the same through a yet undetermined DNA damage response akin to the *E. coli* paradigm. We found that *recA* and all of the multiple error-prone DNA polymerase V (Pol V) genes, those organized as *umuDC* operons and unlinked, are induced upon DNA damage in a RecA-mediated fashion. Consequently, we found that the frequency of rifampin-resistant (Rif^r) mutants is dramatically increased upon UV treatment, alkylation damage, and desiccation, also in a RecA-mediated manner. However, in the *recA* insertion knockout strain, in which we could measure the *recA* transcript, we found that *recA* was induced by DNA damage, while *uvrA* and one of the unlinked *umuC* genes were somewhat derepressed in the absence of DNA damage. Thus, the mechanism regulating the *A. baumannii* DNA damage response is likely different from that in *E. coli*. Notably, it appears that the number of DNA Pol V genes may directly contribute to desiccation-induced mutagenesis. Sequences of the *rpoB* gene from desiccation-induced Rif^r mutants showed a signature that was consistent with *E. coli* DNA polymerase V-generated base-pair substitutions and that matched that of sequenced *A. baumannii* clinical Rif^r isolates. These data strongly support an *A. baumannii* DNA damage-inducible response that directly contributes to antibiotic resistance acquisition, particularly in hospitals where *A. baumannii* desiccates and tenaciously survives on equipment and surfaces.**

Acinetobacter baumannii is a Gram-negative coccobacillus that has quickly become a major nosocomial pathogen in hospitals worldwide, particularly infecting critically ill and immunocompromised patients in intensive care units (1, 2). With its tenacious resistance to desiccation and disinfectants (1), it is able to live on hospital equipment, including plastics, fabrics, and dry surfaces, for long periods of time (3–7). Due to *A. baumannii*'s ability to readily gain multiple antibiotic resistances (2, 8), there is now a high incidence of multidrug-resistant strains in many hospitals, which are sometimes resistant to every antibiotic available to clinicians (9–13). Therefore, there is an increasing need to understand the underlying mechanisms that permit *A. baumannii* to readily evolve in the hospital environment. Though horizontal gene transfer and homologous recombination are important for *A. baumannii* to gain antibiotic resistance (2, 14), it is unclear how *A. baumannii* regulates, if at all, systems that govern recombination and mutagenesis.

A well-understood mechanism by which *Escherichia coli* and possibly other bacteria can become resistant to antibiotics is through the elevated expression of gene products that increase mutagenesis (15, 16). The *E. coli* SOS response, a well-characterized global transcriptional response triggered by DNA damage, replication stress, or antibiotics (17, 18), ultimately helps cells survive poor environmental conditions. The SOS response induces over 40 genes (19) involved in DNA repair (17), mutagenesis (16, 17, 20, 21), homologous recombination (22), virulence (23), and tolerance and persistence to fluoroquinolones (24).

In *E. coli*, DNA damage (17) or other effectors, such as nucleotide starvation (25), trigger DNA replication fork arrest, which in turn signals induction of the SOS gene response. RecA initiates the response by coating single-stranded DNA that accumulates at stalled replication forks, forming a nucleoprotein filament. Also known as RecA*, this filament promotes autocleavage of LexA, the global transcriptional repressor of the SOS gene network, through an endowed coprotease activity. It is LexA proteolysis which ultimately permits the expression of SOS-regulated genes (17). RecA is also necessary for homologous recombination (26) and participates in the DNA damage tolerance pathway by forming complexes with translesion synthesis (TLS) DNA polymerases DinB (or DNA polymerase IV [Pol IV] [27]) and DNA Pol V (27–29). *A. baumannii* encodes a predicted *recA* gene that when knocked out sensitizes it to DNA damage and a number of different stressors (30). Moreover, *recA* and *ddrR* (encoding a protein of unknown function) are induced upon UV irradiation in *Acinetobacter baylyi* ADP1 (31, 32), a nonpathogenic strain of *Acinetobacter*, suggesting a key role for RecA in mechanisms involved in stress survival. Nevertheless, efforts to identify a global DNA damage response in *Acinetobacter* have not been pursued. The lack of a LexA homologue in this genus has undoubtedly hindered efforts to identify such a response (33).

Damaged DNA must be either repaired or tolerated for a cell to survive. UvrA is one of the first gene products in which elevated expression can be detected upon DNA damage in the *E. coli* DNA damage response (19). This enzyme is part of the nucleotide excision repair (NER) pathway that detects DNA-distorting lesions, e.g., those produced by UV irradiation (34), and recruits the NER

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Address correspondence to Veronica G. Godoy, v.godoycarter@neu.edu.
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components to repair them. The *E. coli* DNA damage response also induces error-prone Y-family TLS DNA polymerases, Pol V and DinB, as well as B-family DNA Pol II, to perform DNA synthesis past replication-stalling lesions that have been left behind on the template DNA. These lesions stall DNA replication because they cannot be used as the template by replicative DNA polymerases. Y-family DNA polymerases have a relatively open active site compared to replicative DNA polymerases, permitting the accommodation of damaged bases. In addition, they lack an exonuclease activity, which enables other DNA polymerases to proofread DNA synthesis. Because of these features, Y-family DNA polymerases are generally more error prone on undamaged DNA than replicative, high-fidelity DNA polymerases (21, 35–38). This low-fidelity DNA synthesis increases mutagenesis and can lead to acquisition of antibiotic resistance through the modification of certain gene products (15, 16). The mutation signatures of DNA Pol V and DinB are base-pair substitutions and −1 frameshifts, respectively (27, 39, 40). Notably, sequenced clinical *A. baumannii* strains from different locations worldwide have multiple mutations that result in quinolone resistance (41–43), possibly the result of base-pair substitutions made by mutagenic Y-family DNA polymerases.

Y-family DNA polymerases are evolutionarily conserved from bacteria to humans (44). DNA Pol V ($UmuD'_2C$) is composed of the catalytic enzyme UmuC and a homodimer of the accessory protein UmuD′. UmuD′ is the product of the coprotease activity of RecA* on UmuD; it is a 24-residue amino-terminal truncation of full-length UmuD. The error-prone DNA Pol V is known to bypass UV-induced DNA lesions, and it is responsible for most UV-induced mutagenesis; because of this, *umuD* and *umuC* are highly regulated in *E. coli* to minimize the intracellular concentration of active DNA Pol V (17). *A. baumannii* is capable of UV-induced mutagenesis, and it has also been observed that it carries multiple *umuD* and *umuC* genes (45, 46). It has been assumed that these genes are responsible for the mutagenesis. However, since there are multiple *umuD* and *umuC* genes, it is not yet known whether one or all of them are expressed upon DNA damage.

Therefore, we sought to assess whether a common response to DNA damage exists in *A. baumannii* by determining whether *E. coli* canonical DNA damage genes (e.g., *recA*, *uvrA*), as well as the multiple error-prone DNA polymerase genes, are induced upon DNA damage. We also investigated induced mutagenesis, an output of the DNA damage response, and assessed the impact of having multiple *umuD* and *umuC* genes. In this report, we present evidence that supports the existence of an *A. baumannii* inducible DNA damage response in which RecA plays a major regulatory role. We demonstrate that this response increases mutagenesis and is one of the mechanisms used by *A. baumannii* to acquire antibiotic resistances under clinically relevant DNA-damaging conditions.

MATERIALS AND METHODS

Strains and growth conditions. *A. baumannii* ATCC 17978 (47) and ATCC 19606 (48) were purchased from the American Type Culture Collection (ATCC). The isogenic *A. baumannii* ATCC 17978 *recA*-deficient mutant (*recA*::Km) was the generous gift of the G. Bou lab (Universitario A Coruña, Spain). All GenBank accession numbers, including those of strains used for *in silico* analyses, are shown in Table 2. *A. baumannii* and *E. coli* cultures were routinely grown at 37°C in Luria broth (LB) or on LB agar. MICs were determined using a standard liquid broth dilution method (49). For all strains, 100 μg ml^{-1} of rifampin (Rif; Calbiochem) and 30 μg ml^{-1} of kanamycin (Km; Sigma) were used.

TABLE 1 Oligonucleotides used in this study

Oligonucleotide	Sequence (5′ to 3′)
umuDC(0636–0637)-F	GGCTGAAAATCCAGATTAC
umuDC(0636–0637)-R	CATTGCCATCATTCGAGG
umuDC(1173–1174)-F	CGTTATGTTGATGAACAATG
umuDC(1173–1174)-R	GTCAATGGCTTAAAGCAG
umuD(1389)-F	GTGAAATGGAGGCGATATGCCAAAG
umuD(1389)-R	CGTTGTTCGGATGAACCTGCTGTATC
umuC(2008)-F	GCAGATTTCAGTTAATGAGTAAGGG
umuC(2008)-R	CGTGAGACCACACATCCATC
umuC(2015)-F	CGAATTTTTGCACTCGTTGAC
umuC(2015)-R	GGTTCACCCATCTTAATTCC
dinB-F	ATGCGCAAAATCATTCATATCG
dinB-R	CTCATGGACATGGCAGAGCG
uvrA-F	TGAGCCAAAGTCATATCCGTATTCG
uvrA-R	GCCGAAAGTGATTCGACATAACG
recA-F	GCATTACAAGCCGCTTTGAGCC
recA-R	CTCAGCATCAATGAAGGCACATGTAC
16S-337F	GACTCCTACGGGAGGCAGCAG
16S-518R	GTATTACCGCGGCTGCTGG
rpoB-1441F	GAGCGTGCTGTTAAAGAGCG
rpoB-2095R	CTGCCTGACGTTGCATGT
dinB-up-F	GCGACTGAAGGCGGTGATTATA
dinB-down-R	CAGTTCCGGCTTCAGCAAGTAAGC
dinB-int-F	CTCGCTTGGACTCCTGTTGATGAAGAAGCT GTTTTAGTTCAC
dinB-int-R	AGCTGGCAATTCCGACGTCTCGAGGCTG TCAGTCCGGTTTG
dinB-nest-F	GGTTAAAAGCACGCGAACATGG
dinB-nest-R	CTACACTGGTGTCATCAGCGAG
kan-F	AGACGTCGGAATTGCCAGCT
kan-R	ATCAACAGGAGTCCAAGCGAG

Homology searches and sequence alignments. *A. baumannii* protein sequences were obtained from the NCBI protein-protein BLAST search engine (50) using *E. coli* protein sequences as query. Genomic sequences that were not annotated were hand curated accordingly. The genomic organizations of ATCC 17978 *umuDC* operons were determined by finding the predicted open reading frames (ORFs) of the genes of interest in the available genome sequence. Protein sequences were aligned using the multiple-sequence-alignment tool of CLC Main Workbench (CLC Bio). Gene locus tags for these *A. baumannii* ATCC 17978 genes are as follows: *umuD*(A1S_0636) and *umuC*(A1S_0637), *umuD*(A1S_1174) and *umuC*(A1S_1173), *umuD*(A1S_1389), *umuC*(A1S_2008), *umuC*(A1S_2015), and *dinB*(A1S_0186).

Construction of *A. baumannii* ATCC 17978 *dinB*::Km. The *dinB*::Km insertion knockout was created using a method developed by Aranda et al. (30) with some modifications. An amplicon of approximately 3,000 bp was constructed by splicing by overlap extension PCR (51). This fragment contains a kanamycin resistance gene insertion at bp 414 to 612 (resulting in a 198-bp deletion) of the *A. baumannii* ATCC 17978 *dinB* gene (see Table 1 for oligonucleotide sequences). The Km resistance gene was amplified by PCR from pUA66 (52) using the kan-F and kan-R oligonucleotides (Table 1). *dinB*-int-R and *dinB*-nest-F (Table 1) were used to amplify the 5′ end of the *dinB* gene and approximately 550 bp upstream of *dinB*. *dinB*-int-F and *dinB*-nest-R (Table 1) were used to amplify the 3′ end of the *dinB* gene and approximately 500 bp downstream of *dinB*. Finally, using *dinB*-nest-F and *dinB*-nest-R (Table 1), the three pieces were joined together by PCR. All PCRs were carried out using Go*Taq* 2× master mix (Promega). This 3,000-bp product was ligated into the pGEM-T Easy vector (Promega) using T4 DNA ligase (Promega), and

the resulting *dinB*::Km plasmid was introduced into *A. baumannii* ATCC 17978 cells by electroporation at 1.8 mV for 5 ms following standard *E. coli* protocols (53). *A. baumannii dinB*::Km colonies were confirmed by sequencing (Tufts Core Facility) using chromosomal flanking oligonucleotides *dinB*-up-F with *dinB*-down-R, *dinB*-up-F with kan-R, and *dinB*-down-R with kan-F (Table 1). Kanamycin was used at 35 μg ml^{-1} for selection in *A. baumannii* and plasmid maintenance in *E. coli*.

UV, MMS, and ciprofloxacin treatment. Saturated cultures of *A. baumannii* ATCC 17978 (~10^9 cells; parental) and *A. baumannii* ATCC 17978 *dinB*::Km were diluted 1:1,000 in LB and grown for 2.5 h. They were then subcultured for 2 h three consecutive times by diluting cultures 1:50 each time to ensure that cells were in exponential phase. *A. baumannii* ATCC 17978 *recA*::Km cultures were grown similarly, with the exception that the final growth cycle was 4 h. For UV treatment, 10-ml saturated cultures were spun down and resuspended in an equal volume of SMO (100 mM NaCl, 20 mM Tris-HCl, pH 7.5), and 2-ml samples were evenly placed in a sterile glass petri dish. Samples were irradiated in the dark under a UV germicidal lamp with 270 J m^{-2} for the parental and *dinB*::Km strains or 5 J m^{-2} for the *recA*::Km strain, resulting in approximately 2 to 20% survival. Parallel samples of the parental strain were also irradiated with 100 J m^{-2}.

For methyl methanesulfonate (MMS) and ciprofloxacin treatments, cultures were grown to exponential phase as described for UV treatment. MMS (25 mM, 1× MIC; Sigma) or 6 μg ciprofloxacin ml^{-1} (10× MIC; Sigma) were used to treat the parental and *dinB*::Km cultures for 1 h. In addition, parental strain cultures were treated for 2 and 3 h with ciprofloxacin. For *A. baumannii* ATCC 17978 *recA*::Km cultures, 0.8 mM MMS (1× MIC) or 1 μg ciprofloxacin ml^{-1} (10× MIC) was used. After treatment, which resulted in 10-fold killing for all strains used after 1 h, cells were spun down and washed in SMO two times.

Semiquantitative RT-PCR. UV-treated samples were incubated for 1 h prior to RNA extraction to allow gene expression. Total RNA was obtained by following the RNA Protect and RNeasy protocols (Qiagen). Absence of DNA was verified by carrying out a PCR with Go*Taq* 2× master mix (Promega) and the same oligonucleotide sets described below for reverse transcription-PCR (RT-PCR) (Table 1) at the highest concentration of total RNA used for RT-PCR (100 ng). The total RNA concentration was measured by a spectrophotometer at A_{260} (NanoDrop 2000; Thermo Scientific). Equal amounts of total RNA (100 ng) from treated and untreated samples were 10-fold serially diluted and used as the template for the SuperScript III one-step RT-PCR system with Platinum *Taq* (Life Technologies) kit. The concentrations of the serially diluted total RNA were measured within the NanoDrop spectrophotometer's limit of detection of 1 ng μl^{-1} and were determined to be within approximately 10% of the predicted concentration. PCR conditions were followed per the manufacturer's recommendations. Oligonucleotides (Table 1) were designed to be specific for amplifying either the unique junctions between *umuD* and *umuC* in the *umuDC* operons or to the unlinked *umuC*, *umuD*, *dinB*, *uvrA*(A1S_3295), *recA*(A1S_1962), and 16S rRNA (A1S_r01) open reading frames. cDNA was separated by electrophoresis in 1% agarose (SeaKem) gels. Gel images were analyzed using ImageJ (version 1.46r) software (Wayne Rasband, NIH). The software provides a measurement of the thickness and intensity of the separated electrophoresis bands. The area of each band was determined to learn the specific mRNA concentration present at each dilution from treated and untreated samples; the concentration was in turn divided by the total RNA dilution factor. Changes in relative expression were thus calculated.

Spontaneous and induced mutagenesis. For all mutagenesis assays, bacterial cultures were started with ≤100 cells to reduce the probability of preexisting mutants in the starting inoculum. For UV-induced mutagenesis, samples were treated as described for UV treatment (270 J m^{-2} for the parental strain), with the exception that cultures were grown one time at a 1:50 dilution from the starting saturated culture. After treatment, samples were immediately diluted 1:10 in LB medium-containing flasks wrapped in tin foil and grown to saturation. Then, the appropriate cell dilutions were deposited on LB plates with and without rifampin to assess, respectively, the number of rifampin-resistant (Rif^r) mutants and the total number of CFU. Colonies were counted after 24 h of incubation. Mutation frequency was calculated by dividing the number of Rif^r mutants by the total number of CFU. Spontaneous Rif^r mutants from untreated saturated cultures were determined as described above. Statistical significance was calculated using a Student *t* test.

For MMS-induced mutagenesis, cultures were grown and treated as described for MMS treatment, with the exception that cultures were grown one time at a 1:50 dilution directly from the saturated cultures. After the 1-h treatment, washed cultures were diluted 1:3 in LB medium and grown to saturation. Rif^r mutation frequency was determined as described above.

The protocol used for desiccation-induced mutagenesis is a modification of the one used by Aranda et al. (30). Samples of saturated cultures (0.5 ml) were deposited onto sterile 0.45-μm-pore-size, black-gridded 47-mm filters (Millipore) by filtration. Filters were dried inside a closed, sterile petri dish at 37°C for 24 h ($recA^+$ strains) or 6 h (*recA*::Km strains). Three- to 5-fold killing was observed for the *A. baumannii* ATCC 17978, *A. baumannii* ATCC 17978 *dinB*::Km, and *A. baumannii* ATCC 19606 strains, and 15-fold killing was observed for the *recA*::Km strain. In addition, an exponential-phase culture of *A. baumannii* ATCC 17978 was desiccated as described above for 24 h, which resulted in 15-fold killing.

Sequencing of Rif^r mutants. Colony PCR was performed according to the Go*Taq* 2× master mix (Promega) protocol on 32 individual desiccation-induced Rif^r mutants from 6 independent *A. baumannii* ATCC 17978 $recA^+$ experiments and also on 10 individual *dinB*::Km Rif^r mutants from 5 independent *A. baumannii* ATCC 17978 *dinB*::Km experiments. Oligonucleotides *rpoB*-1441F and *rpoB*-2095R (Table 1) amplify a 654-bp region of *rpoB* (locus A1S_0287) where Rif^r-inducing base-pair substitutions are frequently located (54). Sequencing (Tufts Core Facility) was carried out using the same oligonucleotide set. The data obtained were analyzed using CLC Main Workbench (CLC Bio).

Immunoblotting. Cells were spun down and lysed with Bugbuster reagent (Novagen) after UV treatment. The total protein concentration was determined for each sample with the Bradford reagent (Bio-Rad) following the manufacturer's protocol. Equal amounts of total protein per sample mixed 1:1 with Laemmli sample buffer (2×; Sigma) were separated by SDS-PAGE on a 4 to 12% bis-Tris gel (Life Technologies) with 1× MOPS (morpholinepropanesulfonic acid) buffer (Life Technologies). After electrophoresis, proteins were transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore), and incubation with primary and secondary antibodies was carried out according to published procedures (53). Bound antibodies were detected with Luminata Crescendo Western horseradish peroxidase substrate (Millipore), followed by autoradiography or imaging on a Typhoon 8600 analyzer (GE Healthcare) using ImageQuant (version 5.2) software (Molecular Dynamics). Gel images were analyzed using ImageJ (version 1.46r) software (Wayne Rasband, NIH; see the previous section). The relative fold change in expression was determined by dividing the obtained intensities by the intensity of the untreated sample.

Polyclonal rabbit anti-DinB antibody, the generous gift of Takehiko Nohmi (55), was affinity purified (56) and diluted 1:100. Polyclonal rabbit anti-UvrA antibody (Covance) was generated using purified UvrA protein (the generous gift of Ben Van Houten) and used at a 1:10,000 dilution. Rabbit polyclonal anti-RecA antibody (Abcam, Cambridge, MA) was used at a 1:10,000 dilution, while the mouse monoclonal anti-RpoB antibody (Abcam, Cambridge, MA) was used at a 1:5,000 dilution.

RESULTS

Most *A. baumannii* genomes encode multiple error-prone DNA polymerase genes organized either as operons or as unlinked genes. We wanted to know if *A. baumannii* regulates the error-prone translesion synthesis (TLS) DNA polymerases in response to DNA damage or environmental stress, because this would ac-

TABLE 2 Comparison of number of putative TLS DNA polymerase genes from select isolates of *A. baumannii*

A. baumannii strain	GenBank accession no.	No. of putative genes			
		umuC	*umuD*	*dinB*	*polB*
ATCC 17978	CP000521	4	3	1	0
TCDC-AB0715	CP002522	3	2	1	0
AB059	ADHB00000000	3	2	1	0
ATCC 19606	ACQB00000000	2	2	1	0
AB0057	CP001182	2	2	1	0
AB058	ADHA00000000	2	1	1	0
ABNIH3	AFTB00000000	2	1	1	0
ACICU	CP000863	2	1	1	0
AYE	CU459141	1	1	1	0
MDR-ZJ06	CP001937	1	1	1	0

count for a yet undetermined mechanism of genomic evolution and antibiotic resistance acquisition in this organism.

To gain insights into the expression, genetic context, and relevance of these predicted TLS DNA polymerase genes in *A. baumannii* ATCC 17978, we searched the sequenced genomes of 10 independent *A. baumannii* isolates (Table 2) for genes whose products show similarity with the *E. coli* TLS DNA polymerases UmuC, DinB, and DNA Pol II and the accessory protein UmuD. This was done using the standard protein-protein BLAST search engine made available by NCBI (50); genomic sequences that were not annotated were hand curated accordingly. Interestingly, we found no *polB* genes (encoding TLS DNA polymerase II) in these genomes (Table 2). As in *E. coli*, *A. baumannii* isolates have only one putative *dinB* gene. DinB homologues from *A. baumannii* share sequence similarity with *E. coli* DinB, with E values being less than or equal to 2×10^{-69}, and were found to have nearly 100% sequence conservation between *A. baumannii* isolates (see Fig. S1 in the supplemental material). Not surprisingly, we discovered that *A. baumannii* DinB is also recognized by *E. coli* polyclonal antibody (see below and Fig. 4).

Because *E. coli* DNA Pol V (composed of $UmuD'_2C$) is extensively regulated to minimize unnecessary mutagenesis (17), it is very surprising that the majority of *A. baumannii* genomes encode multiple, putative *umuC* and *umuD* homologues (Table 2). There is even one isolate, *A. baumannii* ATCC 17978, with four putative *umuC* homologues and three *umuD* homologues. We found that isolates have acquired different combinations of the number of *umuC* and *umuD* genes (Table 2) both on the chromosome and on plasmids (e.g., strain ACICU; Table 2). The total intracellular concentration of active DNA Pol V depends on the expression of these multiple *umuC* and *umuD* genes. However, even if an isolate has acquired numerous *umuC* genes, *A. baumannii* DNA Pol V activity likely depends on enough supporting *umuD* gene products (57). Because *A. baumannii* ATCC 17978 has more copies of both *umuC* and *umuD* genes, it may have the potential for more DNA damage-induced (or DNA Pol V-induced) mutagenesis than the other isolates listed (Table 2).

Conserved catalytic residues of the active site (58) were used to validate *A. baumannii* ATCC 17978 *umuC* gene products' homology to *E. coli* UmuC (see Fig. S2 in the supplemental material). Each of the putative UmuC homologues, annotated in GenBank as either RumB, DNA-directed DNA polymerase, or DNA repair protein, shares sequence similarity with *E. coli* UmuC throughout the protein sequences, with E values being less than or equal to 7×10^{-82} (see Fig. S2 in the supplemental material). Similar E values were found for all putative *A. baumannii umuC* genes listed in Table 2. UmuD protein sequences of all *A. baumannii* isolates share sequence similarity with *E. coli* UmuD, with E values being less than or equal to 7×10^{-18}, in agreement with previous reports (46, 59).

In *A. baumannii* ATCC 17978, we found that the four *umuC* genes are uniquely organized and that the organization is different from that in *E. coli*. Figure 1 diagrams the arrangements of the two *umuDC* operons, the two unlinked *umuC* operons, and the one unlinked *umuD* gene of *A. baumannii* ATCC 17978. There are interesting differences between *A. baumannii* and *E. coli* even within the *umuDC* operons: for instance, in *E. coli*, *umuD* and *umuC* genes overlap by 1 nucleotide (Fig. 1A) (17). In contrast, we found that the ORF of *umuC*(A1S_0637) overlaps the ORF of *umuD*(A1S_0636) by 20 nucleotides and the ORF of *umuC*(A1S_1173) does not overlap the *umuD*(A1S_1174) ORF at all. Instead, the *umuC*(A1S_1173) ORF starts 3 nucleotides after the stop codon of *umuD* (Fig. 1). In *E. coli*, the −1 frameshift within the ORF of the *umuDC* operon is part of the regulation of expression of the *umuD* and *umuC* gene products, resulting in significantly less translation of *umuC* than *umuD* and, thus, a low intracellular concentration of DNA Pol V molecules (17). Therefore, it is likely that these gene arrangements in *A. baumannii* would influence the synthesis of their gene products as well.

Predicted TLS DNA polymerase and other DNA damage response genes are expressed in *A. baumannii* ATCC 17978. We wanted to ascertain whether the predicted multiple *umuC* and *umuD* genes and the single *dinB* gene are expressed in *A. baumannii* ATCC 17978, since this isolate has acquired the most TLS DNA

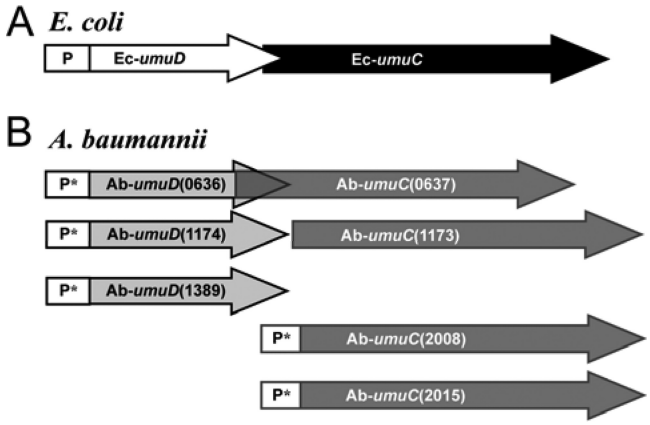

FIG 1 The *A. baumannii* ATCC 17978 predicted *umuC* and *umuD* genes are organized differently in *A. baumannii* ATCC 17978 than they are in *E. coli*. (A) There is one *umuDC* operon in the *E. coli* (Ec) chromosome in which the *umuD* ORF is expressed approximately 10-fold better than *umuC* due to a −1 frameshift between the two ORFs (17). This frameshift in the gene is depicted as overlapping arrows. (B) *A. baumannii* ATCC 17978 (Ab) has two putative *umuDC* operons in an organization similar to the one in *E. coli*, but within the *umuDC*(0636 to 0637) operon there is an overlap between the *umuD* and *umuC* genes of 20 nucleotides (depicted by overlapping arrows). In the *umuDC*(1174 to 1173) operon, we find no overlap between the two predicted genes. There are also two unlinked predicted *umuC* genes and one unlinked predicted *umuD* gene. For easier identification, locus tags ("A1S_" is not included before the numbers) are included as part of each *A. baumannii* gene name. Arrows represent predicted ORFs, and white boxes represent promoter (P) or putative promoter (P*) regions.

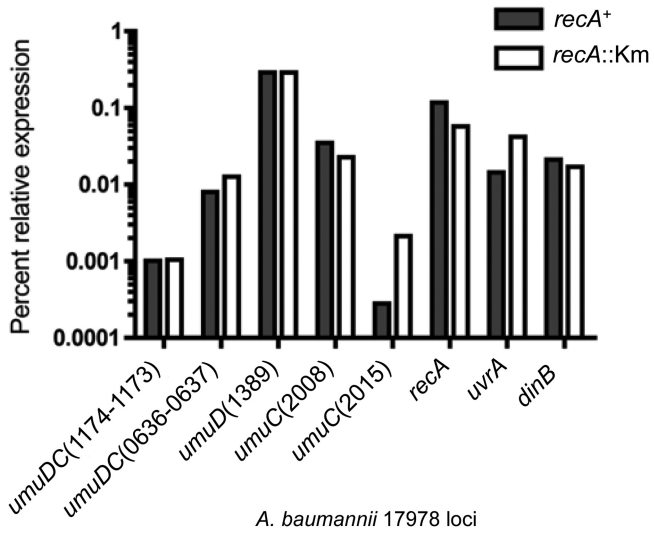

FIG 2 Representative, evolutionarily conserved DNA damage response genes are expressed in *A. baumannii* ATCC 17978. The predicted genes encoding DNA damage response genes are all expressed in the *recA*$^+$ strain, though at different levels. The relative expression of each gene is shown as a percentage of the level of expression of 16S rRNA, a standard housekeeping gene. In the *recA*::Km strain, most genes analyzed had no detectable change in relative basal-level gene expression. Some genes showed modest detectable decreases and modest to moderate increases in expression, which suggests a role for RecA in gene regulation. Semiquantitative RT-PCR was performed on total RNA purified from untreated cultures of *A. baumannii* ATCC 17978. See Materials and Methods for details of this experimental procedure. Gene-specific RT-PCR primers were used to amplify approximately 300 bp of either the unique junctions between the *umuD* and *umuC* genes organized as operons or unique sequences of the unlinked genes. Locus tags from the *A. baumannii* ATCC 17978 genome ("A1S_" is not included before the numbers) are included as part of the *umuD* and *umuC* names. Data from a representative experiment are shown.

polymerases of those sequenced (Table 2). To also examine the role of RecA, if any, in gene expression, we obtained an isogenic *A. baumannii* ATCC 17978 strain with a kanamycin resistance gene cassette inserted within *recA* (*recA*::Km [30]), rendering its gene product functionally inactive. We hypothesized that RecA would play a key role in the induction of the aforementioned genes as well as other DNA damage response genes in *A. baumannii*, despite lacking a discernible LexA. We measured mRNA transcript levels by semiquantitative RT-PCR to determine basal-level gene induction (Fig. 2). Total RNA was purified from untreated *A. baumannii* cells; then, the same amount of starting RNA template was used for subsequent RT-PCRs. The relative mRNA expression levels were thus obtained using gel electrophoresis image analysis (refer to Materials and Methods). Each gene's basal level of expression was calculated as a percentage of the level of expression of 16S rRNA, a standard housekeeping gene, in both the *recA*$^+$ and *recA*::Km strains. This analysis permits the assessment of any differences in the relative basal level of expression between the examined genes. It should be noted here that we were able to measure *recA* expression in the *recA*::Km strain because of the kanamycin resistance cassette insertion (30). The *recA* oligonucleotides are specific to the 5′ end of the gene (first 260 bp), a region that remains intact on the chromosome of the *recA*::Km strain.

We found that the *A. baumannii umuDC* operons, the unlinked *umuD* and *umuC* operons, *dinB*, *uvrA*, and *recA* are expressed because we detected their respective transcripts (Fig. 2). Notably, *umuD*(1389) and *recA* had the highest relative basal level of expression in the *recA*$^+$ strain (Fig. 2). The *umuDC*(0636 to 0637) operon, unlinked *umuC*(2008), *uvrA*, and *dinB* had the second-highest level of relative expression in the *recA*$^+$ strain. Lastly, the *umuDC*(1174 to 1173) operon and unlinked *umuC*(2015) had the lowest relative basal level of expression in the *recA*$^+$ strain, suggesting that these genes may be the most tightly regulated of those analyzed in *A. baumannii* ATCC 17978. In the *recA*::Km strain, we found a similar gene expression profile; however, one surprising difference was evident: *umuC*(2015) and *uvrA* had markedly higher relative basal levels of expression in the *recA*::Km strain than in the *recA*$^+$ strain (Fig. 2). This suggests a role for RecA in the regulation of these genes, possibly an involvement in repression.

***A. baumannii* TLS DNA polymerases are upregulated as part of a RecA-mediated DNA damage response.** *Escherichia coli* and other bacteria manage genomic instability in response to DNA damage or environmental stress by regulating a globally induced response, the SOS gene network (17, 21). The lack of an identifiable LexA homologue has made it difficult to characterize a similar damage response in *Acinetobacter* (33, 46). In the classic *E. coli* DNA damage response, the orchestrated upregulation of stress-response proteins is controlled at the level of transcription (19, 23, 60). We assessed whether we could detect changes in gene expression after treatment with three different DNA-damaging agents: MMS, ciprofloxacin, and UV. These agents are known to induce the DNA damage regulatory system in *E. coli* through various mechanisms. MMS is a cytotoxic DNA alkylating agent that produces replication fork-stalling 3-methyladenine (3-meA) lesions (61). Ciprofloxacin is an antibiotic that is a strong inducer of the SOS response in *E. coli* (24, 62); it causes replication stress because it traps the gyrase-DNA complex and blocks DNA replication, potentiating DNA double-strand breaks (63). UV irradiation is also classically used as a strong inducer of the SOS response (64) because it produces fork-stalling DNA lesions such as thymine-thymine dimers (17). Like *E. coli*, *A. baumannii* is sensitive to killing by UV, and the *recA*::Km strain is extremely sensitive, as predicted (30) (data not shown). *A. baumannii* ATCC 17978 *recA*$^+$ or *recA*::Km strains were each treated with MMS at their respective MICs, and ciprofloxacin was used at a clinically relevant concentration of 10× the MIC. To compare strains with dramatically different sensitivities to DNA-damaging agents, we used doses of drugs or UV treatments in which they had the same viability. Otherwise, cells would either die (e.g., if a UV dose typically used for a *recA*$^+$ strain was used for a *recA*::Km strain) or the treatment would not elicit a response (e.g., if a UV dose typically used for a *recA*::Km strain was used for a *recA*$^+$ strain).

We determined first whether there was induction of *A. baumannii* DNA Pol V genes upon treatment with DNA-damaging agents. In the *recA*$^+$ strain, the levels of expression for all *umuDC* operons and unlinked *umuD* and *umuC* loci increased upon all three treatments (Fig. 3A, black bars). The gene expression profiles differed for each treatment, but the *umuDC*(1174 to 1173) operon had the highest fold increase in expression in each case. We also saw drastic differences in induction between treatments. For instance, *umuC*(2015) was only modestly upregulated upon MMS treatment (~1.5-fold), but upon ciprofloxacin and UV treatment, its expression increased ~10- and ~4-fold, respectively (Fig. 3A). *umuD*(1389) gene expression was induced ~10-

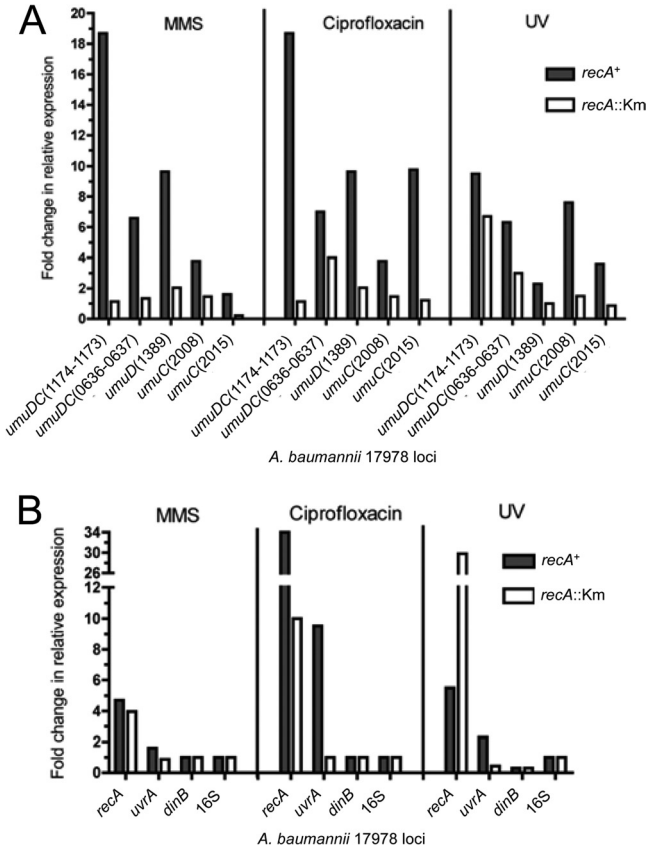

FIG 3 The predicted *A. baumannii* TLS DNA polymerases and other DNA damage response genes are induced by DNA damage and regulated by RecA. (A) Expression of putative DNA polymerase V genes. All *umuD* and *umuC* loci are upregulated upon MMS, ciprofloxacin, or UV light treatment in the *recA*$^+$ strain. In the *recA*::Km strain, most genes have no change in expression, which is denoted as a fold change of 1. We also observed increased expression for some of the genes, though it was lower than that in the *recA*$^+$ strain. (B) Expression of other DNA damage response genes. The three DNA-damaging conditions examined resulted in upregulation of *recA* and *uvrA* in the *recA*$^+$ strain. *uvrA* is regulated by RecA, as shown by its high expression in the *recA*$^+$ strain. Notably, a large increase in *recA* expression is seen in the UV-treated *recA*::Km strain. There is no increase in expression of *dinB* or the 16S rRNA control in either strain. The *recA*$^+$ and *recA*::Km strains were treated with 25 mM and 1.5 mM MMS, respectively, for 1 h; 6 μg ml^{-1} and 1 μg ml^{-1} of ciprofloxacin, respectively, for 1 h; and 270 J m^{-2} and 5 J m^{-2} of UV light, respectively. Semiquantitative RT-PCR was performed on total RNA purified from treated and untreated cultures as described in the Fig. 2 legend and Materials and Methods. Locus tags from the *A. baumannii* ATCC 17978 genome ("A1S_" is not included before the numbers) are included as part of the gene names. Data from a representative experiment are shown.

fold for MMS and ciprofloxacin treatment and only 2-fold upon UV treatment (Fig. 3A).

Conversely, it is apparent that in the *recA*::Km strain, induced levels were either greatly reduced compared to those in the *recA*$^+$ strain or not induced at all (Fig. 3A, white bars). Remarkably, we found that some genes were induced even in the absence of *recA*, as exemplified by the *umuDC*(0636 to 0637) operon during ciprofloxacin and UV treatment and the *umuDC*(1174 to 1173) operon during UV treatment (Fig. 3A).

We next examined the induction of two DNA damage response genes, *recA* and *uvrA*, and the other Y-family DNA polymerase, DinB (or DNA Pol IV). Like the DNA Pol V genes (Fig. 3A), there

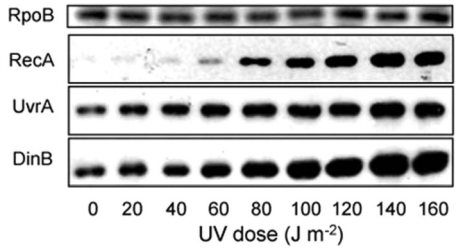

FIG 4 Intracellular concentrations of *A. baumannii* ATCC 17978 DNA damage-inducible proteins increase upon UV irradiation. At 160 J m^{-2}, there was 40-fold more RecA protein, 2.5-fold more UvrA, and 3-fold more DinB than for the untreated samples, while RpoB remained constant. *A. baumannii* cultures were grown to exponential phase, as indicated in Materials and Methods, and irradiated with increasing amounts of UV (J m^{-2}). Equal amounts of whole-cell lysates per treatment were probed with polyclonal anti-RecA, polyclonal anti-UvrA, polyclonal anti-DinB, and monoclonal anti-RpoB antibodies (refer to Materials and Methods). Antibodies used were raised against the *E. coli* proteins. A comparative experiment using the isogenic *recA*::Km strain could not be performed due to its extreme sensitivity to UV irradiation.

was induction of expression of *recA* and *uvrA* in the *recA*$^+$ strain (Fig. 3B, black bars). The induction of *recA* upon ciprofloxacin treatment was quite dramatic (34-fold; Fig. 3B), suggesting that RecA is likely an important part of this response and that ciprofloxacin is a strong inducer of the *A. baumannii* DNA damage response, as it is for *E. coli* (24, 62).

Because we were able to measure *recA* expression in the *A. baumannii recA*::Km strain, we were able to see that its expression in the *recA*::Km strain was almost equal to that in the *recA*$^+$ strain during MMS treatment (Fig. 3B). Upon ciprofloxacin treatment, *recA* was expressed at levels comparatively higher than those of other genes in the *recA*::Km strain (Fig. 3B). It was also expressed approximately one-third as much as was seen in the *recA*$^+$ strain. In contrast, upon UV treatment, *recA* was significantly induced (~30-fold) in the *recA*::Km strain compared to the *recA*$^+$ strain (~5-fold; Fig. 3B), suggesting deregulation of *recA* in the absence of RecA.

No significant changes in expression were observed for *dinB* in either the *recA*$^+$ or *recA*::Km strain, which was similar to the results for the 16S rRNA control (Fig. 3B). In a time course with ciprofloxacin or UV treatment, we found no detectable differences in the levels of induction of many genes, including *dinB*, in comparison to the results shown in Fig. 3 (*recA*$^+$ strain; data not shown).

In response to persistent DNA damage or replication stress, transcript upregulation should coincide with an increase in protein levels (17, 19). We tested for a change in abundance of three DNA damage-inducible proteins in response to UV-induced damage. We selected RecA, UvrA, and DinB, because each of these is encoded by a single gene in *A. baumannii* ATCC 17978. DinB was of particular interest since we were unable to see a detectable increase in transcript level. We predicted that antibodies raised against the *E. coli* proteins would recognize the respective *A. baumannii* homologues, given the similarity in their predicted primary sequences.

Increasing levels of all three proteins in response to increasing doses of UV irradiation were observed in *A. baumannii* ATCC 17978 (Fig. 4). The relative increase in RecA protein expression at 160 J m^{-2} compared to that under the untreated condition was 40-fold; for UvrA it was 2.5-fold, and for DinB it was 3-fold

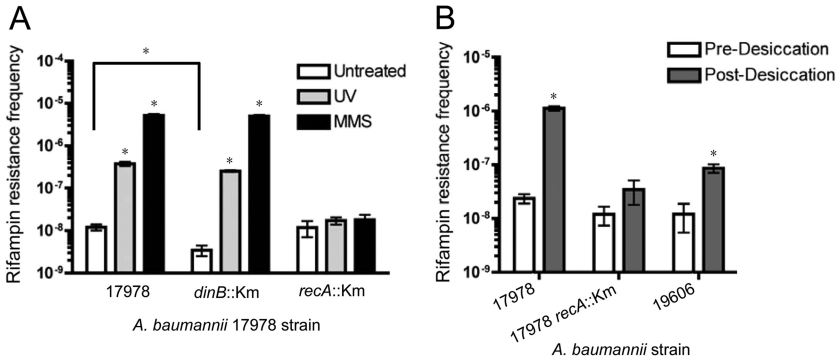

FIG 5 Mutation frequency is elevated upon treatment with DNA-damaging agents or upon desiccation in a *recA*-dependent manner. (A) The *A. baumannii* ATCC 17978 strain has a higher frequency of rifampin-resistant mutants upon both UV and MMS treatment than untreated cultures. There is no significant increase in induced mutation frequency for the isogenic *recA*::Km strain. The isogenic *dinB*::Km strain shows a modest but significant decrease (3.5-fold) in spontaneous mutants than the parental strain but has the same frequency of induced rifampin-resistant mutants as the parental strain upon both treatments. There is also no significant difference in the spontaneous mutation frequency between untreated *recA*::Km and *dinB*::Km strains. Error bars represent the standard errors of the means for at least 3 independently tested cultures, and statistical significance was determined using a Student *t* test. A statistically significant increase in mutation frequency between treated and untreated cultures ($P \leq 0.02$) is indicated (*). (B) *A. baumannii* ATCC 17978 has a dramatically increased frequency of rifampin-resistant mutants after desiccation only in a *recA*$^+$ background. The *A. baumannii* ATCC 17978 *recA*::Km strain showed no difference in predesiccation to postdesiccation rifampin-resistant mutants ($P = 0.2$). *A. baumannii* ATCC 19606, a strain containing fewer isogenic *umuD* and *umuC* genes than the *A. baumannii* ATCC 17978 strain, has an increased desiccation-induced Rif^r frequency but fewer Rif^r mutants than the *A. baumannii* ATCC 17978 *recA*$^+$ strain. A statistically significant increase in mutation frequency between postdesiccation and predesiccation cultures ($P < 0.01$) is indicated (*). *A. baumannii* ATCC 17978 and ATCC 19606 cells were desiccated for 24 h, resulting in 3- to 5-fold killing compared to that for nondesiccated cells. Cells were then rehydrated in LB medium, outgrown, and deposited on plates with rifampin (100 μg ml^{-1}). The *recA*::Km strain, treated for 6 h, was killed 15-fold compared to the level of killing for nondesiccated cells. *A. baumannii* ATCC 17978 *recA*$^+$ cultures at 15-fold killing showed no difference in mutation frequency compared to that for the cultures that resulted in 3- to 5-fold killing (not shown). Error bars represent the standard errors of the means for at least 5 independently tested cultures. Statistical significance was determined using a Student *t* test.

(Fig. 4). No change was observed in the housekeeping protein RpoB, the RNA polymerase β subunit (Fig. 4). Although the use of different antibodies precludes comparison of the amplitude of induction of the three proteins, the simultaneous increase in abundance of all three in response to DNA damage is consistent with a DNA damage regulatory program in *A. baumannii*. While a change in the expression of *dinB* at the level of transcription was undetectable (Fig. 3B), the observable increase in protein over time strongly suggests that DinB is induced upon DNA damage.

Taken together, these data provide evidence for a bona fide DNA damage-inducible response in *A. baumannii* with TLS as a key component. The induction of a host of genes, including the multiple DNA polymerase V components, was shown using a DNA alkylating agent, UV irradiation, and treatment with an antibiotic frequently used by clinicians at clinically relevant concentrations. High-level induction of these genes is dependent on RecA, but the data also suggest that the role of RecA in *A. baumannii* gene regulation is different from the *E. coli* paradigm. These results are also consistent with the hypothesis that *A. baumannii* may induce this DNA damage response as a possible mechanism for genomic evolution upon multiple stressors. We therefore sought to gain evidence for the role of the DNA damage response in *A. baumannii*-induced mutagenesis.

***A. baumannii recA*-dependent DNA damage response contributes to induced mutagenesis.** We set forth to test whether the *A. baumannii recA*-dependent DNA damage response is responsible for DNA damage-induced mutagenesis by using an established Rif^r assay (65). Rifampin is an antibiotic frequently coupled with colistin and used by clinicians to treat multidrug-resistant *A. baumannii* infections (54). Rifampin targets the β subunit of the bacterial RNA polymerase holoenzyme, RpoB. Only base-pair substitutions, i.e., not frameshifts, in the *rpoB* gene lead to select residue changes in the target site of RpoB, decreasing the effectiveness of rifampin binding (65). These base-pair substitutions can be the result of error-prone DNA polymerase, such as DNA Pol V (39). *A. baumannii* clinical Rif^r isolates have been shown to have mutations in *rpoB* (54), validating this assay for use in *A. baumannii*. Both parental strain *A. baumannii* ATCC 17978 and the *recA*::Km isogenic strains were tested for induced mutagenesis by selecting for Rif^r mutants after exposure to UV and to the alkylating agent MMS. We also constructed an *A. baumannii* ATCC 17978 *dinB*::Km insertion knockout strain to assess the impact of DinB on induced mutagenesis. TLS DNA polymerase gene products are necessary in *E. coli* for both survival and induced mutagenesis in cells that have accumulated UV- and MMS-induced DNA lesions (17, 61, 62), and we know (see previous sections) that these genes are induced by treatment with these reagents in *A. baumannii*.

As shown in Fig. 5A, in the parental *recA*$^+$ strain there was a dramatic increase (~30-fold for UV; ~400-fold for MMS) in the frequency of DNA damage-induced Rif^r mutation frequency (UV, gray bars; MMS, black bars) compared to the spontaneous Rif^r mutation frequency (white bars). No significant increase in the MMS- or UV-induced Rif^r mutation frequency was observed for the *recA*::Km strain (Fig. 5A). Interestingly, a significantly lower spontaneous mutation frequency (3.5-fold; $P < 0.01$) was found for the *dinB*::Km strain than the parental strain (Fig. 5A), and it was also not statistically significantly different from that for the *recA*::Km strain ($P > 0.05$). UV- and MMS-induced mutation frequencies for the *dinB*::Km strain were the same as those for the parental strain; however, the fold increase of induced mutation frequencies compared to spontaneous mutation frequencies was larger than that for the *dinB*$^+$ strain (70-fold for UV, 1,400-fold for MMS).

Together, these data demonstrate that rifampin resistance can

be acquired through the *recA*-dependent DNA damage response in *A. baumannii*, likely resulting from DNA base-pair substitutions in the *rpoB* gene (54). The data for the *dinB*::Km strain also suggest a role for *A. baumannii* DinB in generating spontaneous mutations and emphasize that the multiple DNA Pol V enzymes likely have a greater role in induced mutagenesis.

Desiccation-induced mutagenesis is *recA* dependent. From these data, we became intrigued by the possibility that *A. baumannii* may be able to mutate in the hospital setting as the result of environmental processes likely to produce DNA damage. It is known that *A. baumannii* is able to survive on hospital equipment for long periods of time and has considerable desiccation tolerance (3, 4). Desiccation and desiccation-rehydration cause various DNA lesions, including alkylation, oxidation, cross-linking, base removal, and strand breaks (66); and it has been reported that *A. baumannii* ATCC 17978 *recA*::Km cells are sensitive to desiccation stress (30). It is likely that *A. baumannii* cells on the surfaces of hospital equipment incur these types of desiccation-induced DNA lesions. We hypothesized that these DNA lesions would result in elevated mutagenesis when cells are rehydrated. We simulated desiccation-induced DNA damage by drying *A. baumannii* cells on filters for a period of time, resulting in standardized killing (see Materials and Methods). As expected and in agreement with previous findings (30), *recA*::Km cultures were more sensitive to drying than the parental cultures (data not shown). Cells were rehydrated and grown in rich liquid medium to assess the frequency of Rif^r mutants. As seen in Fig. 5B, the mutation frequency postdesiccation (gray bars) compared to that predesiccation (white bars; spontaneously arising mutations only) was significantly increased (~50-fold) in the *A. baumannii* ATCC 17978 *recA*$^+$ strain. No significant increase in mutation frequency was observed in the *A. baumannii* ATCC 17978 strain lacking *recA* postdesiccation ($P = 0.2$). The postdesiccation mutation frequency of *A. baumannii* ATCC 17978 *dinB*::Km matches the frequency of *A. baumannii* ATCC 17978 (data not shown), and we can again infer that there is a lessened role for *A. baumannii* DinB in induced mutagenesis and a greater role for DNA Pol V enzymes. Together, these results correlate with the results of DNA alkylation-induced mutagenesis (MMS; Fig. 5A), since it is probable that cells incur DNA alkylation lesions from desiccation (66).

Because *A. baumannii* ATCC 17978 has 4 predicted *umuC* genes and 3 predicted *umuD* genes (Fig. 1 and Table 2), we expected that a strain with fewer TLS genes would result in fewer Rif^r mutants upon desiccation rehydration. As a proof of concept, we used the strain *A. baumannii* ATCC 19606, which possesses 2 predicted *umuC* loci (HMPREF0010_03135 and HMPREF0010_00311) that are the same as those present in *A. baumannii* ATCC 17978 (A1S_1173 and A1S_2008, respectively). The 2 predicted *A. baumannii* ATCC 19606 *umuD* loci, HMPREF0010_00986 and HMPREF0010_03136, are also present in the *A. baumannii* ATCC 17978 genome as A1S_1389 and A1S_1174, respectively. Moreover, we have shown that these common loci were induced upon DNA damage (Fig. 3). We compared the frequency of Rif^r mutants after desiccation between these two strains. Like *A. baumannii* ATCC 17978, we found that there were significantly more Rif^r mutants for *A. baumannii* ATCC 19606 postdesiccation than predesiccation (~7-fold; $P < 0.01$; Fig. 5B). Remarkably, this increase is significantly less (~7-fold) than the increase observed for *A. baumannii* ATCC 17978 (Fig. 5B), even though both strains are comparably sensitive to desiccation. Therefore, these data suggest a correlation between the number of genes encoding error-prone DNA Pol V and the number of desiccation-induced Rif^r mutants.

TABLE 3 Mutation signatures of desiccation-induced *A. baumannii* ATCC 17978 Rif^r mutants

Mutation type and *rpoB* nucleotide change[d]	RpoB amino acid substitution	Mutation frequency (%) ($n = 42$)
Transversions		
1564 **C**AG → **A**AG	522 Gln → Lys[a]	7
1565 C**A**G → C**T**G	522 Gln → Leu[a]	10
1573 **G**AC → **T**AC	525 Asp → Tyr[b]	5
1574 G**A**C → G**T**C	525 Asp → Val[c]	5
1603 **C**AT → **G**AT	535 His → Asp[a]	2
1604 C**A**T → C**T**T	535 His → Leu[b]	12
1619 T**C**T → T**A**T	540 Ser → Tyr[b]	12
1741 **A**TC → **T**TC	581 Ile → Phe[a,b]	14
Total		67
Transitions		
1565 C**A**G → C**G**G	522 Gln → Arg[a,b]	12
1603 **C**AT → **T**AT	535 His → Tyr[a]	2
1619 T**C**T → T**T**T	540 Ser → Phe[a]	7
1625 C**T**T → C**C**T	542 Leu → Pro[a,b]	10
1696 **C**GT → **T**GT	566 Arg → Cys[a,c]	2
Total		33

[a] Novel *Acinetobacter* substitution.
[b] The mutation was also found in an *A. baumannii* ATCC 17978 *dinB*::Km strain.
[c] The mutation was unique to *A. baumannii* ATCC 17978 *dinB*::Km strain.
[d] Boldface and underscoring indicate the nucleotide changes.

We then tested the hypothesis that the *A. baumannii* ATCC 17978 *recA*$^+$ desiccation-induced Rif^r mutants were the result of *rpoB* base-pair substitutions. The *rpoB* gene from 32 individual colonies was sequenced, and it was found that all isolates had indeed acquired mutations in this gene (Table 3). Sequence analysis revealed single base-pair substitutions that resulted in amino acid substitutions. Our data coincide with published data for clinical Rif^r isolates containing amino acid substitutions for aspartic acid at position 525, histidine at position 535, serine at position 540, leucine at position 542, and isoleucine at position 581 (54). At these positions, we found the recognized D525Y, H535L, and S540Y substitutions (54), as well as a number of novel substitutions that are indicated in Table 3. We also found new substitutions of the glutamic acid in position 522 for lysine, leucine, or arginine.

In addition, the *rpoB* sequence from 10 *A. baumannii dinB*::Km desiccation-induced Rif^r mutants was sequenced. Many of the same mutations as those in the *dinB*$^+$ strain were found, including amino acid substitutions at positions 522, 525, 535, 540, 542, 566, and 581 (Table 3). Two mutations, D525V and R566C, were also found to be unique to the *dinB*::Km strain. The majority of mutations in the *dinB*::Km strain were transversions (7 out of 10), as were the majority of mutations in the *dinB*$^+$ strain (21 out of 32). Analysis of the total sequences of the *dinB*$^+$ and *dinB*::Km strains combined revealed the majority (67%; 28 out of 42) of base-pair substitutions to be transversions (Table 3), a signature of DNA Pol V in *E. coli*, and all but one listed substitution (A-to-G transition) are also known to be DNA Pol V generated (45).

DISCUSSION

A. baumannii is desiccation resistant, which permits long-term survival and transmission in hospital environments. It also quickly becomes multidrug resistant and has thus become a major worldwide health concern (1–4, 8). It is clear that homologous recombination, horizontal gene transfer, and plasmids play a role in antibiotic resistance acquisition (1, 2, 14), though the underlying regulatory mechanisms, if any, have remained unknown. A global response to DNA damage or harsh environmental conditions has been shown to play a key function in antibiotic and virulence acquisition in other organisms (23, 67), but it has been unclear whether such a response exists in *A. baumannii*. In this study, we present evidence for a bona fide *A. baumannii* global DNA damage-inducible response and identify this response to be one important mechanism of antibiotic resistance acquisition.

It has been unclear why *A. baumannii* isolates have acquired, most likely through horizontal gene transfer (46), multiple *umuDC* operons and unlinked *umuC* genes or *umuD* genes (Fig. 1 and Table 2). This is in stark contrast to *E. coli*, which highly regulates a single *umuDC* operon to minimize the intracellular concentration of active DNA Pol V (17). We found that these multiple DNA Pol V gene components are all expressed at different levels in *A. baumannii* ATCC 17978 (Fig. 2) and induced upon DNA damage (Fig. 3A). Different DNA-damaging agents caused distinct expression of the multiple *umuD* and *umuC* genes (Fig. 3A), consistent with an idea in which the multiple DNA Pol V enzymes may have different lesion-bypass abilities (and mutation signatures; Table 3). Thus, these possibly provide *A. baumannii* ATCC 17978 with multiple alternatives to cope with DNA damage. The unlinked *umuD*(1389) is ubiquitously present in all the *A. baumannii* genomes analyzed (Table 2). Its role in *A. baumannii* DNA damage response is likely similar to its role in *E. coli*. Indeed, this *umuD* gene product is most closely similar to the *A. baylyi umuD* gene product shown to be cleaved in *E. coli* in response to DNA damage (59), suggesting that its role in the DNA Pol V complex might be similar to that of *E. coli* UmuD′.

We found that DinB is also induced by DNA damage, based on a detectable increase in protein levels upon UV treatment (Fig. 4), but we were unable to detect increased *dinB* transcript levels (Fig. 3B) even over a time course of treatment (data not shown). We do not yet understand the reason for this discrepancy. We tested whether *A. baumannii* DinB activity is conserved, and we found that *E. coli dinB*::Km is complemented by *A. baumannii dinB* on a low-copy-number plasmid (see Fig. S3 in the supplemental material). Like *E. coli* DinB, which accurately bypasses N^2-furfuryl-dG lesions generated by nitrofurazone (68, 69), complementation with plasmid-borne *dinB* rescues cells from nitrofurazone-induced death (see Fig. S3 in the supplemental material). In contrast and to our surprise, *A. baumannii dinB* does not complement *E. coli dinB*::Km upon treatment with alkylating agents (see Fig. S3 in the supplemental material). In addition, *A. baumannii dinB*::Km cells are neither sensitive to alkylating agents (data not shown) nor more or less mutagenic upon treatment than $dinB^+$ cells (Fig. 5A). These results suggest that *A. baumannii* DinB has lesion bypass activities different from those of *E. coli* DinB and also provide more support for our hypothesis that mutagenesis and TLS are dominated by the DNA Pol V enzymes in *A. baumannii* ATCC 17978, especially considering that there is no DNA Pol II in the *A. baumannii* sequences analyzed (Table 2).

Here we provide evidence for RecA regulating the induction of *A. baumannii* DNA damage response genes (Fig. 3). RecA is essential to mount a DNA damage response in *E. coli* (17) and is necessary for *A. baumannii* to survive DNA damage and general stress (30). Interestingly, we also observed RecA-independent induction of some genes, and RecA may also have an autoregulatory role (Fig. 3B). The precise mechanistic role of RecA in the regulation of the *A. baumannii* DNA damage response remains unknown, as does the yet unidentified LexA-like transcriptional repressor. DNA damage responses vary from bacterium to bacterium (23, 32, 70, 71), so it is possible that (i) a protein unidentifiable by primary and secondary structure has evolved a similar function as LexA or (ii) there is no LexA-like repressor and the regulation in *A. baumannii* is different from that in *E. coli*. While both of these options are currently being investigated, this study suggests that the latter is the most likely. In agreement with this idea, in *A. baumannii* we found no homologue of DinI, a protein that turns off the SOS response in *E. coli* by inhibiting the LexA cleavage promoted by RecA nucleoprotein filament (21). We also failed to complement a *lexA*-deficient strain of *E. coli* with plasmids containing *A. baumannii* genes encoding LexA-like candidates, including *umuD*(1389) (A. MacGuire and V. G. Godoy, unpublished data). *umuD*(1389) may still have a regulatory role in *Acinetobacter* spp., as it does in *A. baylyi*, a notion put forth by Hare et al. (59).

A. baumannii is notorious for readily incorporating foreign DNA, such as transposons, insertion sequence elements, and antibiotic resistance-encoding islands, into its genome (1, 2, 45). Therefore, it has the ability to acquire antibiotic resistances possibly from a wide range of bacteria. In combination with error-prone DNA polymerases, both inherent and acquired through these means, *A. baumannii* could evolve new resistances when faced with environmental stress by generating base-pair substitutions in a variety of cellular targets (13, 42, 43). Our finding that *A. baumannii* mutates upon desiccation-rehydration (Fig. 5B) not only is novel but also has obvious implications in the clinical setting: improper disinfection of *A. baumannii* from surfaces could lead to desiccation-induced mutagenesis. Importantly, current methods of disinfection are lacking in their ability to kill *A. baumannii* or hinder further antibiotic resistance acquisitions. Use of UV light as a sterilizing agent in hospitals (72–75) may even promote mutagenesis (Fig. 5A) if not done properly. Incorporation of a RecA inhibitor (76, 77), for example, into new disinfectants may be a viable option in the near future as novel inhibitors continue to be discovered and patented (78, 79). This would impede the DNA damage response, suppressing both induced mutagenesis and homologous recombination in the hospital and thus limiting evolution of antibiotic resistance (80–82). Another intriguing use for a bacterial RecA inhibitor includes combining it with antibiotic treatment as a combination therapy, thereby increasing bacterial susceptibility and the therapeutic effects of the antibiotic (82).

In summary, we have uncovered a mechanism that may aid *A. baumannii* in genomic evolution and acquisition of antibiotic resistance. This global DNA damage response has hallmark features of responses that are well understood; however, it is clear that the system in place is by no means conventional. Elucidation of the more intricate details of this system will further efforts to combat this deadly opportunistic pathogen.

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