A multidrug resistance plasmid contains the molecular switch for type VI secretion in *Acinetobacter baumannii*

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Infections with *Acinetobacter baumannii*, one of the most troublesome and least studied multidrug-resistant superbugs, are increasing at alarming rates. *A. baumannii* encodes a type VI secretion system (T6SS), an antibacterial apparatus of Gram-negative bacteria used to kill competitors. Expression of the T6SS varies among different strains of *A. baumannii*, for which the regulatory mechanisms are unknown. Here, we show that several multidrug-resistant strains of *A. baumannii* harbor a large, self-transmissible resistance plasmid that carries the negative regulators for T6SS. T6SS activity is silenced in plasmid-containing, antibiotic-resistant cells, while part of the population undergoes frequent plasmid loss and activation of the T6SS. This activation results in T6SS-mediated killing of competing bacteria but renders *A. baumannii* susceptible to antibiotics. Our data show that a plasmid that has evolved to harbor antibiotic resistance genes plays a role in the differentiation of cells specialized in the elimination of competing bacteria.

antibiotic resistance | *Acinetobacter baumannii* | T6SS | bacterial secretion | plasmid

Antibiotic-resistant bacteria that cause hospital-acquired infections are a mounting concern for health care systems globally (1). Multidrug-resistant (MDR) *Acinetobacter baumannii* is emerging as a frequent cause of difficult-to-treat nosocomial infections, and some isolates are resistant to all clinically relevant antibiotics (2, 3). *A. baumannii* is often isolated from polymicrobial infections and therefore spends at least a part of its time competing with other bacteria (4). Antagonistic interactions between bacteria manifest in a variety of different ways (5), and the type VI secretion system (T6SS) is a potent weapon used by many Gram-negative bacteria to kill competitors (6–8). The multicomponent T6SS apparatus facilitates a dynamic contact-dependent injection of toxic effector proteins into prey cells (9, 10), and expression of cognate immunity proteins prevents self-inflicted intoxication (9, 11). The T6SS is composed of several conserved proteins involved in the formation of the secretory apparatus (12, 13). One of these components, hemolysin-coregulated protein (Hcp), forms hexameric tubule structures that are robustly secreted to the culture supernatants in bacteria with an active T6SS, allowing it to be used as a molecular marker for T6SS activity (6, 14).

T6SS is a dynamic apparatus (15). Its biogenesis follows energetically costly cycles of assembly/disassembly, and therefore, in most bacteria, T6SS appears to be exquisitely regulated. T6SS is silenced in most strains and only activated under specific conditions, such as an attack from another bacterium or in environments leading to membrane perturbations (16–19). Many *Acinetobacter* spp. encode the genes for a T6SS, including *Acinetobacter noscomialis* and *Acinetobacter baylyi*, which possess a constitutively active antibacterial T6SS (20–24). *A. baumannii* strains have been shown by us and others to secrete Hcp (21, 25), but to our knowledge a T6SS-dependent phenotype has not been ascribed to this species. Furthermore, our previous results showed that Hcp secretion is highly variable between *A. baumannii* strains, with some isolates carrying an inactive system (21). The precise regulatory mechanism(s) underlying T6SS suppression in some *A. baumannii* is unknown.

Here, we show that a large resistance plasmid of *A. baumannii* functions to repress the T6SS by encoding negative regulators of its activity. Analysis of colonies from a clinical isolate showed that the plasmid is readily lost in a subset of the population. This leads to the activation of the T6SS, which imparts the ability to kill other bacteria, with the simultaneous loss of antibiotic resistance. We propose that the differentiation into T6SS+ MDR– and T6SS– MDR+ phenotypes may constitute a novel survival strategy of this organism.

Results

Individual Colonies of a MDR Clinical *A. baumannii* Isolate Show an On–Off T6SS Phenotype That Correlates with a Loss of DNA and Antibiotic Resistance. To determine the regulatory mechanisms involved in *A. baumannii* T6SS, the Hcp secretion profile of an MDR clinical isolate that caused a recent outbreak (26) was assessed by an Hcp-ELISA (21). We found that individual colonies from a single patient isolate (Ab_{04}) displayed two contrasting Hcp secretion profiles (Fig. 1*A*), which were verified by Western blot (Fig. 1*B*). Colonies displaying robust Hcp secretion profiles were considered T6SS+ (Ab_{04}T6+), whereas those with no detectable Hcp secretion were considered T6SS– (Ab_{04}T6–).

Significance

Although the multidrug-resistant (MDR) bacterium *Acinetobacter baumannii* is a serious threat for health care systems worldwide, very little is known about the mechanisms that have facilitated its rise as a successful pathogen. Our work demonstrates that multiple MDR *A. baumannii* strains regulate the expression of their type VI secretion system (T6SS), an antibacterial apparatus used to kill other bacteria, by harboring a large, self-transmissible resistance plasmid containing T6SS regulatory genes. Through spontaneous plasmid loss, *A. baumannii* activates its T6SS and is able to outcompete other bacteria. However, this comes at a cost, as these strains lose resistance to antibiotics. This mechanism constitutes an apparent survival strategy by *A. baumannii* and provides insights into the pathobiology of this important pathogen.

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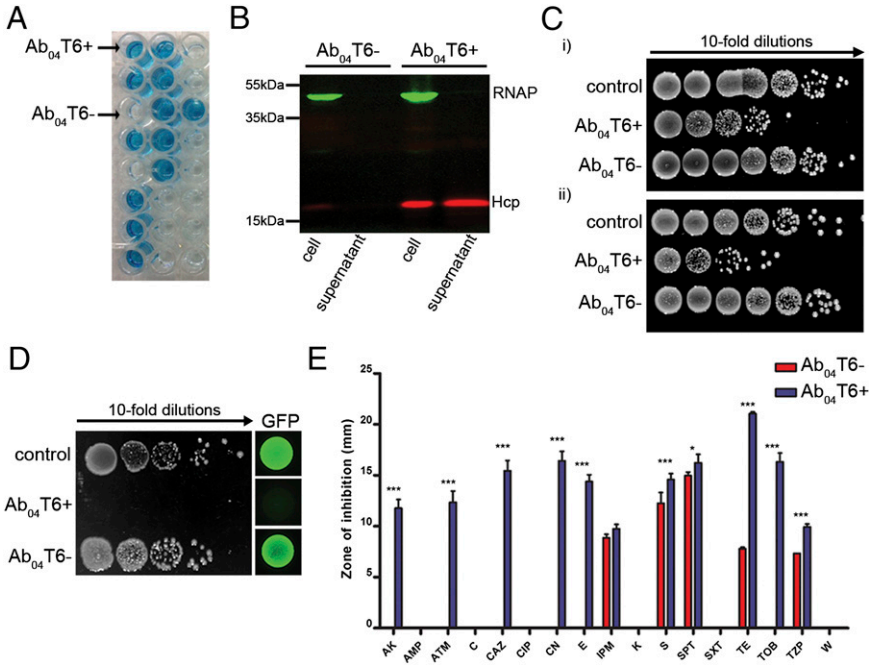

Fig. 1. Outbreak isolate *A. baumannii* Ab_{04} displays an on/off T6SS phenotype concomitant with a loss of antibiotic resistance. (*A*) Detection of Hcp secretion from individual colonies of *A. baumannii* Ab04 (Ab_{04}) by Hcp-ELISA. Ab_{04}T6+ and Ab_{04}T6– labels indicate the typical readout of colonies giving rise to robust or undetectable levels of Hcp secretion, respectively. (*B*) Hcp secretion (red) profiles of Ab_{04}T6+ and Ab_{04}T6– colonies were confirmed by Western blot on whole cells and supernatants, with RNA polymerase (RNAP; green) as the lysis control. (*C*) Recovery of surviving clinical isolates of *E. coli* (*i*) or *K. pneumoniae* (*ii*), coisolated during *A. baumannii* outbreak, after coincubation with Ab_{04}T6+, Ab_{04}T6–, or control strain. (*D*) Recovery of surviving *E. coli* MG1655R after incubation with Ab_{04}T6–, Ab_{04}T6+, or a rifampicin-sensitive *E. coli* strain (control). GFP images were produced using an *E. coli* strain constitutively expressing GFP as prey, and representative images from a single experiment are shown. (*E*) Antibiotic susceptibilities of Ab_{04}T6– and Ab_{04}T6+ as assessed by disk diffusion assay. Antibiotic abbreviations are listed in *Materials and Methods*. ***$P < 0.001$, *$P < 0.05$.

Ab_{04} caused a clonal outbreak that originated from an index patient who was also coinfected with *Escherichia coli* and *Klebsiella pneumoniae* (26). Ab_{04}T6+, but not Ab_{04}T6–, caused a considerable reduction in survival of these *E. coli* and *K. pneumoniae* coisolates in competition assays (Fig. 1*C*). The killing ability of Ab_{04}T6+ was further confirmed with common laboratory *E. coli* strains as prey (Fig. 1*D*). Note that, although bactericidal activity is not formally shown in the competition assays, we use the term "killing," as broadly used for *Acinetobacter* T6SS activity in previous studies (16, 23).

To identify the genetic difference(s) responsible for the discrepancy in T6SS phenotypes, we used Illumina sequencing to generate draft genomes for Ab_{04}T6– and Ab_{04}T6+. Analysis of the de novo assembled genomes revealed that Ab_{04}T6+ contained a noticeably smaller genome than Ab_{04}T6–, lacking a total of ∼170 kb of DNA. Some of the genes encoded by this DNA contained putative antibiotic resistance genes. We determined that Ab_{04}T6+ lost resistance to several classes of clinically important antibiotics, including β-lactams (aztreonam and ceftazidime), aminoglycosides (gentamicin, amikacin, and tobramycin), the macrolide erythromycin, and tetracycline (Fig. 1*E* and Fig. S1). These results suggested that Ab_{04}T6+ had undergone some form of DNA loss, leading to antibiotic susceptibility and T6SS activation.

DNA Loss Leading to T6SS Activation and Antibiotic Susceptibility Is Widespread in *A. baumannii*. We speculated that the DNA missing in Ab_{04}T6+ may be present in other *A. baumannii* strains. We used a combination of PCR and bioinformatic methods to identify other *A. baumannii* strains harboring this additional DNA. Two strains were identified by a positive PCR specific for the missing DNA (Fig. S2). These were the sequenced and well-characterized reference strain *A. baumannii* ATCC 17978 (Ab_{17978}), which was isolated in the early 1950s, before the introduction of many common antibiotics, and is considered a relatively drug-sensitive strain, and a recent MDR clinical isolate from Argentina, *A. baumannii* 1438 (Ab_{1438}). In agreement with the PCR results, homology searches of *A. baumannii* genome sequences revealed several other strains possessing this DNA, including Ab_{17978} (Table S1). Using the Hcp-ELISA, bacteria from Ab_{17978} and Ab_{1438} displaying an on/off phenotype for T6SS were isolated (Fig. S3). Although the T6SS locus was present in both cell types, the T6+ variants did not yield the PCR product detectable in the T6– strains (Fig. S2). In contrast to their T6– counterparts, Ab_{17978}T6+ and Ab_{1438}T6+ efficiently killed *E. coli* in competition assays (Fig. S3). This killing was dependent on a functional T6SS, as Ab_{17978}T6+ lacking essential T6SS components *hcp* or *tssM* did not kill *E. coli* (Fig. 2). Because Ab_{04}T6+ had lost antibiotic resistance, we compared the resistance profiles of Ab_{1438}T6–/T6+ and Ab_{17978}T6–/T6+. Ab_{1438}T6+ showed a significant decrease in

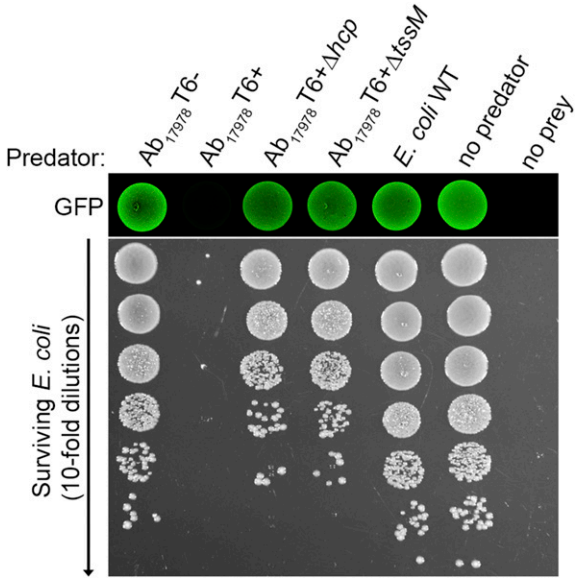

Fig. 2. Bacterial killing is dependent on a functional T6SS. Survival of GFP-expressing *E. coli*, as assessed by fluorescence and antibiotic selection, after incubation with various strains of Ab_{17978}, including the T6SS mutants Ab_{17978}Δ*hcp* and Ab_{17978}Δ*tssM*. *E. coli* WT indicates the parental strain lacking the GFP expression vector and is the no prey control. *E. coli* was selected for in serial dilutions using kanamycin.

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resistance to several antibiotics compared with Ab_{1438}T6–, and Ab_{17978}T6+ lost resistance to the combination of sulfamethoxazole/trimethoprim (S/T) compared with Ab_{17978}T6– (Figs. S1 and S3).

Although the *A. baumannii* T6SS effector–immunity pairs have not been characterized, activation of the T6SS in the T6+ strains could pose a threat to neighboring T6– sister cells. Through competition assays between T6+/T6– counterparts, we found that T6– cells were not affected by their T6+ kin (Fig. S4). This could indicate that *A. baumannii* is not capable of self-targeting or that the immunity proteins protecting from T6SS-mediated attacks are produced even under T6– conditions. Given findings in *Vibrio cholerae*, in which immunity proteins are transcribed constitutively and independently of other T6SS genes (27), we favor the latter hypothesis. This is further supported by our experiments showing that T6+ *A. baumannii* is able to kill nonkin T6– cells (Fig. S5), which implies that lack of kin-cell killing is due to immunity and not an inability to target another *A. baumannii* cell. Furthermore, this suggests that effector–immunity pairs are diverse among *A. baumannii* isolates.

Loss of a Conserved, Conjugative Resistance Plasmid Results in T6SS Activation. To fully elucidate the genetic changes underlying the observed phenotypes, we sequenced the genomes of Ab_{04}T6+, Ab_{04}T6–, Ab_{17978}T6+, and Ab_{17978}T6– using PacBio long read technology (28). Ab_{04}T6– and Ab_{04}T6+ genomes were completely closed and identical, except for the presence of a 170-kb plasmid (pAB04-1) present only in Ab_{04}T6– (Fig. 3*A*). Similarly, the only detectable difference between Ab_{17978}T6– and Ab_{17978}T6+ was the presence of a 150-kbp plasmid (pAB3) in Ab_{17978}T6– (Fig. 3*A*). Interestingly, pAB3 was assembled as part of the chromosome in the original genome sequencing of Ab_{17978} (29). This may be the result of prior genome assembly errors or possibly plasmid integration in the chromosome, although we observed no evidence of integration in our sequence data. Additionally, Illumina sequencing reads from Ab_{1438}T6–, but not from Ab_{1438}T6+, aligned onto pAB04-1 and pAB3 with considerable sequence coverage (Fig. S6).

pAB04-1 and pAB3 are highly similar over much of their sequence and seem to share a common backbone that includes a putative conjugative T4SS. pAB04-1 contains a large island with several antibiotic resistance genes that are absent in pAB3. DNA sequences corresponding to similar plasmids are present in many other recent MDR isolates of *A. baumannii* (Fig. 3*B*). Considering that pAB3 is from an "old" isolate, this suggests that pAB3 could be considered as an ancestral form of these current plasmids, which encode more resistance genes (Fig. 3 *A* and *B* and Table S1). Through conjugation experiments, we determined that pAB3 could be transferred from Ab_{17978}T6– to Ab_{17978}T6+, resulting in gain of antibiotic resistance and suppression of the T6SS in transconjugants (Fig. S7). This indicates that the T4SS contained in pAB3 is functional and that this plasmid can be disseminated among *Acinetobacter* strains.

To estimate the frequency of plasmid loss, we plated Ab_{17978} in the presence of T/S (to ensure plasmid maintenance) and then inoculated single colonies into 96-well plates lacking antibiotics. Wells exhibiting diverse Hcp secretion levels were observed (Fig. S8). About 100 clones of the bacteria contained in representative wells were plated in the presence and absence of antibiotics, and the number of antibiotic-sensitive bacteria was counted, with plasmid loss confirmed by PCR. We estimated that, in our

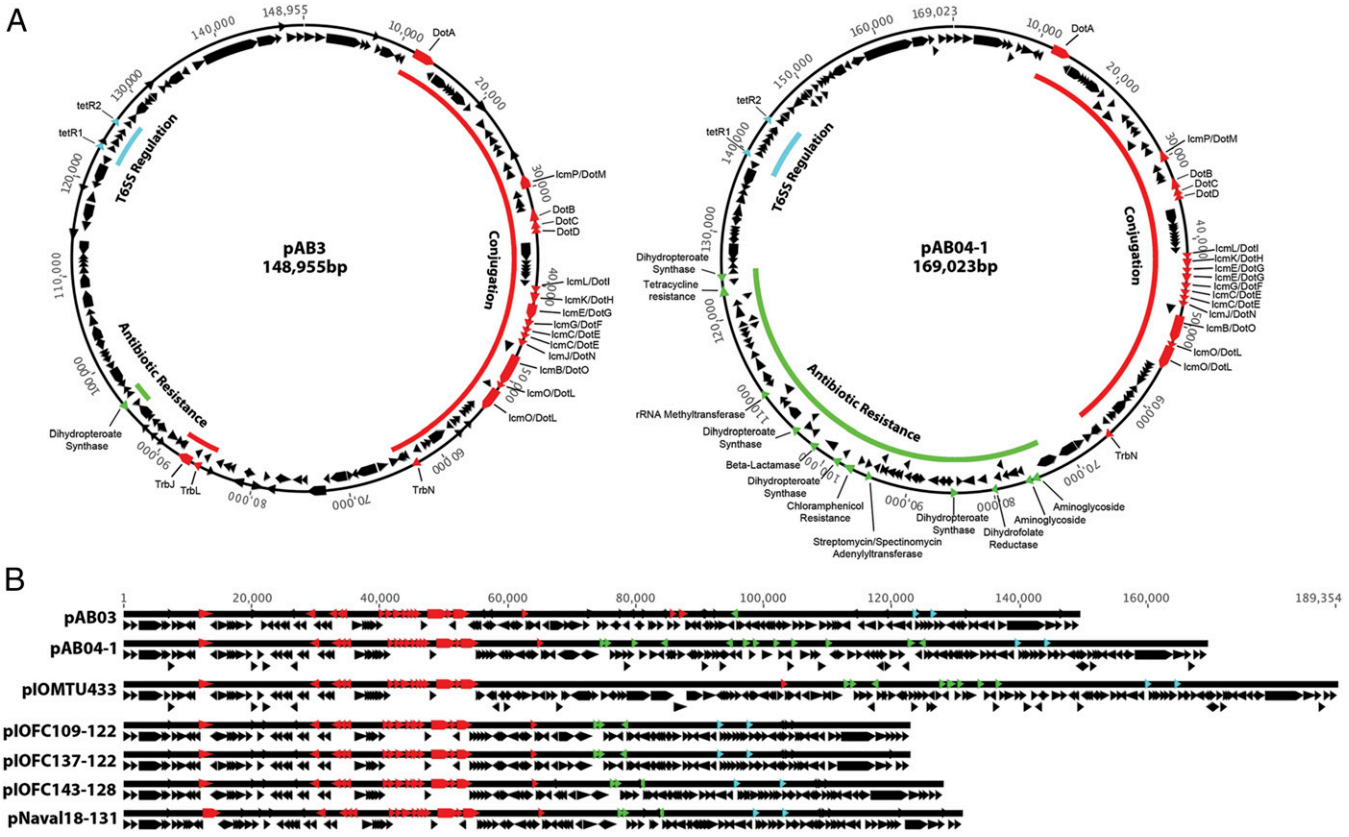

Fig. 3. *A. baumannii* plasmids share common structural features. (*A*) Assembled plasmids from PacBio sequencing of Ab_{17978}T6– (pAB3, accession no. CP012005) and Ab_{04}T6– (pAB04-1, accession no. CP012007) and other plasmids taken from GenBank (*B*), highlighting common conjugation (red), antibiotic resistance (green), and T6SS regulation (blue) loci.

experimental conditions, the percentage of cells lacking the plasmid ranges from 0% to 5% of a given population.

Two Plasmid-Encoded TetR-Like Regulators Suppress the *A. baumannii* T6SS. Because of their similar backbone, we reasoned that the plasmids possessed by T6– *A. baumannii* strains likely contained a genetic element that was responsible for T6SS repression. Each plasmid encodes several conserved predicted regulator genes, including an *hns*-like gene (locus tag ACX61_19730) and two *tetR*-like regulators, *tetR*-like1 and *tetR*-like2 (*tetR1* and *tetR2*, locus tags ACX61_19730 and ACX61_19655, respectively) (Fig. 3 *A* and *B*). H-NS proteins are a family of global regulators that bind A-T–rich regions and are usually used to silence horizontally acquired genes (30). Proteins within the H-NS family have been suggested to be involved in T6SS regulation (31, 32). TetR-like regulators play a broad role in many aspects of prokaryotic physiology (33), and members of this family have been implicated in T6SS regulation (34). Close homologs of TetR1 and TetR2 are only present in *A. baumannii* harboring pAB04-1/pAB3-like plasmids. The *hns*, *tetR1*, and *tetR2* genes were cloned and ectopically expressed in the three T6+ strains to test their effect on T6SS. We observed no change in Hcp expression or secretion in Ab_{04}T6+ carrying the *hns*-like gene (Fig. S9). In contrast, introduction of TetR1 or TetR2 dramatically decreased expression and secretion of Hcp in all T6+ strains (Fig. 4*A*). Furthermore, TetR1 and TetR2 abolished Hcp expression and secretion in *A. baumannii* ATCC 19606 and *A. baylyi* ADP1, both of which possess a constitutively active T6SS under laboratory conditions but had no effect on *V. cholerae* T6SS (Fig. S9). In killing assays, T6+ strains expressing either TetR1 or TetR2 showed an impaired ability to kill *E. coli*, consistent with a defect in Hcp secretion (Fig. 4*B*).

Discussion

In this study, we uncovered the molecular mechanisms leading to the repression of T6SS in *A. baumannii.* Our work suggests an explanation for the differences in T6SS activity observed among different strains of *A. baumannii* (21). By screening different colonies from a MDR clinical isolate, we discovered that *A. baumannii* cells can present an on/off T6SS phenotype that is controlled by the presence or absence of a resistance plasmid that carries the repressors of the secretion system. Indeed two repressors belonging to the TetR family, both individually capable of repressing the T6SS, are encoded in the plasmid. We have identified this plasmid in clinical isolates from geographically diverse locations around the world and also in the Ab_{17978}, one of the most commonly used strains in laboratories that was isolated in the 1950s. Upon plasmid loss, which occurs frequently in the absence of selection, the cells differentiate into T6+ bacteria specialized in the elimination of competing bacteria. Those cells that keep the plasmid maintain the T6SS in a silent state but retain the ability to resist antibiotics. It is conceivable that a ligand(s) for the TetR proteins exists that would relieve T6SS repression without the loss of the resistance plasmid. However, our data suggest that *A. baumannii* loses this plasmid readily without antibiotic selection, indicating plasmid loss is a true mechanism for T6SS activation. In the human host, plasmid loss has been documented in *A. baumannii* strains isolated from the same patient over time (35). Furthermore, the finding that this plasmid can be conjugated from a T6– cell to a T6+ cell raises the possibility that the plasmid can be disseminated back into those cells that have lost it. In a previous work, we reported that although Hcp secretion was detectable in *A. baumannii* 17978, T6SS was not used to kill other bacteria (21). We believe that the use of a mixed population T6SS+/T6SS– in that study masked the activity of the T6SS in this strain. It is

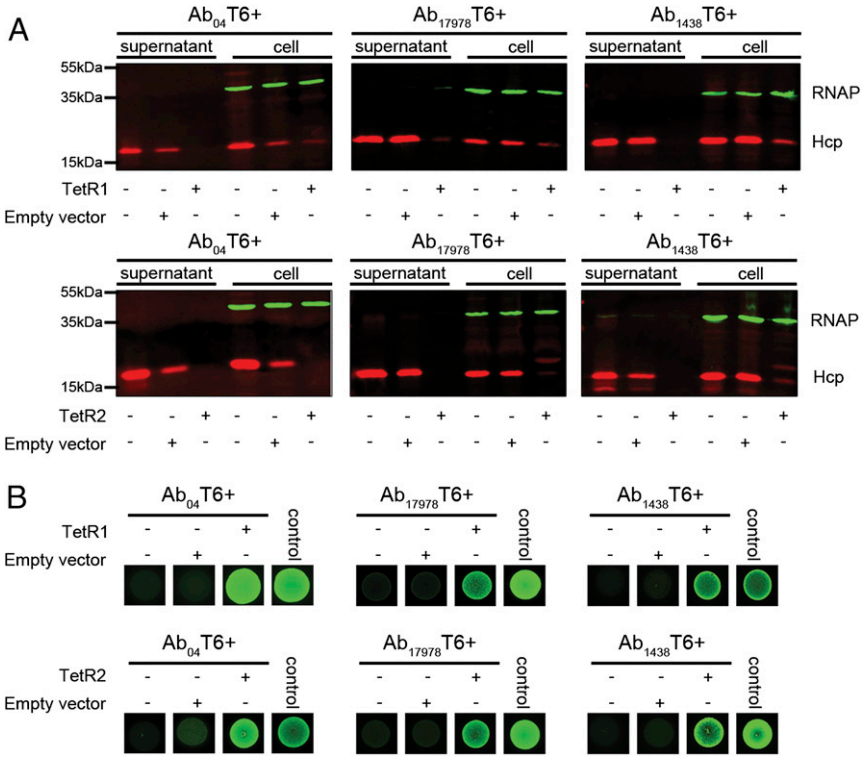

Fig. 4. Plasmid-encoded TetR1 and TetR2 repress the T6SS. (*A*) Detection of Hcp (Red) expression and secretion in Ab_{04}T6+, Ab_{17978}T6+, and Ab_{11438}T6+ expressing TetR1 (*Upper* panels) or TetR2 (*Lower* panels), with RNA polymerase (RNAP; green) as a lysis control. (*B*) Survival of GFP-expressing *E. coli*, as assessed by fluorescence, after coincubation with TetR1 (*Upper* panels) or TetR2 (*Lower* panels) expressing *A. baumannii* strains. Control spot consists of GFP-expressing *E. coli* incubated with non-GFP parental strain, and representative images from a single experiment are shown.

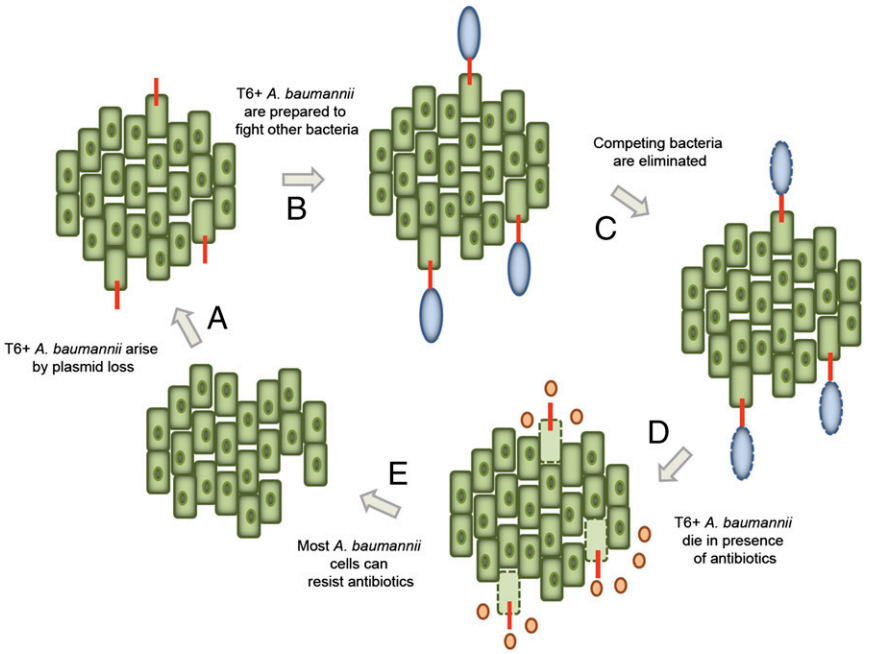

Fig. 5. A model for MDR and T6SS in *A. baumannii*. *A. baumannii* harbors a MDR plasmid that encodes repressors of T6SS. In the absence of antibiotics, this plasmid is lost in a subset of the population and results in T6SS activation (*A*). The activation of the T6SS prepares *A. baumannii* for competition (*B*) and imparts the ability to kill other bacteria that may try to enter the same environment (*C*). Upon (re)introduction of antibiotics, plasmid-less *A. baumannii* will die (*D*), and the rest of the *A. baumannii* cells will be resistant and ensure survival of the population (*E*).

conceivable that other reports using strains harboring the plasmid may also be biased by the use of mixed populations.

The observation that the TetR repressors can act on T6SS in a wide range of *A. baumannii* strains and species suggests they operate on a conserved component found across *Acinetobacters.* This may not be surprising, given the high sequence conservation of T6SS loci in these organisms (21, 22). It remains unknown whether *A. baumannii* strains with a constitutively active T6SS, like *A. baumannii* ATCC 19606 and *A. baumannii* SDF (21, 25) (which do not harbor a similar plasmid), at some point (whether during laboratory culture or before isolation) lost an analogous plasmid to those described here, leading to T6SS activation, or whether this plasmid was independently acquired by strains like Ab_{17978} and Ab_{04}, silencing their previously active T6SS. The finding that these plasmids are highly conserved and apparently only present in *A. baumannii* suggests that this method of regulation is restricted to *Acinetobacter.* Our data also show that T6– *A. baumannii* are resistant to T6SS-mediated attack from T6+ sister cells but not from nonkin T6+ *A. baumannii*. This indicates that the immunity proteins involved in preventing self-intoxication are produced even when the secretory apparatus itself is not expressed, analogous to the scenario described in *V. cholerae* (27). However, at this time the effector–immunity pairs of *A. baumannii* have not been characterized, and as such direct experimental evidence of this remains to be seen.

It has been demonstrated that antibiotic resistance-carrying plasmids can impose a fitness cost on their bacterial hosts (reviewed in ref. 36). Furthermore, as has been shown for *Salmonella typhimurium*, expression of a secretion system can be costly and reduce the competitive fitness of an organism in environments where the secretory apparatus is not beneficial (37). Our results suggest that *A. baumannii* has partitioned two phenotypes: an ability to resist being killed by antibiotics, and an ability to kill using its T6SS. For the strains and conditions tested in this study, these phenotypes are mutually exclusive. It is tempting to speculate that *A. baumannii* has evolved the strategy of carrying the T6SS repressors in a frequently lost MDR plasmid as a response to fitness defects imposed by harboring both a large resistance plasmid and a constitutively active T6SS. We suggest a model for the relationship between MDR and T6SS that allows *A. baumannii* to maintain both systems while avoiding potential deleterious effects (Fig. 5). When MDR *A. baumannii* is not under the threat of antibiotics, such as in the inanimate hospital environment or an untreated polymicrobial infection, there is an increased likelihood of encountering competitors. In this instance, repression of T6SS is relieved in a subset of the bacterial population by plasmid loss, allowing *A. baumannii* to actively attack other bacteria. Under conditions where antibiotics are present, MDR *A. baumannii* may derive enough of a survival advantage from antibiotic resistance alone that an active T6SS is neither necessary nor beneficial (35). In fact, genome sequencing of several recent MDR *A. baumannii* isolates revealed that some lack a full T6SS locus, suggesting some strains have inactivated their secretion system in favor of antibiotic resistance (35, 38, 39). The plasmid present in the old isolate Ab_{17978} strain encodes a single antibiotic resistance. The fitness cost of this does not seem to justify the use of the plasmid as a molecular switch for T6SS; however, it is possible that loss of the T6SS-repressing plasmid provides other advantage(s). The plasmids encode about 150 genes, including several other regulators such as H-NS, whose functions remain to be elucidated. It is conceivable that these control other important metabolic pathways or virulence mechanisms in *A. baumannii*. The antibiotic cassettes appear to be a later addition to the plasmid, and the fact that in only a few decades the number of antibiotic resistance cassettes has increased from 1 up to 11 demonstrates that insertion of resistance cassettes in an "easy-to-lose" plasmid containing the repressors of T6SS is a very efficient strategy to accumulate MDR. An alternative view is that encoding T6SS repressors in the plasmid prevents T6SS-mediated killing of potential recipients, facilitating plasmid propagation among different *Acinetobacter* strains. In the context of hospital environments, the encounter of *A. baumannii* with antibiotics is inevitable, and therefore plasmid loss could be regarded as an altruistic mechanism to differentiate cells specialized for elimination of competing bacteria. The interplay between T6SS and antibiotic resistance may constitute an important survival strategy for this nosocomial pathogen.

Materials and Methods

The bacterial strains used in this study are listed in Table S2. Hcp-ELISAs were performed as previously described (21). T6+ strains (Ab_{04}T6+, Ab_{17978}T6+,

Ab_{1438}T6+) were isolated by Hcp-ELISA. Bacterial killing assays were performed as described in *SI Materials and Methods*. Antibiotic resistance profiles were determined by disk diffusion. Antibiotics tested were amikacin (AK), ampicillin (AMP), aztreonam (ATM), chloramphenicol (C), ceftazidime (CAZ), ciprofloxacin (CIP), gentamicin (CN), erythromicin (E), imipenem (IMP), kanamycin (K), streptomycin (S), spectinomycin (SPT), S/T (SXT), tetracycline (TE), tobramycin (TOB), piperacillin/tazobactam (TZP), and T. The sequence data for the Ab_{17978} strain described here and its plasmid pAB3 have been deposited in the GenBank database under accession numbers CP0120004 and CP012005, respectively. The Ab_{04} strain and its associated plasmid pAB04-1 (as well as a second plasmid, pAB04-2) are available under accession numbers CP012006, CP012007, and CP012008, respectively. The DNA libraries for genomic DNA extracted from Ab_{04}T6–, Ab_{04}T6+, Ab_{17978}T6–, and Ab_{17978}T6+ (DNeasy Blood and Tissue Kit, Qiagen) were prepared following the Pacific Biosciences 20 kb Template Preparation Using BluePippin Size-Selection System protocol. We screened our *Acinetobacter* strain library (~15 isolates) using primer pairs Node_182F/Node_182R (Table S3) to identify other strains carrying plasmids similar to pAB04-1/pAB3. These primers target a conserved region on the plasmid. We bioinformatically identified other strains by BLAST, using the full pAB04-1/pAB3 plasmids as the query against both the nucleotide collection (nr) database and the whole genome shotgun (wgs) database. All primers used are listed in Table S3. For conjugation experiments, Ab_{17978}T6– was used as the donor and a modified strain of Ab_{17978}T6+ was used as the recipient. A spontaneous rifampicin-resistant mutant of Ab_{17978}T6+ was isolated, into which pBAVMCS (providing kanamycin resistance) was introduced by electroporation, generating Ab_{17978}T6+ (Rif^R; pBAVMCS). A full description of methods is available in *SI Materials and Methods*.

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