RESEARCH LETTER

Toxin–antitoxin (TA) systems are prevalent and transcribed in clinical isolates of *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus*

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Abstract

The percentage of bacterial infections refractory to standard antibiotic treatments is steadily increasing. Among the most problematic hospital and community-acquired pathogens are methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* (PA). One novel strategy proposed for treating infections of multidrug-resistant bacteria is the activation of latent toxins of toxin–antitoxin (TA) protein complexes residing within bacteria; however, the prevalence and identity of TA systems in clinical isolates of MRSA and PA has not been defined. We isolated DNA from 78 MRSA and 42 PA clinical isolates and used PCR to probe for the presence of various TA loci. Our results showed that the genes for homologs of the *mazEF* TA system in MRSA and the *relBE* and *higBA* TA systems in PA were present in 100% of the respective strains. Additionally, reverse transcriptase PCR analysis revealed that these transcripts are produced in the clinical isolates. These results indicate that TA genes are prevalent and transcribed within MRSA and PA and suggest that activation of the toxin proteins could be an effective antibacterial strategy for these pathogens.

Introduction

Initially discovered on plasmids, toxin–antitoxin (TA) systems were termed 'plasmid-addiction' modules to describe their role in plasmid maintenance through a post-segregational killing mechanism (Gerdes *et al.*, 1986; Hayes, 2003). TA systems ensure plasmid maintenance in the bacterial host population through the differential stability of the stable toxin and labile antitoxin, both encoded by the plasmid. When present, the plasmid enables the continued expression of antitoxin, which binds to and inactivates the toxin. However, if the plasmid is lost during cell division, the antitoxin protein is rapidly degraded and not replenished, thus releasing the stable toxin to kill the bacterial cell.

TA genes are also found on bacterial chromosomes, although their precise role in this setting is debated (Keren *et al.*, 2004; Buts *et al.*, 2005; Gerdes *et al.*, 2005; Engelberg-Kulka *et al.*, 2006; Szekeres *et al.*, 2007; Nariya & Inouye, 2008). Two of the most well-studied TA systems are MazEF and RelBE encoded by the *Escherichia coli* chromosome. The MazEF system in *E. coli* may function as an irreversible mediator of cell death under stressful conditions (Amitai *et al.*, 2004) or as a modulator of translation to induce a reversible state of bacteriostasis (Pedersen *et al.*, 2002; Christensen *et al.*, 2003). RelBE modulates the stringent response induced by amino acid starvation (Christensen *et al.*, 2001), causing global translation inhibition and leading to bacteriostasis (Pedersen *et al.*, 2002, 2003). Similar to plasmid-encoded systems, chromosomal TA modules derive their intrinsic killing/growth inhibition ability from a shift in the balance towards free toxin (Christensen *et al.*, 2004).

Exploitation of the inherent toxicity of TA systems has been proposed as a novel antibacterial target, as activation of the latent toxin via direct TA complex disruption or some alternative mechanism would result in bacterial cell death (Engelberg-Kulka *et al.*, 2004; DeNap & Hergenrother, 2005; Alonso *et al.*, 2007; Williams & Hergenrother, 2008). However, a prerequisite for the success of this strategy is the

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identification of clinically important bacteria that would be susceptible to a compound that activates TA systems. Surveys of clinical isolates to determine the prevalence and identity of TA systems could support and guide the development of this strategy by establishing which TA loci are most frequently encountered and would thus serve as the best target candidates.

One such survey discovered that TA systems were frequently encoded on plasmids carried by vancomycin-resistant enterococci (VRE) (Moritz & Hergenrother, 2007). The observation that TA systems are ubiquitous and functional on plasmids in VRE (Moritz & Hergenrother, 2007; Sletvold *et al.*, 2007; Halvorsen *et al.*, 2011) raises the possibility that other pathogenic bacteria may also harbor the genes for TA systems.

A bioinformatics survey of 126 prokaryotic genomes identified TA loci belonging to the seven known TA gene families in the completed genomes of three *Staphylococcus aureus* strains (Pandey & Gerdes, 2005). The genomes of all three *S. aureus* strains studied contained two loci belonging to the *relBE* gene family and one locus belonging to the *mazEF* gene family, which was later demonstrated to be a functional TA module in *S. aureus* (Fu *et al.*, 2007). The toxin, $MazF_{Sa}$, is a sequence-specific endoribonuclease that inhibits cell growth when expressed in both *E. coli* and *S. aureus* (Fu *et al.*, 2009; Zhu *et al.*, 2009). The $MazEF_{Sa}$ system is cotranscribed with the alternative transcription factor σ^B under certain stress conditions (Donegan & Cheung, 2009).

Additionally, the bioinformatics survey identified three TA loci on *Pseudomonas aeruginosa* (PA) strain PAO1, *relBE*, *parDE*, and *higBA* (Pandey & Gerdes, 2005). Although no additional work has been published on these TA systems, functional homologs have been described in other pathogenic bacteria, including RelBE in *Streptococcus pneumoniae* (Nieto *et al.*, 2006), *Yersinia pestis* (Goulard *et al.*, 2010) and *Mycobacterium tuberculosis* (Yang *et al.*, 2010); ParDE in *Vibrio cholerae* (Yuan *et al.*, 2011); and HigBA in *V. cholerae* (Christensen-Dalsgaard & Gerdes, 2006; Budde *et al.*, 2007), *Proteus vulgaris* (Hurley & Woychik, 2009), and *Y. pestis* (Goulard *et al.*, 2010).

While the analysis of sequenced genomes has been informative, there are no data on the prevalence and identity of TA loci in a large cadre of methicillin-resistant *S. aureus* (MRSA) and PA clinical isolates. In the current study, we find that *mazEF*, *relBE*, *higBA*, and *parDE* are widespread in collections of MRSA and PA clinical isolates.

Materials and methods

Bacterial strains

Clinical isolates of MRSA were obtained from three medical centers and the Network on Antimicrobial Resistance in S. *aureus* (NARSA) for a total of 78 strains. The medical centers were Carle Foundation Hospital (Urbana, IL), Memorial Medical Center (Springfield, IL), and Delnor Community Hospital (Geneva, IL). The clinical isolates of PA designated CI01–CI20 were obtained from the sputum of 20 different cystic fibrosis patients at Carle Foundation Hospital, as described previously (Musk *et al.*, 2005). The remaining 22 PA clinical isolates were a kind gift from Cubist Pharmaceuticals Inc. (Lexington, MA) and had been obtained from various acute infections over eight geographically diverse clinical sites in the United States.

Multiple-locus variable number of tandem repeats analysis (MLVA)

To assess the clonality of the clinical MRSA and PA isolates, basic molecular typing was performed by PCR-based MLVA described previously (Sabat *et al.*, 2003; Vu-Thien *et al.*, 2007). For MRSA, a minor modification was made to the reported protocol, in that a greater amount of *Taq* polymerase was added to the PCR mix (5 U) and 6 μL of PCR products were analyzed in 1.8% Low-Range Ultra agarose (Biorad) for 3 h at 6.5 V cm^{-1}. For PA, 10 of the 15 minisatellites described by Vu-Thien and colleagues were analyzed (ms142, ms211, ms 212, ms213, ms214, ms215, ms216, ms217, ms222, ms223) and 1 μL of PCR products was electrophoresed in 2.0% Molecular Biology Grade agarose (Fisher) at 10 V cm^{-1}. For MRSA or PA, respectively, TIFF or JPEG files of the MLVA gel images were visually evaluated with BIONUMERICS software (Applied Maths) and a dendrogram of banding patterns was constructed using the Dice or Pearson coefficients, respectively, and the unweighted-pair group method using average linkages.

PCR analysis

For all MRSA and PA strains, PCR amplification was performed from purified total DNA. Gene-specific internal primers were used to amplify the $mazEF_{Sa}$, $relBE_{Pa}$, $parDE_{Pa}$, and $higBA_{Pa}$ TA genes and separate intergenic primers were used to amplify the upstream and downstream flanking regions. The oligonucelotide sequences of the primers are listed in Table 1, and Fig. 1 depicts the homologous region of the primers for the PCR-based screen and the flanking region primers. PCR amplification was carried out in a DNA thermal cycler (PTC-200, MJ Research Inc.) under reaction conditions as described previously (Moritz & Hergenrother, 2007) with a lowering of the annealing temperature to 49 °C for most primers. PCR amplified products were analyzed by agarose gel electrophoresis in 1% agarose and stained with ethidium bromide.

Reverse transcriptase (RT)-PCR analysis

RT-PCR was performed using the SuperScript One-Step RT-PCR with Platinum Taq kit (Invitrogen). The primers used to amplify the *mazEF*$_{Sa}$ and *parDE*$_{Pa}$ sequences for RT-PCR are the same as those designed for PCR analysis, whereas RT-PCR for *higBA*$_{Pa}$ and *relBE*$_{Pa}$ was performed with separate specific intragenic primers designed from the *P. aeruginosa* PAO1 sequence. The sequences of all primers used in RT-PCR are listed in Table 1 and the homologous regions are depicted in Fig. 1. The extracted total RNA (up to 40 ng) was used in RT-PCR as well as PCRs with Platinum *Taq* DNA polymerase (Invitrogen) to detect DNA contamination. Reverse transcription and PCR amplification were carried out in a DNA thermal cycler (PTC-200, MJ Research Inc.) under reaction conditions as described previously (Moritz & Hergenrother, 2007), with modifications made to the PCR annealing temperature as follows: 55 °C for *mazEF*$_{Sa}$, 58 °C for *relBE*$_{Pa}$, and 50.8 °C for both *higBA*$_{Pa}$ and *parDE*$_{Pa}$. RT-PCR amplification products were analyzed by agarose gel electrophoresis in 1% agarose and stained with ethidium bromide.

Results

MLVA of clinical isolates

MRSA isolates (collected from the three medical centers and NARSA) and PA isolates (collected from Carle Foundation Hospital and from Cubist Pharmaceuticals) were analyzed by the DNA-based typing method, MLVA, to assess intraspecies relatedness. Although the MLVA for the 17 NARSA strains had been characterized previously, eight of these isolates were included in the MLVA for comparison (see Materials and methods). For PA, two standard laboratory strains (PAO1 and PA14) were included for comparison.

The experimental variation between duplicate experiments was determined for the MLVA profile using five

Table 1. Gene-specific primers used for PCR and RT-PCR analysis

TA system	Primer sequence (5′–3′)	Length (nt)	Application
mazEF$_{Sa}$	(+)ATCATCGGATAAGTACGTCAGTTT	408	PCR, RT-PCR
	(−)AGAAGGATATTCACAAATGGCTGA		
parDE$_{Pa}$	(+)GCGGCTGACCTGGATTTATC	556	PCR, RT-PCR
	(−)CCAAGCAGTAGCGGATCAATTG		
relBE$_{Pa}$	(+)CAGGGGGTAATTTCGACTCTG	505	PCR
	(−)ATGAGCACCGTAGTCTCGTTC		
higBA$_{Pa}$	(+)CTCATGTTCGATCTGCTTGC	469	PCR
	(−)CAATGCTTCATGCGGCTAC		
relBE$_{Pa}$	(+)CGCAGTACCTGGAAAGGCAGC	349	RT-PCR
	(−)GCCTTTAACCCGAAACGGG		
higBA$_{Pa}$	(+)GGCCAACATAGCATCAGGATC	305	RT-PCR
	(−)GGACGTATCAAAGTAACGCCC		
mazEF$_{Sa}$ Up	(+)GTCTTGAACACATCTTCACGCG	753	PCR, flanking
	(−)GCGAAAATACCGACACATGTAGAG		
mazEF$_{Sa}$ Down	(+)GCTTCGTTCGCTAGGGAGAG	556	PCR, flanking
	(−)CTACAAGCGGGTGAGTCTGTAA		
parD$_{Pa}$ Up	(+)CGGTGATCTTTGCCAACACTAAG	679	PCR, flanking
	(−)CTTCCGCTCAGCATATGACTC		
parE$_{Pa}$ Down	(+)TGAGTCTTCTGGGGGTGCTG	676	PCR, flanking
	(−)GGAATTCCACACCATCCGC		
relE$_{Pa}$ Up	(+)CCGGAAAAAGCGCGAGAAAGC	548	PCR, flanking
	(−)GGGGGCTGCAATGAGCCTG		
relB$_{Pa}$ Down	(+)GTGCTCATTTTCTGATCAACTTCG	646	PCR, flanking
	(−)GTGACGCTCTCCGACAGCTTC		
higA$_{Pa}$ Up	(+)GATCCGACCCCTTCCGTCTAAACG	823	PCR, flanking
	(−)GTAGCCGCATGAAGCATTG		
higB$_{Pa}$ Down	(+)CAGGTGGAGAGCGCAGGTC	712	PCR, flanking
	(−)CAATTGTCCCAACGCCTCCTTCG		
higA$_{Pa}$ Up-PA7	(+)GTTTGCCACGTTTGCATGCAG	803	PCR, flanking
	(−)CGCTCAGTTCTGGATGAATCTCC		
higB$_{Pa}$ Down-PA7	(+)GCATCGCCGATTCCAAGTG	655	PCR, flanking
	(−)GCAACGTGTGTTCGTCACC		

+, sense primer; −, antisense primer.

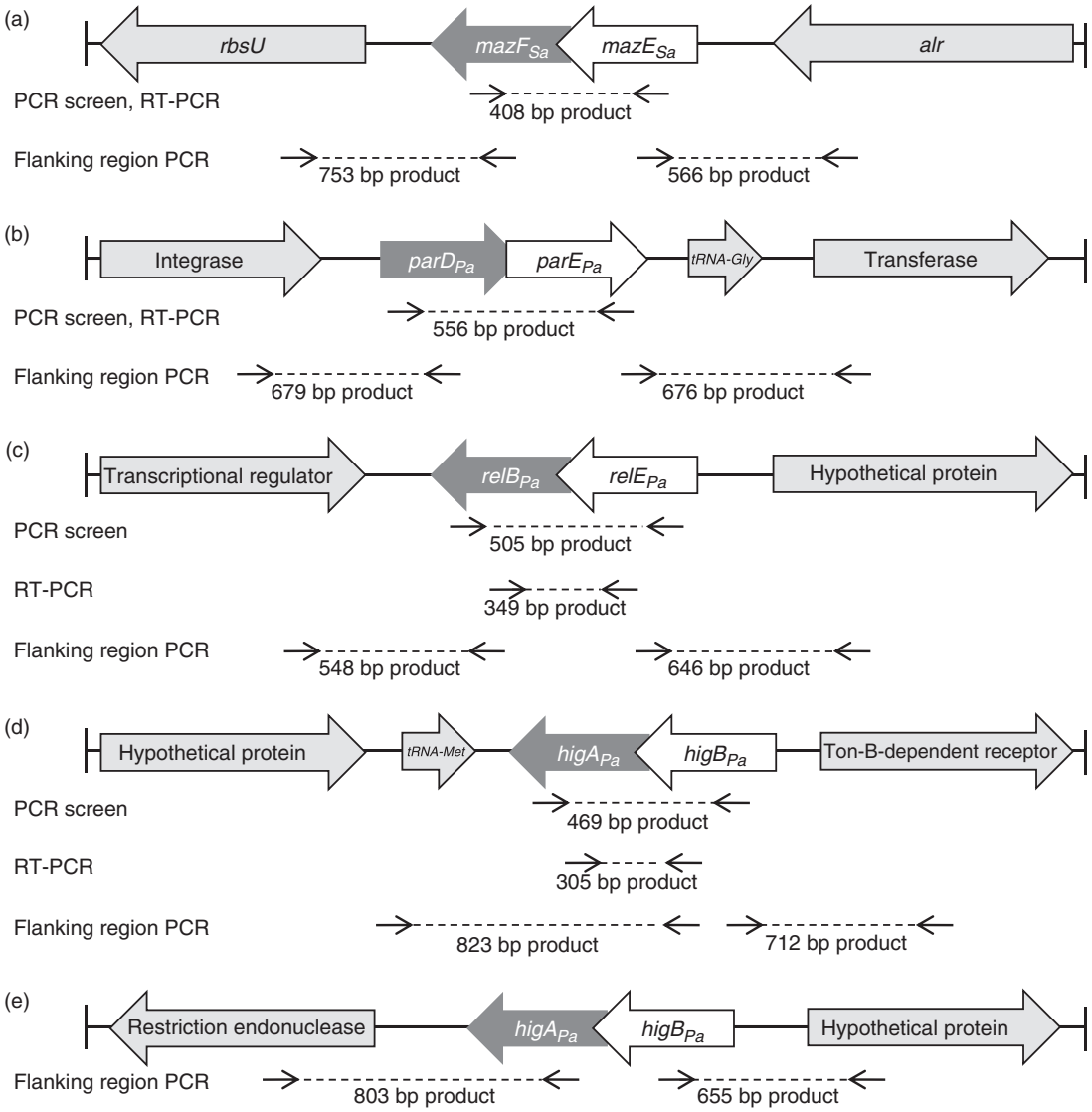

Fig. 1. Locations of primary homology for primers used in PCR screen, RT-PCR and flanking region PCR. (a) Primer sequences were based off the *Staphylococcus aureus* COL genome. The same internal primers were used to amplify a region of *mazEF*$_{Sa}$ for both the PCR-based screen and RT-PCR. Flanking *mazEF*$_{Sa}$ are the genes *rbsU* and *alr*. Primers were designed to amplify the sequences from *rbsU* to *mazF*$_{Sa}$ and from *mazE*$_{Sa}$ to *alr*. (b–d) Primer sequences were based off the *Pseudomonas aeruginosa* PAO1 genome. (b) The same internal primers were used to amplify a region of *parDE*$_{Pa}$ for both the PCR-based screen and RT-PCR. Flanking *parDE*$_{Pa}$ are genes encoding an integrase, a tRNA and a transferase. Primers were designed to amplify the sequence between the integrase gene and *parD*$_{Pa}$ and between *parE*$_{Pa}$ and the transferase gene. (c) Separate sets of internal primers were used to amplify regions of *relBE*$_{Pa}$ for the PCR-based screen and for RT-PCR. Flanking *relBE*$_{Pa}$ are genes encoding a transcriptional regulator and a hypothetical protein. Primers were designed to amplify the sequence from the transcriptional regulator gene to *relB*$_{Pa}$ and from *relE*$_{Pa}$ to the hypothetical protein gene. (d) Separate sets of internal primers were used to amplify regions of *higBA*$_{Pa}$ for the PCR-based screen and for RT-PCR. Flanking *higBA*$_{Pa}$ are genes encoding a hypothetical protein and a Ton-B dependent receptor. Primers were designed to amplify the sequence from the hypothetical protein gene to *higA*$_{Pa}$ and from *higB*$_{Pa}$ to the Ton-B dependent receptor gene. (e) In *P. aeruginosa* PA7, *higBA*$_{Pa}$ is flanked by genes encoding a restriction endonuclease and a hypothetical protein. Primers were designed to amplify the sequence from the restriction endonuclease gene to *higA*$_{Pa}$ and from *higB*$_{Pa}$ to the hypothetical protein gene.

MRSA or five PA isolates and applied to establish a cutoff value of 91% or 97%, respectively, for typing strains with identical DNA banding patterns. Using the 91% cutoff, 46 MLVA patterns were defined out of the 69 MRSA strains evaluated. Applying a 75% similarity value generated 13 clusters from 56 strains, excluding 13 strains from these clusters (Fig. 2a). Isolates belonging to the same cluster differed by up to four bands. MLVA of the clinical *S. aureus* isolates using either cutoff value revealed that the majority of isolates do not belong to the same clusters as US *S. aureus*

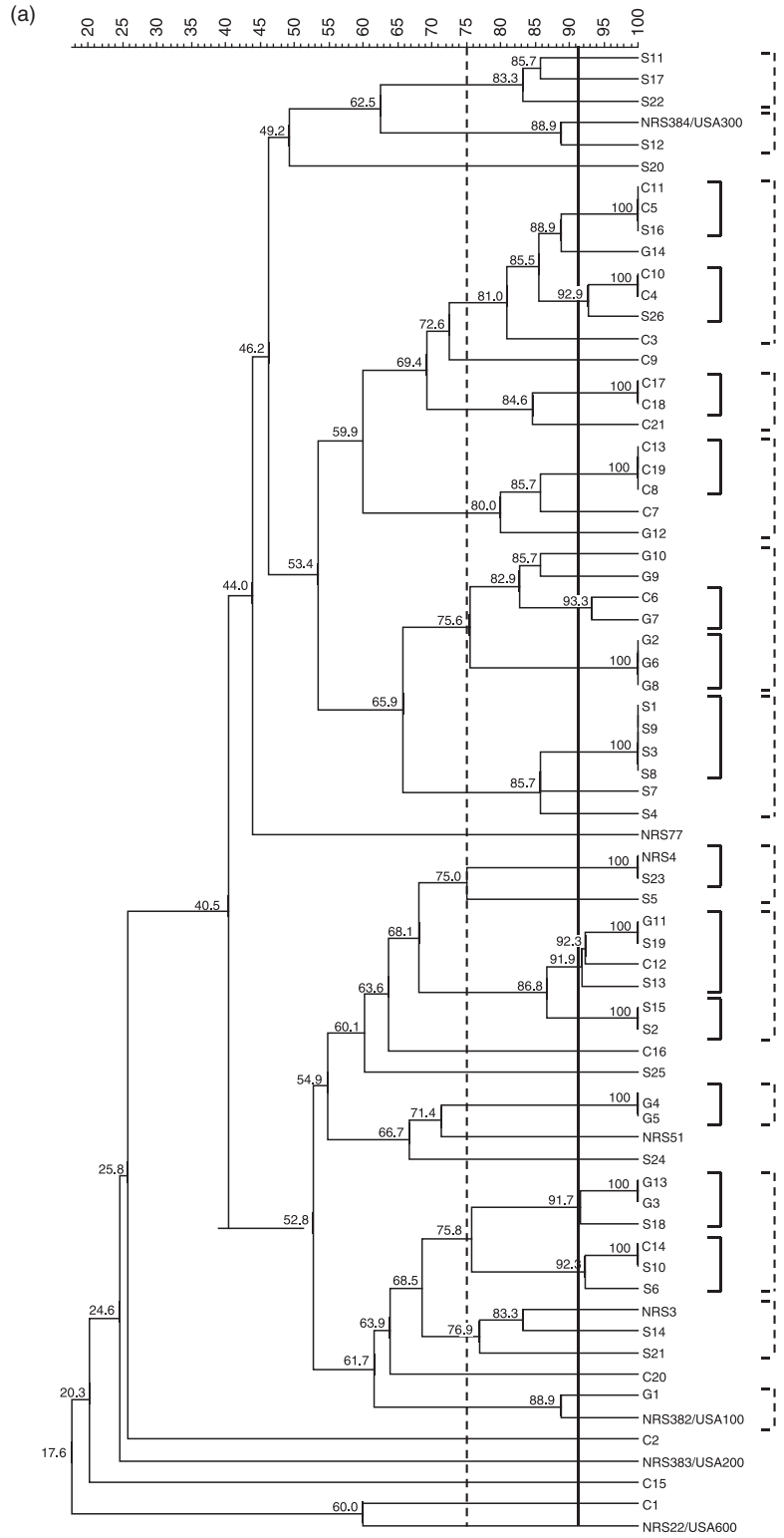

Fig. 2. MLVA of the MRSA and PA clinical isolates. The 91% or 97% clonal cutoff value and 75% similarity cutoff value are indicated by solid and dashed vertical lines, respectively, for MRSA (a) and PA (b). The clusters generated are shown in corresponding solid and dashed brackets alongside the dendrogram.

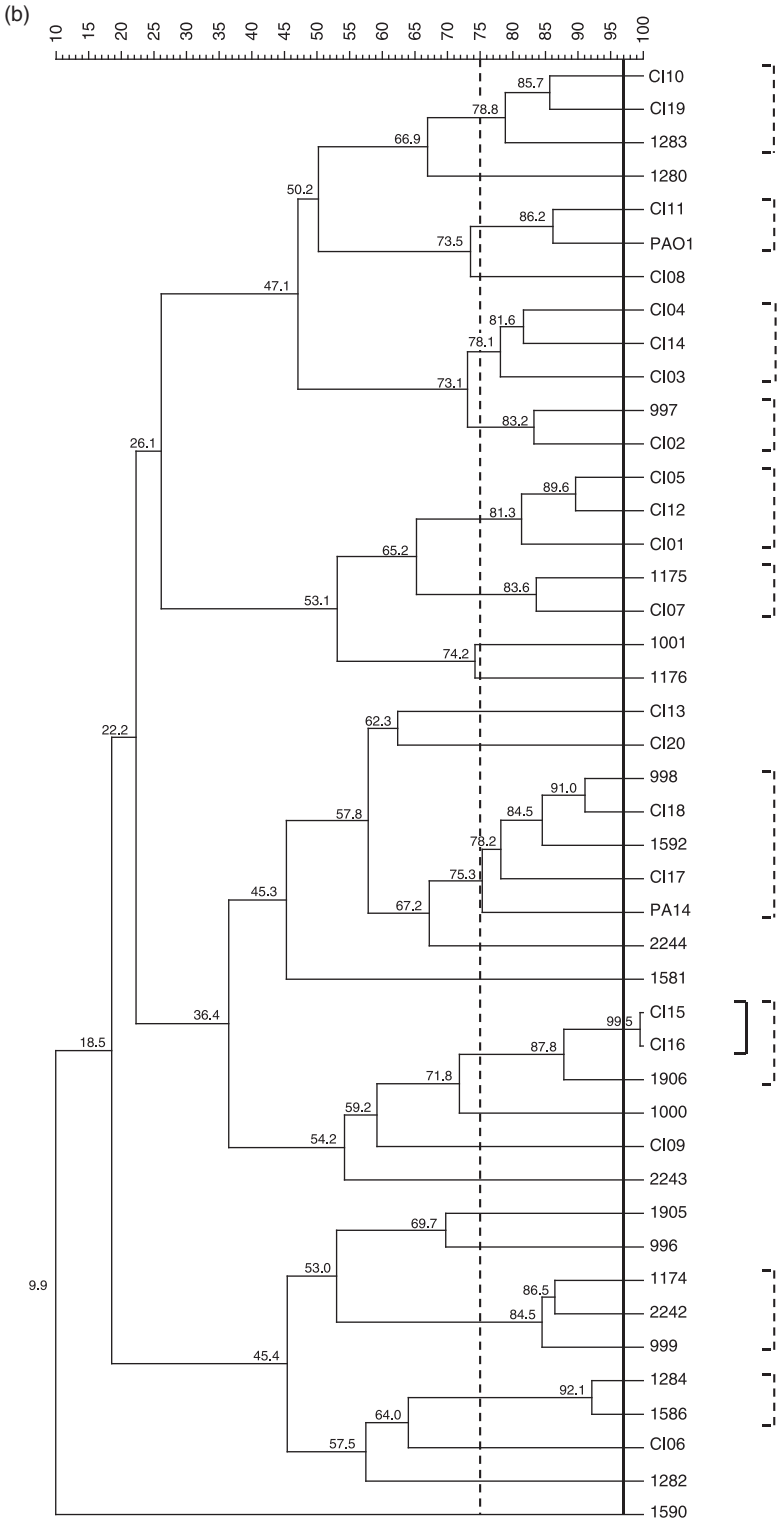

Fig. 2. (Continued)

clones (USA100, USA200, USA300, and USA600) and suggest that the isolates collected from the Illinois area are not clonal.

For PA, using the 97% cutoff value, 43 MLVA banding patterns were formed out of the 44 strains. When a cutoff value of 75% was applied, 10 clusters were generated comprising 28 strains, and 26 MLVA banding patterns were discerned (Fig. 2b). Strains that group according to these two cutoff values are in a variety of clusters, demonstrating that the isolates studied were not clonal.

Presence of TA genes

Armed with the knowledge that the collection of MRSA and PA clinical isolates were sufficiently diverse, an effort was made to define the prevalence of TA genes in the strains. For the MRSA isolates, gene-specific PCR primers were used to amplify the genes for the *mazEF* homolog (called $mazEF_{Sa}$) observed on the *S. aureus* COL genome (Pandey & Gerdes, 2005). The PA isolates were probed for homologs of the *higBA*, *parDE* and *relBE* systems identified in PA strain PAO1 (Pandey & Gerdes, 2005). The oligonucleotide sequences of all PCR primers used to amplify TA genes are listed in Table 1 and the homologous regions are represented in Fig. 1.

Total DNA preparations from each of the 78 MRSA and 42 PA strains were analyzed by PCR, and results were designated as positive if a distinct band was observed at the expected size on an agarose gel. The PCR screen revealed that the $mazEF_{Sa}$ TA system was present in all MRSA isolates (78/78, 100%). For the 42 PA isolates, $relBE_{Pa}$ (42/42, 100%) and $higBA_{Pa}$ (42/42, 100%) were ubiquitous, whereas $parDE_{Pa}$ (13/42, 30%) was less prevalent. Supporting Information Table S1 contains a complete list of all MRSA and PA isolates and the TA genes detected by PCR. Comparison of the MLVA genotypes of PA strains that carry $parDE_{Pa}$ showed that these strains are dispersed throughout the dendrogram, indicating that there is no correlation between genome relatedness and carriage of $parDE_{Pa}$.

DNA sequencing was performed on ~10% of all PCR products. For the MRSA isolates, sequenced PCR products revealed strong sequence identity (95.6–99.5%) to the reference TA system sequence ($mazEF_{Sa}$ alignments are shown in Fig. S1). For the PA isolates, sequenced PCR products also revealed strong sequence identity (97.8–100%) to the reference TA system sequences [$higBA_{Pa}$, 99.4% average identity; $parDE_{Pa}$, 99.6% average identity; and $relBE_{Pa}$, 98.7% average identity); alignments are shown in Figs S2–S4].

TA gene localization

It was next investigated whether the TA genes were located on a plasmid or the chromosome of the MRSA and PA isolates. The sequences directly upstream and downstream of the $mazEF_{Sa}$ and $relBE_{Pa}$ TA genes are highly conserved among the completed *S. aureus* and PA genomes in the National Center for Biotechnology Information (NCBI) Genome database, whereas the flanking regions of $parDE_{Pa}$ and $higBA_{Pa}$ are conserved in *P. aeruginosa* PAO1, LESB85 and UCBPP-PA14, but are different in strain PA7. Primers were designed (Table 1 and Fig. 1) to amplify the sequences flanking the TA genes based on the conserved sequence in *S. aureus* strains and in *P. aeruginosa* strains PAO1 and PA7. In this experiment the presence of a PCR product would suggest chromosomal location of the TA systems.

PCR analysis revealed that in 100% (78/78) of the MRSA isolates, the regions upstream and downstream of the $mazEF_{Sa}$ genes were amplified with the flanking region primers, suggesting a chromosomal location with sufficient homology to the *S. aureus* reference strains in the NCBI database. In the PA isolates, both flanking regions of the $parDE_{Pa}$ genes in all isolates (13/13, 100%) were amplified using primers homologous to the PAO1 reference sequence. The flanking regions of nearly all $relBE_{Pa}$ genes (41/42, 97%) were amplified, except for strain 1284, for which no flanking region could be amplified. Amplification was observed for the downstream sequence of every $higBA_{Pa}$ loci (42/42, 100%) as well as for the region upstream of $higBA_{Pa}$ except for in 10 strains (32/42, 76%). For these 10 strains, PCR was performed with various primers designed based on the PAO1 reference sequence, as well as primers designed to probe the upstream sequence of $higBA_{Pa}$ observed in *P. aeruginosa* PA7; however, no product was amplified in any of these cases. All results from the flanking region PCR are listed in Table S2.

DNA sequencing was performed on $>$ 10% of the PCR products to confirm the identity of the amplified sequence. Sequenced PCR products revealed a strong sequence identity for the $mazF_{Sa}$ upstream and downstream regions (91.5–98.6%) compared with the reference sequence from the *S. aureus* COL genome (Fig. S5). The flanking region PCR products of $parDE_{Pa}$ (92.6–98.2%), $relBE_{Pa}$ (96.2–99.4%), and $higBA_{Pa}$ (91.8–99.4%) also showed strong sequence identity to the reference *P. aeruginosa* PAO1 sequence (Figs S6–S8).

Transcription of TA genes

To determine whether the TA systems were transcribed by the clinical isolates, RT-PCR was performed with total RNA isolated from $>$ 10% of strains shown by PCR to contain the genes for each TA system. The oligonucleotide sequences of all primers used for RT-PCR are listed in Table 1, and Fig. 1 depicts the regions of homology. The $mazEF_{Sa}$ transcript was detected from the total RNA of all nine MRSA strains probed by RT-PCR (Fig. 3a). Similarly, the

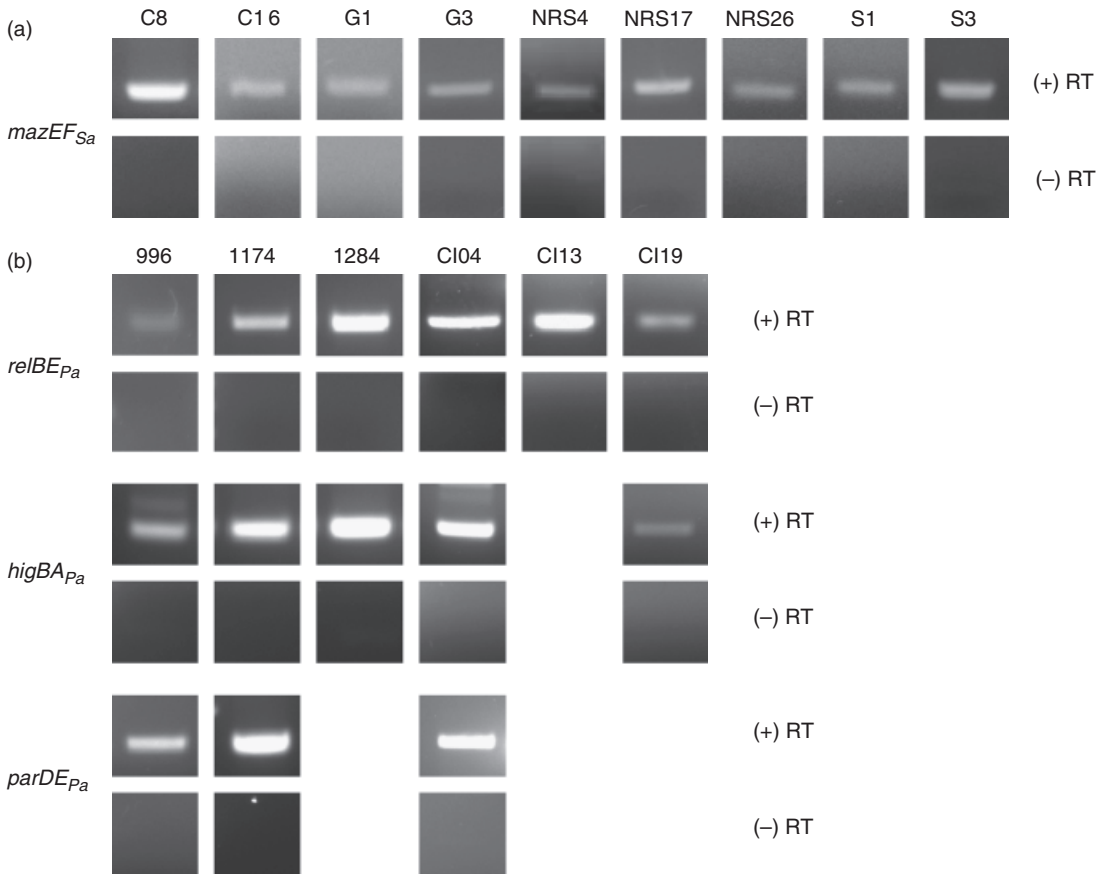

Fig. 3. RT-PCR analysis of MRSA and *Pseudomonas aeruginosa* clinical isolates. RT-PCR with primers complementary to the genes encoding each TA system indicates that transcripts are produced in the clinical isolates of (a) MRSA and (b) PA, as shown in the (+) RT row. Controls for DNA contamination, in which the reverse transcriptase is omitted from the reaction mix, yield no product, as shown in the (−) RT row. For each strain, the (+) RT and (−) RT reactions were analyzed on the same agarose gel, although they are depicted separately. Clinical isolates analyzed are indicated by the strain number above each column.

transcripts for $relBE_{Pa}$ (6/6), $higBA_{Pa}$ (5/5), and $parDE_{Pa}$ (3/3) transcripts were detected in all PA strains probed by RT-PCR (Fig. 3b). For all samples, no amplification products were observed in the absence of reverse transcriptase, confirming that the products seen by RT-PCR were due to the presence of the TA transcript in the clinical isolates and not DNA contamination (Fig. 3a and b).

Discussion

Bioinformatics analyses of published prokaryotic genomes have demonstrated the pervasive nature of TA loci (Makarova *et al.*, 2009); however, little effort has been made to survey large collections of clinical bacterial strains for the presence and functionality of TA systems. Herein we use PCR to determine that $mazEF_{Sa}$ is ubiquitous in a collection of MRSA clinical isolates, and $higBA_{Pa}$ and $relBE_{Pa}$ are ubiquitous in a collection of PA clinical isolates, whereas $parDE_{Pa}$ is less commonly observed. This PCR method is complementary to the whole genome sequencing that has previously been used to examine the presence of TA systems in MRSA and PA, and the results reveal the value of inspecting large numbers of clinical isolates in the manner. For example, of the three sequenced PA clinical isolates that have been analyzed, PA14 does not have the genes for $parDE_{Pa}$, whereas PAO1 and PA7 do (Makarova *et al.*, 2009). However, the results presented herein show that PA clinical isolates that cluster with PA14 (via MLVA) are just as likely to have the genes for $parDE_{Pa}$ as those PA strains that do not cluster with PA14.

Assessment of the flanking sequence of the TA systems in MRSA and PA revealed that the chromosomal location was conserved across all strains carrying $mazEF_{Sa}$ and $parDE_{Pa}$, in nearly all strains for $relBE_{Pa}$ and in the majority of strains for $higBA_{Pa}$. The inability to amplify the upstream sequence of $higBA_{Pa}$ in 10 strains suggests that the upstream sequence has diverged or that the *higBA* loci of these 10 strains is located elsewhere; however, the conservation of the

downstream sequence implies that *higBA*$_{Pa}$ is chromosomally encoded.

Defining the identity of TA systems in clinical isolates satisfies the first requirement in validating TA systems as a viable antibacterial target. However, it is imperative to establish which TA systems are transcribed in clinical isolates. Thus RT-PCR analysis was performed to determine whether the TA systems were transcribed. Importantly, it was shown by RT-PCR that *mazEF*$_{Sa}$, *higBA*$_{Pa}$, *relBE*$_{Pa}$, and *parDE*$_{Pa}$ were transcribed in strains that carried the genes. Collectively, the results presented herein indicate that the TA genes detected in the MRSA and PA strains reside on the chromosome and are active TA modules.

It has been suggested that activation of TA systems could be an attractive antimicrobial strategy, as the released toxin would kill the host bacterial cell (Engelberg-Kulka *et al.*, 2004; DeNap & Hergenrother, 2005; Gerdes *et al.*, 2005; Alonso *et al.*, 2007; Williams & Hergenrother, 2008). While the presence of TA systems in sequenced prokaryotic genomes has been established, before this work the prevalence of TA systems in clinical isolates of MRSA and PA was unknown. In addition, this is the first time that the *mazEF*$_{Sa}$, *higBA*$_{Pa}$, *parDE*$_{Pa}$, and *relBE*$_{Pa}$ transcripts have been shown to be produced in these bacteria, and one of the few examples of demonstrated transcription of any TA genes from a clinical isolate. Given the results of a previous study showing that TA systems are ubiquitous in VRE (Moritz & Hergenrother, 2007), and the current survey showing that TA systems are also highly prevalent and transcribed in MRSA and PA, it appears that these problematic bacterial pathogens would indeed be susceptible to TA-based antibacterial strategies. Specifically, activation of MazEF$_{Sa}$ should be considered for MRSA, and activation of RelE$_{Pa}$ or HigBA$_{Pa}$ appear to be attractive strategies against PA.

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Authors' contribution

J.J.W. and E.M.H. contributed equally to this work.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Alignment of $mazEF_{Sa}$ sequences.
Fig. S2. Alignment of $parDE_{Pa}$ sequences.
Fig. S3. Alignment of $relBE_{Pa}$ sequences.
Fig. S4. Alignment of $higBA_{Pa}$ sequences.
Fig. S5. Alignment of $mazEF_{Sa}$ upstream (a) and downstream (b) flanking sequences.
Fig. S6. Alignment of $parDE_{Pa}$ upstream (a) and downstream (b) flanking sequences.
Fig. S7. Alignment of $relBE_{Pa}$ upstream (a) and downstream (b) flanking sequences.
Fig. S8. Alignment of $higBA_{Pa}$ upstream (a) and downstream (b) flanking sequences.
Table S1. Presence of TA Systems in MRSA and *Pseudomonas aeruginosa*.
Table S2. Flanking regions of TA Systems in MRSA and *Pseudomonas aeruginosa*.

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