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White and blue light induce reduction in susceptibility to minocycline and tigecycline in Acinetobacter sp. and other bacteria of clinical importance.

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Abstract:	Minocycline (MIN) and tigecycline (TIG) are antibiotics currently used for treatment of multi-drug resistant nosocomial pathogens. In this work, we show that blue light, as well as white light, modulate susceptibility to these antibiotics in a temperature-dependent manner. The modulation of susceptibility by light depends on the content of iron, resulting an increase in iron in a reduction in antibiotic susceptibility both under light as well as in the dark, though the effect was more pronounced in the latter condition. We further provide insights into the mechanism by showing that reduction in susceptibility to MIN and TIG induced by light is likely triggered by the generation of 1O2, which, by an yet unknown mechanism, would ultimately lead to the activation of resistance genes such as those coding for the efflux pump AdeABC. The clinical relevance of these results may rely in surface-exposed wound infections, given the exposure to light in addition to the relatively lower temperatures recorded in these type or lesions. We further show that the modulation of antibiotic susceptibility not only occurs in A. baumannii but also in other microorganisms of clinical relevance such as Escherichia coli or Staphylococcus aureus. Overall, our findings allow us to suggest that MIN and TIG antibiotic treatments may be improved by the inclusion of an iron chelator, a condition that in addition to keeping the wounds in the dark would increase the effectiveness in the control of infections involving these microorganisms.

White and blue light induce reduction in susceptibility to minocycline and tigecycline in *Acinetobacter* sp. and other bacteria of clinical importance.

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Abstract

Minocycline (MIN) and tigecycline (TIG) are antibiotics currently used for treatment of multi-drug resistant nosocomial pathogens. In this work, we show that blue light, as well as white light, modulate susceptibility to these antibiotics in a temperature-dependent manner. The modulation of susceptibility by light depends on the content of iron, resulting an increase in iron in a reduction in antibiotic susceptibility both under light as well as in the dark, though the effect was more pronounced in the latter condition. We further provide insights into the mechanism by showing that reduction in susceptibility to MIN and TIG induced by light is likely triggered by the generation of $^1O_2$, which, by an yet unknown mechanism, would ultimately lead to the activation of resistance genes such as those coding for the efflux pump AdeABC. The clinical relevance of these results may rely in surface-exposed wound infections, given the exposure to light in addition to the relatively lower temperatures recorded in these type or lesions. We further show that the modulation of antibiotic susceptibility not only occurs in *A. baumannii* but also in other microorganisms of clinical relevance such as *Escherichia coli* or *Staphylococcus aureus*. Overall, our findings allow us to suggest that MIN and TIG antibiotic treatments may be improved by the inclusion of an iron chelator, a condition that in addition to keeping the wounds in the dark would increase the effectiveness in the control of infections involving these microorganisms.

INTRODUCTION

Acinetobacter baumannii has emerged as a nosocomial pathogen of increasing clinical importance over the course of the last decades. It has established itself within the hospital niche, where its ability to persist in the nosocomial environment despite unfavorable conditions such as desiccation, nutrient starvation, and antimicrobial treatments appears key in its success as a pathogen (Mussi *et al.*, 2010). In this sense, recent findings from our group have shown that light governs many processes related to its ability to persist and live in the environment, as well as key determinants involved in its pathogenesis (Mussi *et al.*, 2010). In fact, we found that blue light inhibited motility and the formation of biofilms and pellicles in *A. baumannii* cells cultured at 24°C in liquid broth, and enhanced the ability of the bacteria to kill the filamentous form of the eukaryotic fungus *Candida albicans* (Mussi *et al.*, 2010). By means of biophysics as well as genetic studies we have shown that this response to light depends on BlsA, the only photoreceptor encoded in its genome (Mussi *et al.*, 2010). In addition, we have also shown that the response to light is widespread within other species belonging to the *Acinetobacter* genus, showing that sensing and responding to light is part of the lifestyle of these bacteria (Golic *et al.*, 2013).

The other determining factor in the success of *A. baumannii* as a pathogen is its ability to acquire or rapidly evolve and accumulate resistance mechanisms to antibiotics, where its suitability for genetic exchange is of central importance (Mussi *et al.*, 2011; Snitkin *et al.*, 2011; Tan *et al.*, 2013). In fact, the rates of multidrug resistance in this organism have been increasing among clinical strains lately, resulting in a present panorama where most strains are extensively (XDR) or pan drug resistant (PDR), rendering most available antibiotic treatments useless (Magiorakos *et al.*, 2011). In this sense, the selected treatment for infections caused by *A. baumannii* has for many years been the carbapenem antibiotics such as imipenem (IPM) and meropenem (MEM). However, most *A. baumannii* isolates are now resistant to these antibiotics, even reaching 85% of the strains in our country (on line: http://antimicrobianos.com.ar/2013/10/informe-resistencia-2012-argentina/, last acceded April 19th, 2014). Minocycline (MIN) and its derivative tigecycline (TIG) are tetracycline antibiotics capable of confronting certain MDR-*A. baumannii* infections and therefore, constitute a therapeutic option to treat infections caused by these microorganisms (Bradford *et al.*, 2005; Talbot *et al.*, 2006).

One aspect that has been scarcely studied is related to whether resistance to antibiotics is modulated in response to external signals. Given that the response to light is an important trait modulating *A. baumannii* -as well as other members of the genus *Acinetobacter*- physiopathology (Mussi *et al.*, 2010; Golic *et al.*, 2013), the question arises on whether light modulates antibiotic susceptibility as well.

In this work, we show that light effectively modulates antibiotic susceptibility, in particular, to antibiotics such as MIN and TIG in a temperature-dependent manner. We found that this response is dependent on the culture media, being the content of iron present in the media key for the magnification of the phenotype. Modulation of susceptibility to MIN and TIG by light is not dependent on BlsA, the photoreceptor previously shown to mediate light regulation of motility, biofilm formation and virulence in *A. baumannii*. Rather, the effect would probably involve the generation of $^1O_2$ as a result of light absorption by a photosensitizer molecule which, by an yet unknown mechanism, would ultimately lead to the activation of resistance genes such as those coding for the efflux pump AdeABC. The clinical relevance of these results may rely in surface-exposed wound infections, given the exposure to light in addition to the relatively lower temperatures recorded in these type or lesions. We further show that the modulation of antibiotic susceptibility not only occurs in *A. baumannii* but also in other microorganisms of clinical relevance such as *Escherichia coli* or *Staphylococcus aureus*. In conclusion, our results contribute to the characterization of factors modulating one of the main determinants in the success of important nosocomial pathogens, antibiotic resistance, and provide information valuable for medical practices in the hospital setting.

MATERIALS AND METHODS

Bacterial strains. Thirty-five *A. baumannii* clinical isolates obtained during the period from 1990 to 2013 from patients hospitalized in 12 public nosocomial institutions of major urban centers of Argentina were used in the present study. These strains were used initially to determine whether light modulated antibiotic resistance and to identify the affected antibiotics, and are not further described. In addition, *A. baumannii* type strains ATCC 19606, ATCC 17978 as well as the extensively characterized strain A118 (Ramírez *et al.*, 2011; Traglia *et al.*, 2014) were also used in this study. The clinical strain A118 was the first naturally competent *A. baumannii* strain reported (Ramírez *et al.*, 2010). A118, unlike other clinical isolates, is susceptible to numerous antibiotics, supports replication and stable maintenance of different plasmid replicons and takes up fluorophore labeled oligonucleotides (Ramírez *et al.*, 2010; 2011). This strain showed to be a singleton by MSLT technique. ATCC 19606 is also susceptible to multiple antibiotics with widespread use in most experimental studies, and doesn’t belong to any of the most widespread clonal complexes, as is the case for the A118 strain. Regarding strain A42, it is MDR (Vilacoba *et al.*, 2013), though susceptible to MIN and TIG, and belongs to the international clonal complex 1 (ICL1), which together with the ICL2 are the most widespread clonal complexes of *A. baumannii*.

Moreover, 8 strains representative of non-*baumannii Acinetobacter* species and 7 isolates belonging to other important nosocomial pathogen species were also included (Table 4). The *Acinetobacter* spp. isolates were identified to species level using i) conventional biochemical tests, ii) automated system VITEK2, iii) API 20 NE system (Vitek; bioMérieux, France), iv) matrix-assisted laser desorption ionization– time of flight

(MALDI-TOF) mass spectrometry (MS) (Bruker Daltonik) using of the current BrukerDaltonics 206 database version 3.0 (MBT-BDAL-5627 MSP library) and v) *rpo*B sequence analysis when was required, or obtained from the mentioned sources (indicated in Table 4).

Antibiotic Susceptibility Assays. Antibiotic susceptibility assays were performed according with the procedures recommended by the NCLSI, with the following modifications:

a) Disk Diffusion method. For determination of inhibition halos by the disk diffusion method, plates containing 20 ml of different media: Müeller Hinton (MH; Britania); minimal media BM2 (62 mM potassium phosphate (pH=7.0), 0.5 mM $MgSO_4$, 10µM $FeSO_4$, and 7 mM $(NH_4)_2SO_4$) supplemented with 10 mM sodium succinate; or Luria Bertani (LB; Difco) were prepared. When indicated, LB Difco agar plates were supplemented with $FeCl_3$, or 2,2'-dipyridyl (DIP) to simulate iron-replete or iron-limited conditions; NaCl; or methylene blue (MB), to produce $^1O_2$ in the presence of red light (Galbis-Martínez *et al.*, 2012). The agar plates were inoculated with 100 µl of a culture of each tested strain, which was previously resuspended in physiologic solution and adjusted to OD_{600}= 0.1. It should be noted that the inocula was not swabbed in the plates but administered using Drigalsky spatula, to standardize and homogenize conditions between replicates exposed to light or kept in the dark. Antimicrobial commercial discs (BBL, Cockeysville, MD, USA) containing 10 mg of ampicillin, 30 mg of amikacin, 30 mg of cefepime, 30 mg of cefotaxime, 30 mg of cefoxitin, 30 mg of cephalotin, 30 mg of chloramphenicol, 5 mg of ciprofloxacin, 10 mg of imipenem, 10 mg of gentamycin, 10 mg of meropenem, 100 mg of piperacillin, 5 mg of rifampicin, 15 mg TIG or 30 mg MIN were placed on the surface of plates, which were latter incubated overnight at 24ºC or 37ºC in the dark or under light emitted by nine-LED (light-emitting diode) arrays with an intensity of 6 to 10 µmol photons/m2/s. Each array was built using three-LED module strips emitting blue, green, or red light (Mussi *et al.*, 2010). The assays were performed in triplicate.

Breakpoints defined by the CLSI criteria for MIN in MH solid media consider: susceptible ≥16 mm; intermediate 13-15 mm; resistant ≤12 mm. The breakpoint criteria assumed to determine the TIG phenotype was based on the United States Food and Drug Administration for *Enterobacteriaceae* and considers susceptibility ≥19 mm; intermediate 15-18 mm; resistant ≤14 mm.

b) Minimum Inhibitory Concentration (MIC) determination in liquid media. MIC determination was performed in multi-well microplates using LB Difco broth at 24ºC, conditions that produced maximal differences in the disk diffusion method between light and dark conditions. For these assays, we used minocycline hydrochloride (Sigma; cat. number M9511), which was resuspended in water at a stock concentration of 12.5 mg/ml. The antibiotic was subjected to serial half dilutions starting from 64 µg/ml. In addition, we used TIG (Richet). The antibiotic was prepared in a stock solution of 3 mg/ml in DMSO. In this case, the antibiotic was subjected to serial half dilutions starting from 256 µg/ml. The tested strains were resuspended in physiologic solution and adjusted to OD_{600}= 0.1, then diluted 1/10 in LB Difco media and applied to the wells. Identical microplates were incubated overnight in the dark or under blue light using devices described in the above item. In MH broth, breakpoints for MIN defined by CLSI are: susceptible ≤4 µg/ml; 8 µg/ml intermediate; resistant:≥16 µg/ml. The breakpoint criteria used to determine the TIG phenotype was based on the United States Food and Drug Administration breakpoint criteria for *Enterobacteriaceae* considering susceptibility ≤ 2 µg/ml, intermediate at 4 µg/ml and resistance ≥ 8 µg/ml.

Construction of the *A. baumannii* ATCC 19606.OR isogenic insertion derivative. A genomic fragment containing the *blsA* gene and flanking sequences was PCR amplified using primers BlsA.R/1 and BlsA.F/2 (see Table 1), from ATCC 19606 genomic DNA. The amplicon was cloned into pGemT-easy vector to generate pBLSA. This fragment was subsequently subcloned into the *Eco*RI sites of pKNOCK-Amp, and the resulting plasmid (pKABLSA) was used to construct pKABLSA-Km, in which the pUC4K *Pst*I restriction fragment harboring the DNA kanamycin resistance (Km^r) cassette was inserted into a unique *Nsi*I site within the *blsA* gene. *E. coli* DH5α cells harboring pKABLSA-Km, *E. coli* HB101 cells harboring pRK2073, and *A. baumannii* ATCC 19606 cells were used as donor, helper, and recipient strains, respectively, in triparental conjugations. Transconjugants were selected on Simmons citrate agar plates containing 40 µg/ml Km. Total DNA was isolated from a putative *A. baumannii* ATCC 19606.ORtransconjugant derivative, which was resistant to Km and sensitive to 1000 µg/ml ampicillin (Amp), and used to confirm the nature of the site-directed insertion mutation by PCR with primers BlsA.R/1 and BlsA.F/2.

Transcriptional analysis. A42 or ATCC 19606 cells were grown in LB Difco broth until they reached DO= 0.5 at 24ºC in the presence or absence of blue light. When indicated, the culture media was supplemented with TIG 0.1 µg/ml. Cell pellets were immediately mixed with 2 ml lysis buffer (0.1 M Na acetate, 10 mM EDTA, 1% SDS) in a boilingwater bath. Cell lysates were extracted twice at 60°C with phenol, which was equilibrated to pH 4.0 with 50 mM Na acetate, and then once with chloroform at room temperature. The RNA precipitated overnight at -20°C with 2.5 volumes of ethanol was collected by centrifugation, washed with 70% ethanol, and dissolved in DEPC-treated deionized water. Total RNA samples were treated with RNase-free DNase I. The integrity of the RNA samples was checked by agarose electrophoresis.RNA samples were collected from three different biological samples prepared in triplicate each time.

First-strand cDNA was synthesized with MoMLV-reverse transcriptase following the manufacturer's instructions (Promega, Madison, WI, USA) using 2 µg of RNA and random primers. Relative expression was determined by performing quantitative real-time PCR (qRT-PCR) in an iCycleriQ detection system and the Optical System Software version 3.0a (Bio-Rad, Hercules, CA, USA), using the intercalation dye SYBR Green I (Invitrogen) as a fluorescent reporter, with 2.5 mM $MgCl_2$, 0.5 µM of each primer and 0.04 U/µl GoTaq (Promega). PCR primers used in these experiments are described in Table 1, and were designed with the aid of the web based program "primer3" (http://www.frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) in a way to produce amplicons of 150 to 300 bp in size. A ten-fold dilution of cDNA obtained as described above was used as template. Samples containing no reverse transcriptase or template RNA were included as negative controls to ensure RNA samples were free of DNA contamination. Cycling parameters were as follows: initial denaturation at 94°C for 2 min; 40 cycles of 96°C for 10 s, and 54°C for 15 s; 72°C for 1 min, and 72°C for 10 min. The SYBR Green I fluorescence of the double strand amplified products was measured at 76ºC. Melting curves for each PCR reaction were determined by measuring the decrease of fluorescence with increasing temperature (from 65ºC to 98ºC). The specificity of the PCR reactions was confirmed by melting curve analysis using the software as well as by agarose gel electrophoresis of the products. Amplification efficiency (E) for each gene was determined from the relative standard curve method (Cikoš *et al.*, 2007). The *adeA*, *adeB* and *adeC* transcript levels of each sample were normalized to the *recA* transcript levels for each cDNA sample Relative gene expression was calculated using the Comparative $E^{-\Delta CT}$ method (Livak & Schmittgen, 2001). Each cDNA sample was run in technical triplicate and repeated in at least three independent sets of samples. ANOVA test was used to determine statistical significance.

RESULTS

Blue light modulates antibiotic susceptibility to MIN and TIG in *A. baumannii*.

We systematically performed antibiotic susceptibility assays under blue light or in the dark using a wide collection of clinical strains of *A. baumannii* (see Materials and Methods), to determine if light regulates this trait in addition to modulating motility, biofilm formation, and virulence as previously described by our group (Mussi *et al.*, 2010). The experiments were initially performed at 24°C since photoregulation has been shown to occur at this temperature and not at 37ºC in *A. baumannii* (Mussi *et al.*, 2010).

Our results show that light effectively modulates antibiotic susceptibility in *A. baumannii*. In fact, light produces important differences in the diameters of inhibition zones of MIN and TIG antibiotics between blue light and dark conditions, when the bacteria were cultured in solid LB media. Strains A118, A42 and ATCC 19606 are representatives of isolates that showed the highest differences in the diameters of inhibition zones (between 12 and 14 mm)(Figure 1A and Table 2), and therefore were selected for further studies. Other strains, such as Ab107or ATCC 17978, are examples of strains showing less pronounced differences (Table 2).

Other antibiotics such as IPM and MEM also showed differences between light and dark for some strains, however, to a much lesser extent (data not shown). Despite belonging to the same family, we did not observe differences between light and dark for tetracycline (TET) (Table 2); nor for other antibiotic considered last resource to treat XDR strain such as colistin (COL) (Table 2).

It is important to note that the light-mediated response is not due to the effect of light on cell growth and viability. Cells cultured in LB broth displayed similar growth curves and reached comparable viable counts after incubation for up to 96 h at 24°C in the presence or absence of light (not shown).

Blue light induces important reductions in susceptibility to both MIN and TIG in liquid media.

We were interested in determining whether blue light modulates susceptibility to MIN and TIG also in liquid media. To test this possibility, we performed MIC determinations for MIN and TIG using strains A42, A118 and ATCC 19606 under blue light or in the dark at 24ºC. Our results show again that the bacteria are more resistant to MIN and TIG under blue light than in the dark. For example, we registered changes in MIC values to MIN from <0.125 µg/ml in the dark to 16 µg/ml under blue light in strain A42, or changes from 2 µg/ml in the dark to 128 µg/ml as a result of application of blue light in strain ATCC 19606. These differences in MIC values are very important, ranging from at least 16 to 128 folds between light and dark conditions depending on the strain and antibiotic analyzed (Table 3). These results highlight the importance of the findings reported in this work showing that light significantly reduces bacterial susceptibility to these antibiotics.

Blue light modulation of antibiotic resistance in *A. baumannii* is dependent on the culture media.

Further studies using isolates A118 and ATCC 19606, show that light modulation of antibiotic susceptibility is strongly dependent on the culture media. For instance, our results using strain A118 show that blue light modulation of antibiotic susceptibility is particularly important for MIN and TIG in LB agar (Difco), while the effect is minimized in MH agar (Table 2). When agar blood media (AB) was used, the extent of light modulation of antibiotic susceptibility was intermediate respect to media producing the highest or minimal differences (LB Difco or MH) (Table 2). We also tested the phenotype using the minimal BM2 media. Again, in this media no differences in antibiotic resistance were registered between light and dark conditions for MIN or TIG. Similar results were obtained for ATCC 19606, revealing again strong differences when LB Difco media was employed, which were minimized in MH (Table 2). These results indicate that the presence or absence of some component in the culture media contributes to the amplification of the modulation of susceptibility to MIN and TIG by light.

Blue light modulation of antibiotic susceptibility in *A. baumannii* varies with the content of iron.

We were interested in determining which component of the culture media was responsible for the differences in light modulation of antibiotic susceptibility observed among the different media used. For this purpose, we supplemented LB media with different concentrations of NaCl or $FeCl_3$. The addition of NaCl showed no effect at physiological concentrations (Figure 2D), a condition previously shown to modulate antibiotic resistance to some antibiotics such as aminoglycosides, carbapenems, quinolones, and colistin in *A. baumannii* (Indriati Hood *et al.*, 2010). However, the addition of $FeCl_3$ resulted in a reduction in the effect of light in antibiotic susceptibility. Specifically, the halos of both MIN and TIG were significantly reduced both under light as well as in the dark, though the effect was higher in the dark, resulting in an overall reduction in the difference in the halos between both conditions (Figure 2B). Conversely, the addition of the iron chelator DIP (Penwell *et al.*, 2012), results in a slight amplification in the differences in the inhibition halos between light and dark for these antibiotics (Figure 2C).

Blue light modulation of antibiotic susceptibility in *A. baumannii* depends on temperature.

We also evaluated whether the effect of light on antibiotic susceptibility observed at 24ºC occurred also at 37ºC. Our results show that at this temperature, the effect was significantly reduced or null, indicating that the modulation of antibiotic susceptibility by light occurs mainly at 24ºC. In particular, strain A42 shows only a slight difference between light and dark both for MIN as well as TIG at 37ºC (Figure 3A and E), in contrast to the effect observed at 24ºC. Similar results were obtained for strains A118 and 19606 (Figure 3B, C, D and E). Overall, our results show that modulation of antibiotic susceptibility occurs mainly at environmental temperatures.

White and blue light modulate susceptibility to MIN and TIG in *A. baumannii*.

We initially studied the effect of blue light on antibiotic resistance since we previously found that blue light modulated different physiologic responses related to pathogenesis (Mussi *et al.*, 2010). To characterize the modulation of susceptibility to MIN and TIG by light described in this work further, we studied the effect of different light wavelengths. Our results indicate that white light produced the same effect as blue light at 24°C (Figure 4). This is not surprising given that blue light is a component of white light. On the contrary, red-light illumination showed no differences in antibiotic susceptibilities neither for MIN or TIG, indicating that this light source does not modulate resistance to these antibiotics (Figure 4). Finally, incubation of antibiogram plates in the presence of green light resulted in a partial inhibition compared to that detected with blue light (Figure 4). These results are consistent with the fact that the emission spectra of the blue and red LEDs do not overlap, while the emission spectra of the blue and green LEDs are superimposed. It is noteworthy to mention that white light, the type of light most commonly used in our everyday life, modulates antibiotic susceptibility.

Modulation of antibiotic susceptibility in *A. baumannii* is not dependent on the photoreceptor BlsA.

In order to determine whether the modulation of antibiotic susceptibility is mediated by the photoreceptor BlsA, we constructed a *blsA* mutant in ATCC 19606 strain by insertion of a Kn resistance cassette within its coding sequence, using a similar strategy as the one we used before for the construction of *blsA* mutant in ATCC 17978 (Mussi *et al.*, 2010). When we assayed ATCC 19606 wt as well as its isogenic derivative ATCC 19606.OR (*blsA* mutant), we found that they present the same difference in antibiotic susceptibility both for MIN as well as for TIG under blue light or in the dark (Figure 1B and Table 2), indicating that modulation of antibiotic susceptibility occurred regardless of the presence of BlsA, and therefore, this photoreceptor is not responsible for the observed phenotype. We also assayed whether there existed difference between the mutant and wild type strains in the presence of iron or DIP, to test whether BlsA played a role in modulation by iron, but again, we observed no difference (not shown). Finally, despite ATCC 17978 shows a much less pronounced light-mediated effect in antibiotic resistance than ATCC 19606 strain, we also assayed the 17978.OR (*blsA* mutant) to evaluate the contribution of BlsA to the modulation of antibiotic susceptibility by light in this strain as well. Again, we did not observe any difference between the wild type and the *blsA* mutant regarding antibiotic susceptibility to MIN and TIG under blue light or in the dark (Table 2).

$^1O_2$ triggers reduction in susceptibility to MIN and TIG in *A. baumannii*.

It has been recently reported that light can modulate gene expression in *Myxococcus xanthus* independently of the presence of "traditional" bacterial photoreceptors (Galbis-Martínez *et al.*, 2012; Ortiz-Guerrero *et al.*, 2011). One of these mechanisms is mediated by singlet oxygen ($^1O_2$), which is produced as a result of excitation of protoporphyrin IX (PPIX) by blue light absorption in this microorganism (Galbis-Martínez *et al.*, 2012).

The photosensitizer methylene blue (MB), a phenothiazinium dye which strongly absorbs red but not blue light (absorption range of 550 to 700 nm) (Kochevar & Redmond, 2000; Mellish *et al.*, 2002), has been used to generate $^1O_2$ from molecular oxygen (Berghoff *et al.*, 2009; Lourenco & Gomes, 2009; Galbis-Martínez *et al.*, 2012). Red light irradiation of MB in the presence of oxygen can therefore be used as a means of generating $^1O_2$ that bypasses the blue-light–sensitizer interaction. We therefore studied whether $^1O_2$ could be involved in the modulation of antibiotic resistance by light by investigating the response when MB and red light were applied together in antibiogram plates. As can be observed in Figure 5A, when the bacteria are irradiated by red light, the diameters of inhibition zones both for MIN or TIG are similar to those observed in the dark; i.e., bacterial resistance to MIN and TIG is blind to red light. However, the application of both MB and red light resulted in important reductions in the inhibition diameters (Figure 5B and 5C), consistent with the bacteria becoming more resistant, reaching similar patterns to those observed under blue light illumination (Figure 1A and Table 2). These results suggest that the modulation of susceptibility to MIN and TIG could be mediated by $^1O_{2.}$

Blue light induces the expression of resistance genes to TIG.

In contrast to other antibiotic families, there is little information regarding the mechanisms of resistance to MIN and TIG in *A. baumannii*. The available data indicate the involvement of the efflux pumps Tet(B) and AdeABC in resistance to MIN and TIG in this microorganism, respectively (Ribera *et al.*, 2003; Ruzin *et al.*, 2007; Vilacoba *et al.*, 2013; Rumbo *et al.*, 2013).

Tet(B) is a tetracycline-specific efflux pump able to extrude MIN and TET (Ribera *et al.*, 2003). In the case of TIG, it has been shown that overexpression of *adeABC* as a result of point mutations in the regulatory genes *adeR* or *adeS* or triggered by the incorporation of insertion sequences, would play a role in the development of resistance or in the decrease in susceptibility to this antibiotic (Higgins *et al.*, 201; Rumbo *et al.*, 2013).

Bioinformatic searches into the sequenced genome of A118 strain indicate that the *tet(B)* determinant is not present in this strain. Besides, no *tetB* homologs were retrieved in database searches in strain ATCC 19606, and PCR reactions showed no amplification for this strain. The overall data indicate that light modulation of antibiotic resistance to MIN is dependent on another mechanism different from *tetB.*

We therefore focused on AdeABC, particularly in studying whether the expression levels of *AdeABC* transcripts were induced under blue light, a situation that would provide evidence on the involvement of this mechanism in reduction of susceptibility to TIG mediated by light. To test this hypothesis, we collected ATCC 19606 or A42 cells from exponentially growing cultures performed in LB Difco media incubated under blue light or in the dark at 24ºC. Our results show that the levels of *AdeA* (major fusion protein), *AdeB* (member of the RND superfamily) and *AdeC* (outer membrane component) transcripts are induced aprox. 2-3 folds by light in strain ATCC 19606 (Figure 6A). Most strikingly, in the presence of sub-MIC concentrations of TIG (0.1 µg/ml) in the growth media the difference in expression levels of the *AdeA* and *B* transcripts between light respect to dark conditions was greatly expanded, showing inductions of aprox. 60 and 18 folds, respectively (Figure 6C).

In the case of strain A42, we detected an induction in *AdeA* and *B* transcripts of aprox. 2 folds under blue light respect to dark conditions in LB Difco exponentially growing cells; while *AdeC* transcripts experienced an increace of aprox. 13 folds. When sub-MIC concentrations of TIG (0.1 µg/ml) were applied to the growth media, an increase in *AdeB* and *AdeC* transcripts of 8 and 17 folds were detected between light and dark conditions.

These findings indicate a correlation between induction of the genes coding for key members of the efflux pump AdeABC and the reduction in susceptibility to TIG observed in the presence of light.

Blue light modulates antibiotic susceptibility in other *Acinetobacter* species.

In the last years -with the introduction of new technologies to identify microorganisms in clinical settings- *Acinetobacter* non-*baumannii* species have been increasingly recognized as responsible for intrahospital infections (Mader *et al.*, 2010; Turton *et al.*, 2010; Karah *et al.*, 2011; Sousa *et al.*, 2014). This prompted us to study whether other members within the *Acinetobacter* genus also show light modulation of antibiotic susceptibility. For this purpose, we performed similar experiments to those described above for *A. baumannii* but using strains representatives of different species (n= 7).

Our results show that modulation of antibiotic susceptibility by light, in particular to MIN and TIG antibiotics, is widely distributed among the different species at 24°C, albeit to different extents. In fact, our results showed important differences similar to those observed in the case of *A. baumannii* for MIN and/or TIG in some strains of *A. nosocomialis, A. pitti, A. calcoaceticus*, which belong to the *Acinetobacter calcoaceticus-baumannii* complex (Golic *et al.*, 2013; Sousa *et al.*, 2014), as well as *A. radioresistens* strain Ar181, and *A. lwoffii*. In contrast, the tested strains of *A. oleivorans*, *A. radioresistens* SH164 and *A. soli* showed less pronounced phenotypes (Table 4).

Since in our previous works we showed that modulation of motility occurred at 37ºC for non-*baumannii Acinetobacter* species (Golic *et al.*, 2013), we also evaluated in this work whether modulation of antibiotic resistance occurred also at 37ºC in some of these strains. In the case of *A. radioresistens* strain SH164 no difference was observed for MIN or TIG at 37ºC, while an important difference is observed at 24ºC (Table 4). In the case of *A. calcoaceticus*, the tested strain showed difference mostly for TIG at 24ºC (Table 4), which was significantly reduced at 37ºC (not shown).

Blue light modulates antibiotic susceptibility in other bacterial genres also of clinical importance.

We were interested in studying whether other species belonging to other genres of bacteria that share niches with *A. baumannii*, and also represent a concern in the nosocomial context, also showed modulation of antibiotic susceptibility. For this purpose, we included in our study strains representatives of a group of particular pathogens that has been collectively named ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), as they can cause the majority of hospital infections and "escape" antibiotic treatment by becoming resistant or persistent to antibiotic treatment (Rice, 2010; Boucher *et al.*, 2009; Boucher *et al.*, 2013). These pathogens are responsible for a substantial percentage of nosocomial infections in the modern hospital and represent the vast majority of isolates whose resistance to antimicrobial agents presents serious therapeutic dilemmas for physicians (Rice, 2010; Boucher *et al.*, 2009; Boucher *et al.*, 2013). Again with the aim to identify differences in antibiotic susceptibility between blue light or in the dark, we carried out similar experiments as were described before using the following species: *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *E. cloacae* (Table 4). Among the studied strains, *S. aureus* strain 632 showed remarkable differences between light and dark conditions for TIG and MIN (Table 4). The results obtained show that other members within the ESKAPE group also present modulation of antibiotic susceptibility. In addition, we also show that *E. coli* strain DH5α presents differences in resistance to MIN and TIG under blue light or in the dark (Table 4), spreading further the spectra of bacteria affected by the phenotype.

Discussion

For a long time, bacteria were considered insensitive to light, with the exception of phototrophs, which use sunlight as an energy source (Gomelsky & Hoff, 2011). However, recent studies demonstrated that chemotropic bacteria are also able to perceive this stimulus through photoreceptors, and adjust their behavior accordingly (Mussi *et al.*, 2010, Gomelsky & Hoff, 2011; Golic *et al.*, 2013). In this context, new exciting discoveries have shown that light modulates physiologic responses as diverse and interesting as the general response to stress in *B. subtilis* (Avila-Pérez *et al.*, 2006); the attachment of *Caulobacter crescentus* to glass surfaces (Purcell *et al.*, 2007), the ability of *B. abortus* to replicate within murine macrophages (Swartz *et al.*, 2007), or even traits related to persistence and virulence in the nosocomial pathogen *A. baumannii* (Mussi *et al.*, 2010) or in other members within this genus (Golic *et al.*, 2013). In this work, we extended our previous findings by showing that antibiotic susceptibility is also modulated by light in *A. baumannii*, however through a different mechanism. In particular, our results show that light modulates susceptibility to the antibiotics MIN and TIG.

MIN, and particularly its derivative, TIG, have been during the last years in addition to COL, the only antibiotics to which remain susceptible the *A. baumannii* isolates circulating in our region. These antibiotics have shown high antimicrobial activity against *A. baumannii*, though relevant clinical evidence is still scarce. Yet, it is well known that these drugs have a potential therapeutic benefit in combination treatment with COL and carbapenems (Bradford *et al.*, 2005; Talbot *et al.*, 2006). MIN and TIG are also effective against difficult-to-treat pathogens such as methicillin-resistant *S. aureus*, vancomycin-resistant *Enterococcus* spp., as well as Gram-negative bacterial strains that produce extended-spectrum β-lactamases. However, TIG is only actually approved for the treatment of severe intra-hospital bacterial infections such as severe skin and intra-abdominal infections (Bradford *et al.*, 2005; Talbot *et al.*, 2006). Moreover, no defined susceptibility breakpoints have been established thus far for TIG in *A. baumannii*. In Argentina, the emergence of MIN resistance has been observed in the past few years, varying from 10 to 40% resistance among different centers, and an increasing rate to TIG resistance has also been observed (12, http://antimicrobianos.com.ar/2013/10/informe-resistencia-2012-argentina/).

Surprisingly, light modulation of susceptibility to MIN and TIG does not depend on BlsA, the only "traditional" photoreceptor encoded in the genome of *A. baumannii* (Mussi *et al.*, 2010). The existence of alternative pathways for light sensing that do not depend on traditional photoreceptors is increasingly being recognized. For example, sensing of the light signal that triggers the transcriptional response leading to carotenogenesis in *Myxococcus xanthus* is achieved by two distinct mechanisms, neither of which is based on conventional photoreceptor proteins. In one of them, light is sensed by a photosensitizer molecule, protoporphyrin IX (PPIX) (Ortiz-Guerrero *et al.*, 2011). Blue light interaction with PPIX generates $^1O_2$, which must be transmitted via CarF to trigger inactivation of the anti-sigma factor, CarR, and the consequent liberation of the cognate extracytoplasmic function (ECF) factor, CarQ. Then, CarQ, in association with core RNA polymerase (RNAP) activates transcription from PQRS, the promoter of the regulatory *carQRS* operon, and from PI, the promoter of the carotenogenic gene *crtIb*, underlying light-induced carotenogenesis (Galbis-Martínez *et al.*, 2012). In the second mechanism, the light signal is sensed by a coenzyme B12-based photoreceptor, which specifically dictates the functioning of the repressor CarH in the dark and on exposure to light. CarH contains two B12 binding domains associated with a DNA HTH binding domain, which perceive the light signal and modulate gene expression accordingly (Ortiz-Guerrero *et al.*, 2011). In *A. baumannii*, there is only one protein, methionine synthase, which is involved in the metabolism of methionine that contains a B12-binding domain, which is associated with domains specific to the functioning of the protein. Therefore, light perception by B12 does not seem to be an operating mechanism in *A. baumannii*.

The exact mechanism of light perception and modulation of antibiotic resistance in *A. baumannii* is still unknown and currently under study in our laboratory. However, our results suggest a mechanism by which light is perceived by some photosensitizer molecule, with the concomitant generation of $^1O_2$, as occurs with *M. xanthus*. Afterwards, $^1O_2$ could be transmitted through unknown partners to trigger a possible transcriptional response leading to reduced susceptibility to MIN and TIG. In fact, our results show that light, with a wider effect in the presence of low concentrations of TIG, induces the expression of some key components of an efflux pump, AdeABC, previously shown to be involved in resistance to this antibiotic in *A. baumannii* (Vilacoba *et al.*, 2013; Higgins *et al.*, 2010).

The difference in susceptibility to MIN and TIG between light and dark is maximized under low iron levels while, conversely, almost suppressed in the presence of this ion, indicating that its content is a variable modulating the effect. Perhaps the presence of iron reduces the amount of free photosensitizer, reducing therefore the possibility to generate $^1O_2$. This, as well as other possibilities, are under study in our laboratory. Finally, it should be mentioned that the stimulation of antimicrobial resistance by the presence of iron has been previously reported in *P. aeruginosa* for tobramycin as well as TIG (Oglesby-Sherrouse *et al*., 2014). However, the mechanism for this modulation has not yet been characterized.

Clinical implications of light-induced antibiotic tolerance or reduction in susceptibility.

Given that there is no light within organs or tissues, it does not seem that light plays a significant role in modulation of systemic infections in humans. However, modulation of surface-exposed wound infections by light may be of critical importance, given in addition the relatively lower temperatures recorded in these type or lesions (McGuiness *et al*., 2004; Mussi *et al*., 2010). In this context, it is important to mention that the microorganisms reported here to show modulation of antibiotic susceptibility by light, such as *S. aureus* and *A. baumannii,* are known causative agents of skin infections.

The fact that, as expected, white light modulates antibiotic susceptibility as well implies that the findings reported in this work can be translated to the clinical setting. Also of critical importance is that blue light modulation is observed in liquid media as well, highlighting that it is a general finding and discarding an unspecific effect of the solid media. Most importantly, MIC values showed differences of even 128 folds between blue light respect to dark conditions for some strains. These impressive differences point out the profound influence light can exert on antibiotic susceptibility, as well as the fact that the importance of light as a key environmental stimuli is underestimated. Alternatively, in other strains such as A118, the response to light might enhance the bacterium's ability to persist until conditions are more favorable for growth or until additional resistance determinants can be accumulated.

Finally, our findings allow us to postulate that MIN and TIG antibiotic treatments may be improved by the inclusion of an iron chelator (such as the FDA-approved DSX), a measure that in addition to keeping the wounds in the dark, would increase the effectiveness in the control of infections involving these microorganisms.

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Table 1. Primers used in this study.

Name/No.	**Nucleotide sequence**
BlsA.R/1	5'- GCAATGTCTCACAATTATGT-3'
BlsA.F/2	5'- ATGACCATACAAACATCTAG-3'
TetBF	5'- ATAGGCGCATCGCTGGATTACT- 3'
TetBR	5'- GAACCACTTCACGCGTTGAGAA- 3'
adeA.rtF	5'- GGGCATGTATGTGCGTGTCAAT- 3'
adeA.rtR	5'- ACAACGACTCTGTCACCGACTT- 3'
adeB.rtF	5'- ATTGAGCGCGAATTATCGGGTG- 3'
adeB.rtR	5'- AAGCGAGCTTCTACAGCCTTGA- 3'
adeC.rtF	5'- ACAACCGTGATTTACGGACTGC- 3'
adeC.rtR	5'- TAGGCAGTCATTCCCAAGCCAA- 3'
RecAF.rt	5´- TACAGAAAGCTGGTGCATGG-3´
RecAR.rt	5´- TGCACCATTTGTGCCTGTAG -3´

Table 2. Blue light modulates susceptibility to MIN and TIG in *A. baumannii*, and is dependent on the culture media.

	MIN				TIG				TET	COL	Source
	AB	MH	LB (Difco)	BM2	AB	MH	LB (Difco)	BM2	LB (Difco)	LB (Difco)	
A118											Ramírez *et al.*, 2010
Light	26±1	40±2	28±1	50±2	18±1	23±1	20±1	32±1	32±1	ND	
Dark	30±1	40±2	42±1	50±2	18±1	24±1	28±1	33±1	32±1	ND	
ATCC 19606											ATCC
Light	ND	40±1	30±1	ND	ND	25±1	18±1	ND	30±1	23±1	
DarK	ND	40±1	44±1	ND	ND	27±1	30±1	ND	30±1	21±1	
ATCC 19606 *blsA*											

Light	ND	ND	29±1	ND	ND	ND	17±1	ND	ND	ND	
Dark	ND	ND	44±1	ND	ND	ND	30±1	ND	ND	ND	
A42											(Vilacoba *et al.*, 2013)
Light	ND	36±2	26±1	ND	ND	ND	20±1	ND	26±1	ND	
Dark	ND	38±2	40±1	ND	ND	ND	28±1	ND	28±1	ND	
ATCC 17978											ATCC
Light	ND	32±1	31±1	ND	ND	ND	16±1	ND	ND	ND	
Dark	ND	31±1	37±1	ND	ND	ND	23±1	ND	ND	ND	
ATCC 17978 *blsA*											
Light	ND	ND	31±1	ND	ND	ND	16±1	ND	ND	ND	
Dark	ND	ND	37±1	ND	ND	ND	23±1	ND	ND	ND	
Ab107											This work
Light	ND	29±1	28±1	ND	ND	ND	20±1	ND	17±1	ND	
Dark	ND	30±1	33±1	ND	ND	ND	23±1	ND	18±1	ND	

Diameters of inhibition zones of antibiogram plates performed in the indicated media under blue light or in the dark. L: light; D: dark. MIN: 30 µg. TIG: 15 µg; ND: non-determined. The experiments were repeated at least three times for each condition.

Table 3. Blue light modulates susceptibility to MIN and TIG also in liquid media.

	MIC Minocycline (µg/ml)			MIC Tigecycline (µg/ml)		
Strain	Light	Dark	MIC folds	Light	Dark	MIC folds
A42	16	<0.125	128	64	2	32
A118	4	<0.125	32	32	1	32
ATCC 19606	2	<0.125	16	128	2	64

[a] The MICs were determined by the microdilution method, in accordance with general procedures recommended by the National Committee for Clinical Laboratory Standards. For specific details, please refer to Materials and Methods.

Table 4. Blue light modulates antibiotic susceptibility to MIN and TIG in other species.

24ºC			Source
	MIN	TIG	
A. radioresistens			
SH164L	28±1	17±1	(Seifert *et al.*, 1997)
SH164D	34±1	24±1	
Ar181L	25±2	18±1	This work
Ar181D	35±2	30±1	
A. nosocomialis			
45L	28±2	17±2	This work
45D	42±2	29±2	
A. oleivorans			
DR1L	27±1	19±1	(Jung *et al.*, 2010)
DR1D	31±1	24±1	
A. pittii			
SH024L	27±1	19±1	(Seifert *et al.*, 1997)
SH024D	35±1	27±1	
A. lwoffii			
SH145L	24±2	15±2	(Seifert *et al.*, 1997)
SH145D	39±2	24±2	
A. calcoaceticus			
48L	29±	21±	This work
48D	32±	30±	
A. soli			This work
7L	25±1	18,5±1,5	
7D	32±2	26±4	
E. coli			Gibco-BRL
DH5αL	23±1	18±1	
DH5αD	30±1	26±1	
P. aeruginosa			
802L	24±1	16±1	This work
802D	20±1	18±1	
S. aureus			
632L	28±2	22±1	This work

632D	38±1	32±0,5	
K. pneumoniae			
313L	20±1	22±	This work
313D	25±1	26	
404L	8±1	18±1	This work
404D	8±1	22±1	
E. cloacae			
9L	14±1	16±1	This work
9D	16±1	18±1	
1L	18±2	18±1	This work
1D	20±1	22±1	

Legends to Figures

Figure 1. Light modulates susceptibility to MIN and TIG in *A. baumannii*. Cells of the parental strain ATCC 19606 (A) and the ATCC 19606.OR *blsA* mutant (B) were resuspended in physiologic solution and adjusted to OD_{600}= 0.1. Then, 100 µl of the bacteria were plated on the surface of LB Difco agar plates. Plates were inspected and photographed after incubation overnight (10 to 12 h) in darkness (D) or in the presence of blue light (BL) at 24°C. MIN: 30 µg. TIG: 15 µg. The experiments were repeated at least three times for each condition.

Figure 2. The content of iron influences the effect of light on antibiotic susceptibility. Cells of ATCC 19606 were resuspended in physiologic solution and adjusted to OD_{600}= 0.1. Then, 100 µl of the bacteria were plated on the surface of LB Difco agar plates without supplement (A), supplemented with 200 µM FeCl3 (B), supplemented with 100 µM DIP (C); or with 200 mM NaCl (D). Plates were inspected and photographed after incubation overnight (10 to 12 h) in darkness (D) or in the presence of blue light (BL) at 24°C. MIN: 30 µg. TIG: 15 µg. The experiments were repeated at least three times for each condition.

Figure 3. Light modulation of susceptibility to MIN and TIG occurs at 24ºC and not at 37ºC in *A. baumannii*. Cells of strains A42 (A), A118 (B) and ATCC 19606 (C and D) were resuspended in physiologic solution and adjusted to OD_{600}= 0.1. Then, 100 µl of the bacteria were plated on the surface of LB Difco agar plates. Plates were inspected and photographed after incubation overnight (10 to 12 h) in darkness (D) or in the presence of blue light (BL) at 37ºC (A, B and C) or 24°C (D). MIN: 30 µg. TIG: 15 µg. (E) Quantification of the diameters of inhibition zones of antibiogram plates similar to those

shown in A, B, C and D. BL: blue light; D: dark. The experiments were repeated at least three times for each condition.

Figure 4. White and blue light modulate susceptibility to MIN and TIG in *A. baumannii*. (A) Cells of the parental strain ATCC 19606 were resuspended in physiologic solution and adjusted to OD_{600}= 0.1. Then, 100 µl of the bacteria were plated on the surface of LB Difco agar plates. Plates were inspected and photographed after incubation overnight (10 to 12 h) under different light sources or in the dark at 24°C. MIN: 30 µg. TIG: 15 µg. (B) Quantification of the diameters of inhibition zones of antibiogram plates similar to those shown in A. WL: white light; BL: blue light; GL: green light; RL: red light; D: dark. The experiments were repeated at least three times for each condition.

Figure 5. $^1O_2$ triggers reduction in susceptibility to MIN and TIG in *A. baumannii*. Cells of the parental strain ATCC 19606 were resuspended in physiologic solution and adjusted to OD_{600}= 0.1. Then, 100 µl of the bacteria were plated on the surface of LB Difco agar plates(A) or LB Difco agar plates supplemented with 5 µM MB. Plates were inspected and photographed after incubation overnight (10 to 12 h) in darkness (D) or in the presence of red light (RL) at 24°C.MIN: 30 µg. TIG: 15 µg. (B) Quantification of the diameters of inhibition zones of antibiogram plates similar to those shown in A and B.

Figure 6. Effects of light and sub-MIC concentrations of TIG on *Ade*A, B and C transcript levels. cDNA from *A. baumannii* ATCC 19606 (A and C) or A42 (B and D) cells grown to exponential phase in LB Difco at 24°C in the presence of blue light (L) or in darkness (D) was used as the template for qRT-PCR using *adeA*, *B* or *C* specific primers. Panels C and D show data for cells grown under the same conditions as in panels

A and B, with the difference that 0.1 µg/ml TIG was added to the culture media. Transcription of *recA* was used as a constitutively expressed internal control. Standard deviations of three independent experiments are shown. Asterisks indicate transcript levels statistically different between light and dark conditions. Above the bars are indicated the ratio of induction of each transcript between light vs. dark conditions.

Figure 1

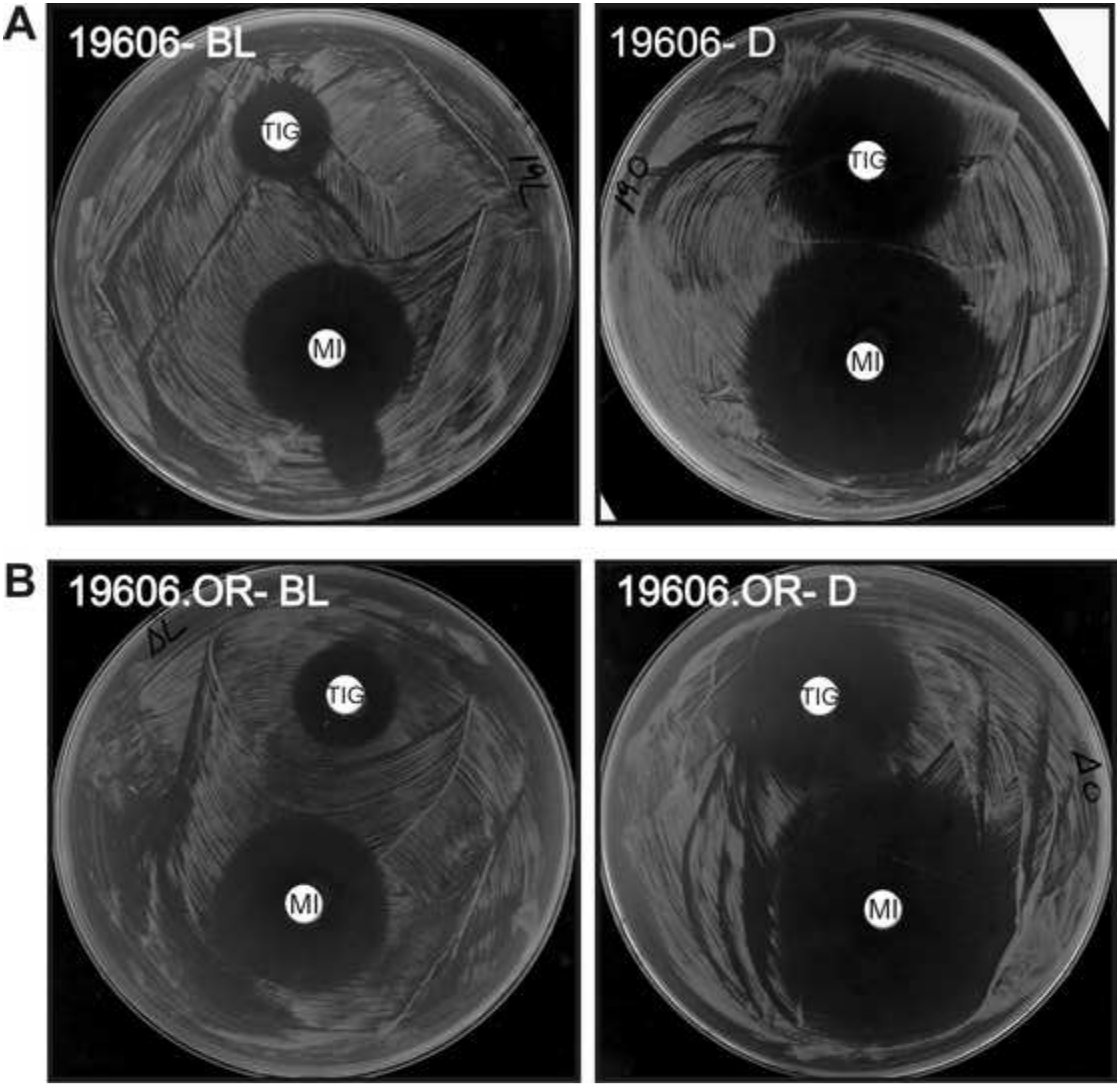
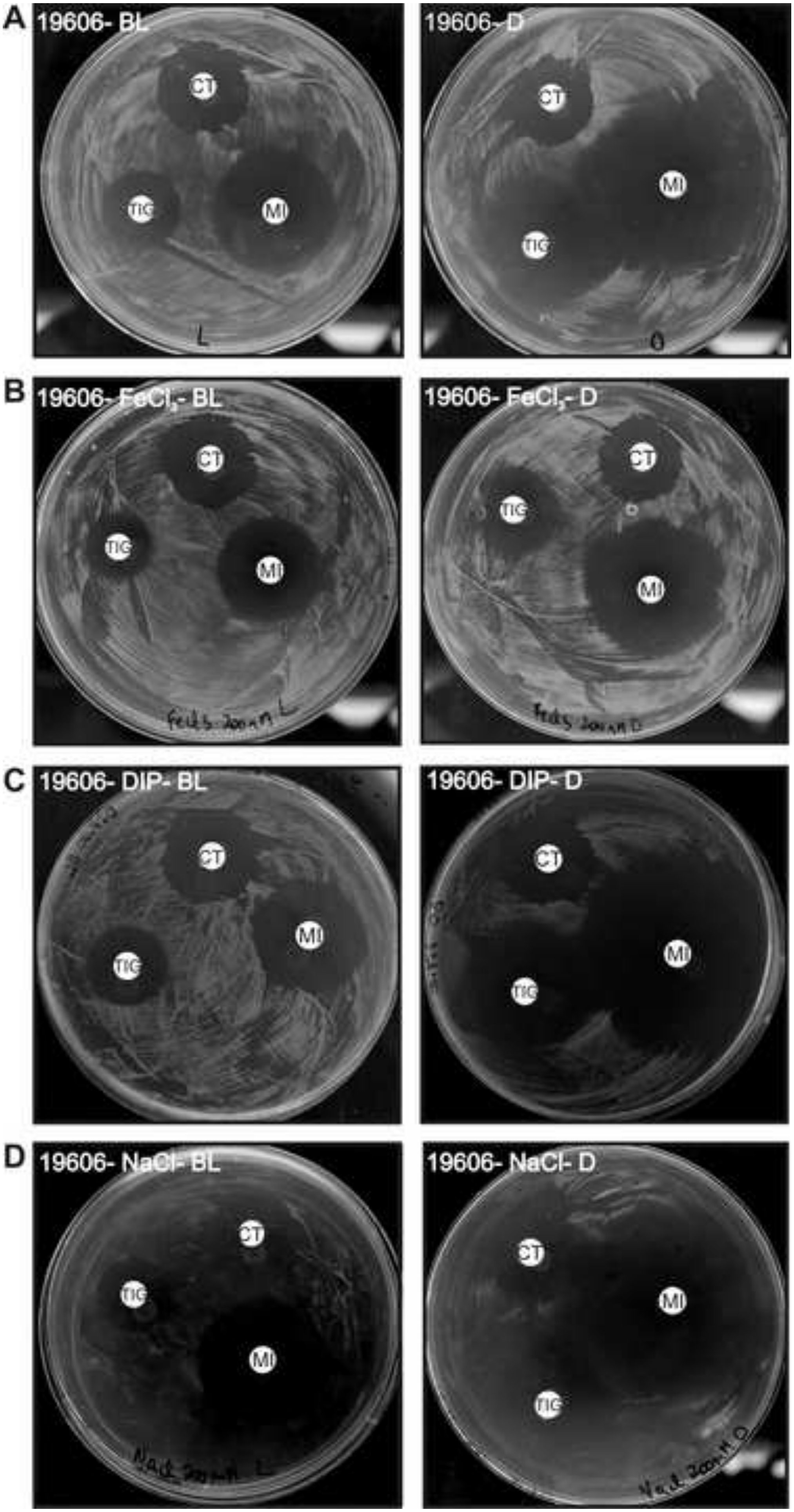

A
19606- BL
TIG
MI
19606- D
TIG
MI
B
19606.OR- BL
TIG
MI
19606.OR- D
TIG
MI

Figure 2


A
19606- BL
CT
TIG
MI
L
19606- D
CT
MI
TIG
D
B
19606- $FeCl_3$- BL
CT
TIG
MI
FeCl3 200mM L
19606- $FeCl_3$- D
CT
TIG
MI
FeCl3 200mM D
C
19606- DIP- BL
CT
MI
TIG
19606- DIP- D
CT
MI
TIG
D
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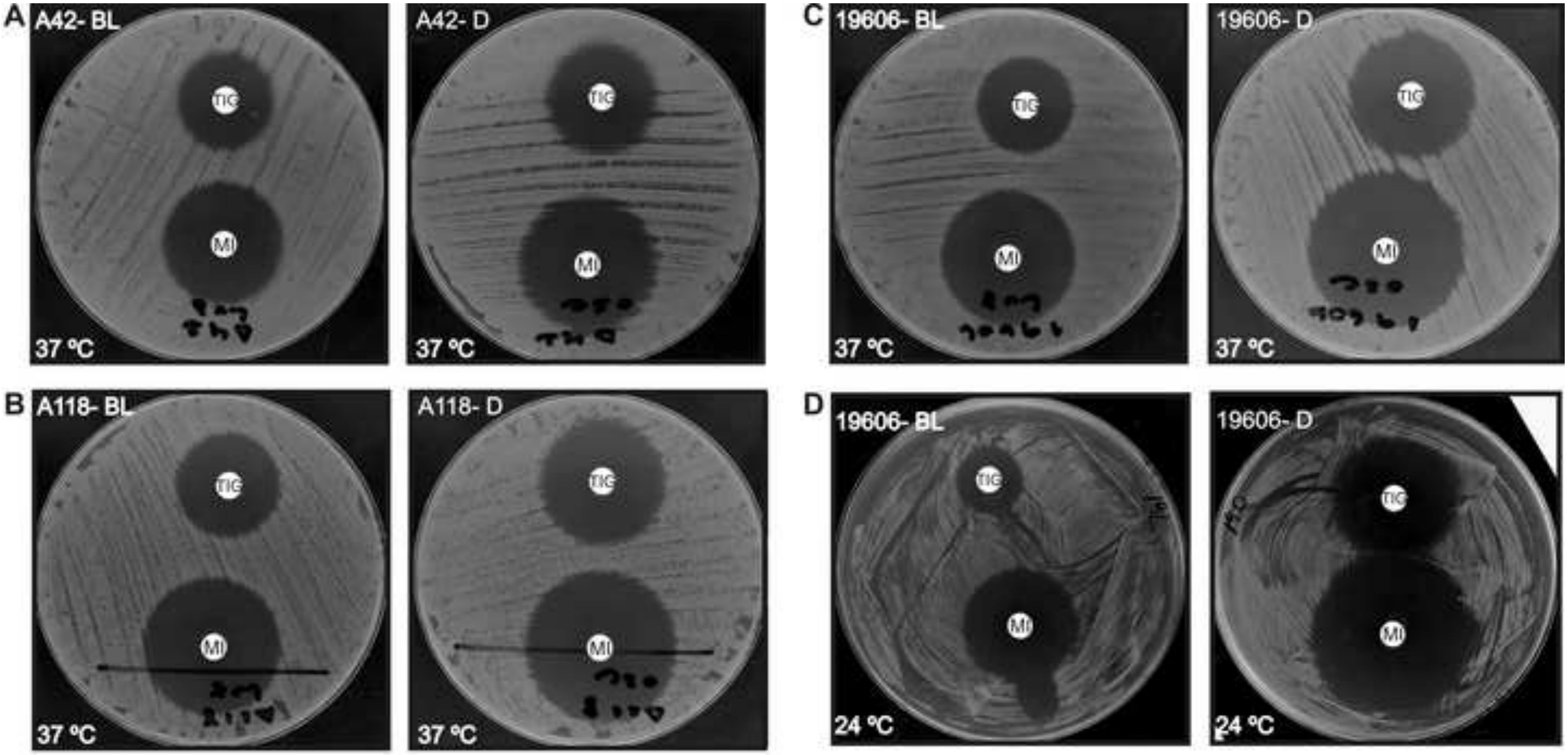
CT
TIG
MI
NaCl 200mM L
19606- NaCl- D
CT
MI
TIG
NaCl 200mM D

Figure 3



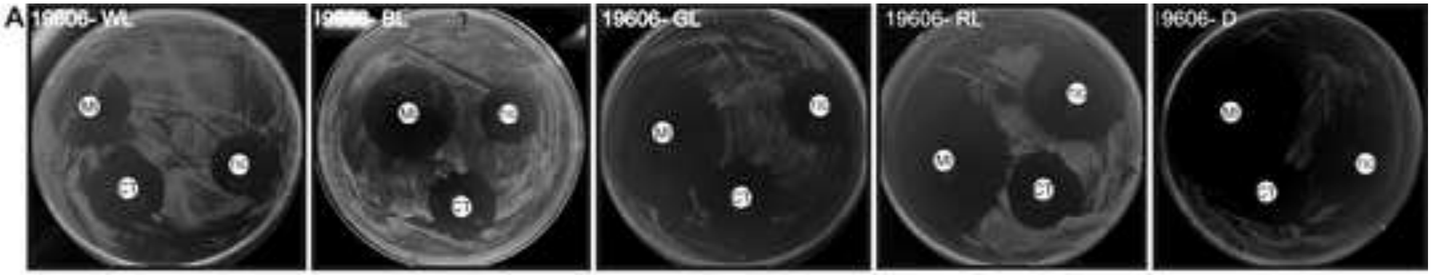
E

Strain- 37°C	MIN	TIG
A42L	28±1	22±1
A42D	31±1	25±1
A118L	33±1	25±1
A118D	35±1	29±2
ATCC 19606L	33±1	24±1
ATCC 19606D	36±1	29±1

Figure 4

Click here to download high resolution image

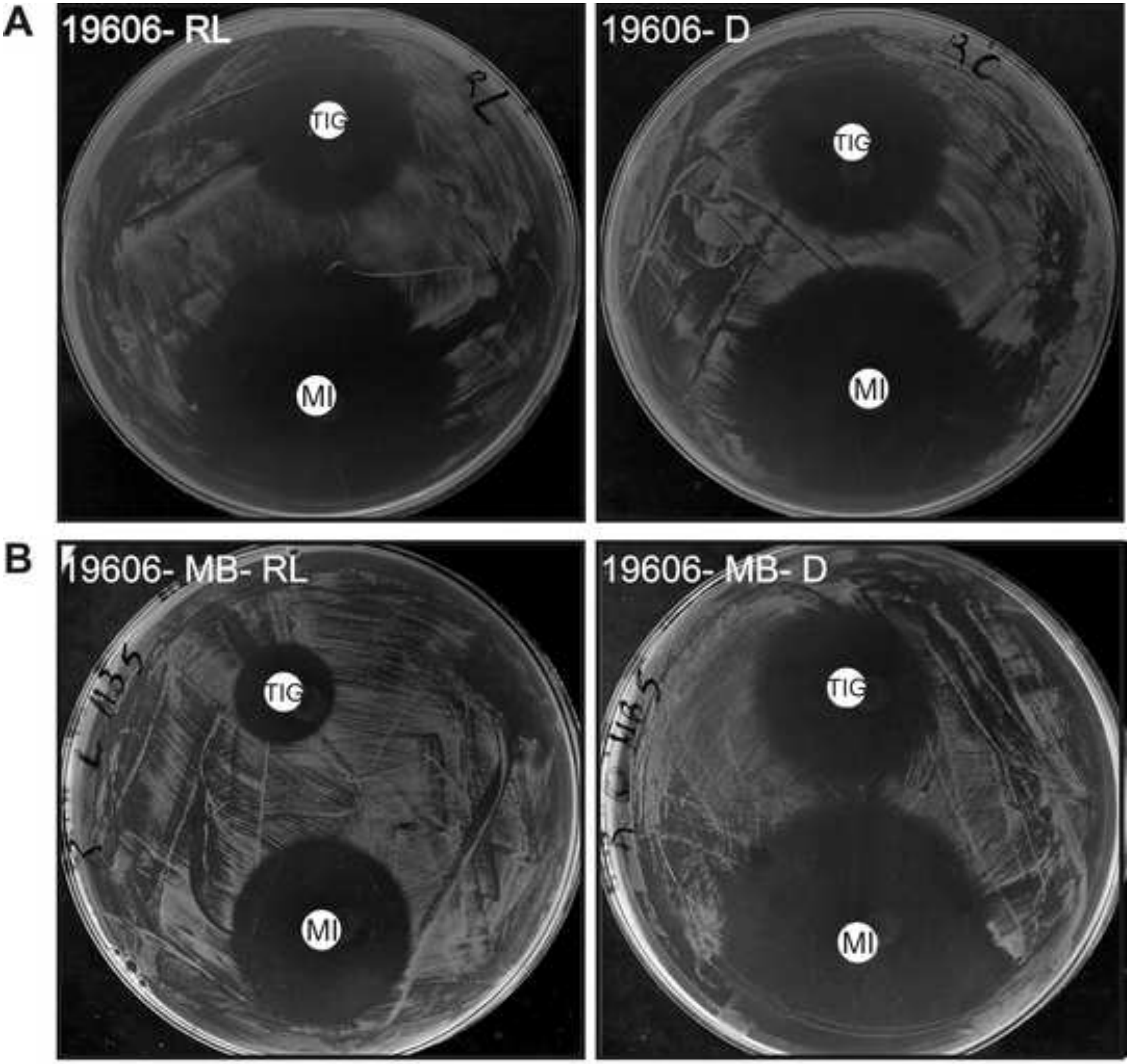
A



B **White and blue light modulate resistance to MIN and TIG in *A. baumannii*.**

LB Difco	MIN	TIG	COL
A. baumannii ATCC 19606			
WL	26±1	19±1	25±1
BL	27±1	18±1	24±1
GL	36±1	21±1	24±1
RL	43±1	30±1	24±1
D	42±2	30±2	22±1

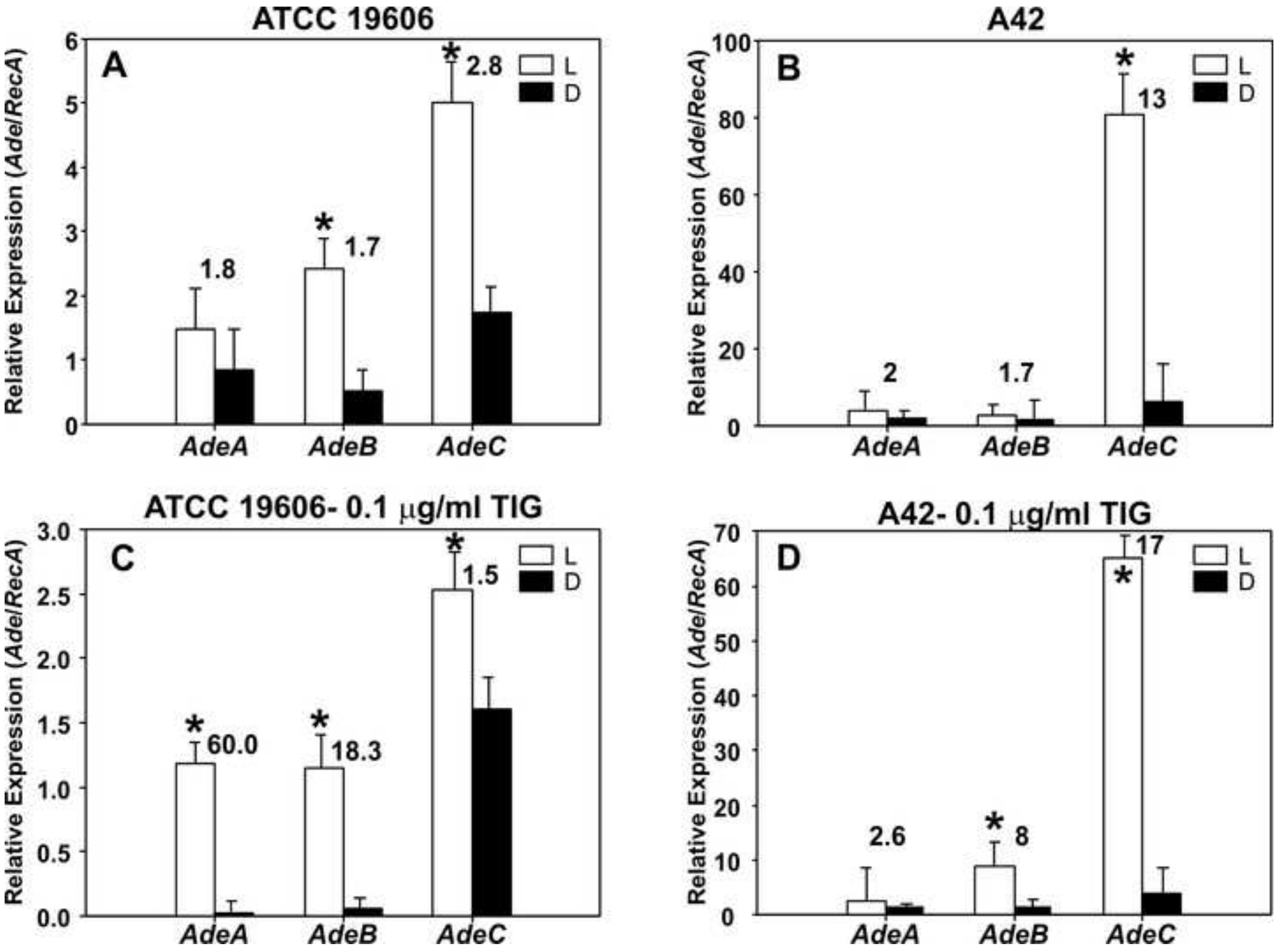
Figure 5



C

	MIN	TIG
ATCC 19606 RL	42±1	28±1
ATCC 19606 D	41±1	30±1
ATCC 19606- MB RL	30±1	16±1
ATCC 19606- MB D	42±1	26±1

Figure 6


ATCC 19606
A
Relative Expression (Ade/RecA)
0
1
2
3
4
5
6
L
D
1.8
*
1.7
*
2.8
AdeA
AdeB
AdeC
A42
B
Relative Expression (Ade/RecA)
0
20
40
60
80
100
L
D
2
1.7
*
13
AdeA
AdeB
AdeC
ATCC 19606- 0.1 μg/ml TIG
C
Relative Expression (Ade/RecA)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
L
D
*
60.0
*
18.3
*
1.5
AdeA
AdeB
AdeC
A42- 0.1 μg/ml TIG
D
Relative Expression (Ade/RecA)
0
10
20
30
40
50
60
70
L
D
2.6
*
8
17
*
AdeA
AdeB
AdeC