The Expression of Type II TA System Genes Following Persister Cell Formation in *Pseudomonas Aeruginosa* Isolates in The Exponential and Stationary Phases

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Title page

The expression of type II TA system genes following persister cell formation in *Pseudomonas aeruginosa* isolates in the exponential and stationary phases

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Abstract

Objectives: Failure of infection therapy in the presence of antibiotics has become a major problem which has been mostly attributed to the ability of bacterial persister cell formation. Bacteria use various mechanisms to form persister cells in different phases, among which is the toxin-antitoxin (TA) systems. This study aimed at investigating the expression of type II TA system genes under the stress of ciprofloxacin and colistin antibiotics in the exponential and stationary phases.

Methods: To determine the effects of ciprofloxacin and colistin on persister cell formation in the exponential and stationary phases of *Pseudomonas aeruginosa* strains, colony counting was performed at different time intervals in the presence of 5-fold MIC of ciprofloxacin and colistin. In addition, the expression of *relBE*, *Xre-COG5654*, *vapBC*, and *Xre-GNAT* genes in *P. aeruginosa* isolates was assessed 3.5 h after antibiotic treatment in the exponential and stationary phases using qRT-PCR.

Results: Our results indicated the presence of persister phenotype of *P. aeruginosa* strains in the presence of 5-fold MIC of ciprofloxacin and colistin compared to the control after 3.5 h of incubation in the exponential and stationary phases. Also, the number of persister cells in the stationary phase was higher than that of the exponential phase. According to the results of qRT-PCR, ciprofloxacin and colistin may induce persister cells by increasing the expression of type II TA systems in stationary and exponential phases.

Conclusion: Ciprofloxacin and colistin may increase the formation of persister cells by affecting the expression of type II TA systems.

Keywords: Persister cell; Antibiotic ciprofloxacin and colistin; *p.aeruginosa*; TA systems; Real-time PCR; exponential and stationary phases

Introduction

Chronic wound infections are mostly due to the formation of a subpopulation of bacteria called “persister cells” which can survive in the presence antibiotics [1]. Formation of these cells can cause recurrent infections, often resulting in treatment failure. Most pathogenic bacteria can form persister cells, providing an opportunity for bacteria to become resistant and making infection treatment more challenging [2, 3]. Several mechanisms contribute to the formation of persister cells, one of which is the TA systems, and in particular type II TA systems. Toxin and antitoxin of type II TA systems are both proteins with a wide distribution in bacteria [4-6]. The mechanism of TA systems is based on a stable toxin and a labile antitoxin located on a plasmid or chromosome. Under stress conditions such as antibiotic exposure, cellular proteases degrade the antitoxins, thereby freeing the toxins to disrupt essential cellular processes (replication, translation, etc.) [7, 8]. Also, TA systems have the ability of controlling the secondary messenger 3',5'-cyclic diguanylic acid in response to stress and might have a role in persister cell formation [9]. For instance, in the model organism *Escherichia coli*, quorum sensing or general stress responses and

TA modules such as HipBA as global regulators have been linked with the regulation of the persister phenotype [9, 10]. *P. aeruginosa* is an opportunistic pathogen in humans. Patients with chronic diseases or immune system defects, such as immunodeficiency or burns, are more susceptible to infections. These infections often become chronic and show high mortality [11]. For instance, *P. aeruginosa* colonizes the lungs of patients with cystic fibrosis and is considered as a major cause of death in these patients [12]. Apart from intrinsic tolerance of *P. aeruginosa* against a broad spectrum of antibiotics, the presence of specialized persister cells makes it almost impossible to completely eradicate these bacterial populations [13].

Therefore, this study aimed to evaluate persister cell formation of *P. aeruginosa* PAO1 and clinical *P. aeruginosa* in the exponential and stationary phases after exposure to ciprofloxacin and colistin antibiotics. Moreover, due to the role of TA systems in persister cell formation, the effects of lethal concentrations of selected antibiotics were analyzed on the expression levels of type II TA systems in the studied isolates.

Materials and Methods

Bacterial strain and growth conditions

In this study, *P. aeruginosa* PAO1 and clinical *P. aeruginosa* isolates were used. The clinical *P. aeruginosa* was isolated from a 54-year-old patient admitted to the burn ward who died of severe burns and infection. (The ethics committee of the Iran University of medical sciences has approved the study protocol (code:IR.IUMS.FMD.REC.1398.161). The patient signed a written informed consent form before died). The exponential phase of the planktonic cells was prepared by the inoculation of 10^5 CFU/ml of bacteria in LB broth and incubation for 3 h at 37 °C. Stationary phase of the planktonic cells was obtained by the inoculation of 10^5 CFU/ml of bacterial cells in LB broth and incubation for 18 h at 37 °C [14].

Identification of type II TA systems

Type II TA systems in *P. aeruginosa* were predicted by TADB database and as shown in Table 1, specific primers were designed using NCBI designing primer tool [15].

Polymerase chain reaction (PCR) was performed to screen the studied isolates for the presence of type II TA system genes, including *relBE*, *Xre-COG5654*, *vapBC*, and *Xre-GNAT*. PCR was prepared in a total volume of 25 µl containing 12.5 µl MasterMix PCR (Ampliqon, Denmark), 1 µl of primers, 2 µl of template DNA and 6.5 µl DDW. Thermal cycling included an initial denaturation step at 95°C for 5 min; 34× (95 °C for 45 s, annealing at 58 °C for 45 s, and extension at 72°C for 30 s), with a final extension step at 72 °C for 5 min. PCR products were analyzed by electrophoresis on 1% (w/v) agarose gel (Merck, Germany) containing DNA safe stain. Agarose gels were visualized by gel documentation (Bio-rad, USA).

Minimum inhibitory concentration (MIC) determination

The microdilution method was used to determine the MIC of ciprofloxacin (Sigma Aldrich) and colistin (Sigma Aldrich) against *P. aeruginosa* isolates according to Clinical and Laboratory

Standards Institute (CLSI-2019). To determine the MICs of ciprofloxacin and colistin (Sigma Aldrich), 100 μ L of overnight bacterial culture (dilution of 1:100 in MHB, set according to 0.5 McFarland) and 512 mg/l of the tested antibiotic were serially diluted (512 mg/l to 0.25 mg/l) in the 96-well microtiter plates and incubated at 37 °C for 18 h. MIC was defined as the lowest concentration of the antibiotic that prevented the visible bacterial growth after 18 h of incubation. MIC values for the studied antibiotics were determined in triplicates [16].

Persistence assay in the exponential and stationary phases with antibiotics ciprofloxacin and colistin

Persister cells of *P. aeruginosa* isolates were investigated by exposing the isolates in the exponential and stationary phases to 5-fold MICs of ciprofloxacin and colistin. To measure the number of persister cells, a single colony of the isolates was inoculated into 5mL LB broth (Merck, Germany) for 24 h. Then, 50 μ l of sub-cultured isolates were re-suspended in 50 mL of LB broth and bacterial cultures were incubated at 37 °C in an incubator shaker (Bio-rad, USA) at 220 rpm until reaching an optical density (at 600 nm) of 0.4 after 3 h of incubation (exponential phase). Also the optical density of 0.6 after 18 h of incubation was designated as the stationary phase. In order to induce persister cells, isolates in the exponential and stationary phases were treated with 5-fold MICs of ciprofloxacin and colistin and incubated at 37 °C in an incubator shaker at 220 rpm for 3.5 h [17] .

Colony counting

The number of formed persister cells was determined by plating the antibiotic-treated cultures on LB agar plates and the subsequent measurement of colony forming unit (CFU) representing the number of cells that survived the antibiotic exposure. For this purpose, samples were washed twice using ice-cold PBS and were added to LB broth, and incubated at 37°C in incubator shaker at 220 rpm. Samples were subsequently diluted in the same buffer (PBS). Samples were plated on LB agar plates and cultured on LB agar for four times every 20 min. Bacterial culture without antibiotics was used as the control [17].

Quantitative real-time polymerase chain reaction

To evaluate the expression of type II TA system genes, RNA of isolates in the exponential and stationary phases was extracted in the presence of 5-fold MIC of ciprofloxacin and colistin. Bacteria were pelleted by centrifugation at 2500 $\times$ g for 15 min. Total RNA was isolated using the RNA extraction kit (Gene all, Korea). NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) and gel electrophoresis analysis were used to assess the concentration, quality, and integrity of the extracted RNA. Extracted RNA was then treated with DNaseI (Roche, Germany) according to the manufacturer's protocols and total RNA was reverse transcribed to cDNA using the cDNA synthesis Kit (Betabairn, Germany).

Finally, quantitative real-time PCR was performed in a Rotor-Gene thermal cycler (Corbett 6000; Australia) using SYBR Green method (AccuPower Green Star qPCR Master Mix; Bioneer; Korea). A total volume of 20 μ l reaction containing 2 μ l of cDNA, 12.5 μ l SYBR Green

MasterMix, 4.5 µl nuclease-free water, and 1 µl of each primer (5 pmol) was subjected to PCR as follows: an initial activation step at 95 °C for 12 min, 40 cycles of denaturation at 95°C for 20 s, annealing at 58 °C for 35 s. The 16s rRNA gene was used as an internal control for normalization of mRNA levels and fold changes. mRNA expression was calculated by the $2^{-\Delta\Delta C_t}$ method [18] .

Statistical analyses

All the statistical analyses were carried out by GraphPad Prism (GraphPad Software, Inc). Student's t-test (for two groups) and analysis of variance (two-way ANOVA) were used. P-value of less than 0.05 was considered as statistically significant.

Table 1. Primers used in this study .

Gene Names		Sequence (5'→3')	Annealing temperature	Product length	Reference
T.relE	F	GGACGCCATCTACGACCATT	58 °C	72	This study
	R	ACGATGTCCTGAACGGCTTT			
T. Xre-COG	F	CAATTGGTCGACACCCTGGA	58°C	177	This study
	R	GTAGAACAGGCTTGGCTCGT			
vapBC */Xre-PIN	F	CCCTGTGGGACGAATACGAG	58°C	76	This study
	R	AGGGTTTCCAGCTTGTCAG			
-/Xre-GNAT	F	CAAGCATGCCTTCGACAACC	58°C	178	This study
	R	GTCGGTGATGCTGTAGACGA			
16S. PSEU	F	GCCCTCAAGTTCGATTTCGC	58°C	99	This study
	R	ATGGAAGATCAGTGGCGTGG			

Result

Detection of type II TA system genes by PCR

The results indicated the existence of all the studied genes (*relBE*, *Xre-COG5654*, *vapBC*, *Xre-GNAT*) coding for the TA systems in the studied isolates.

Minimum inhibitory concentration of *P. aeruginosa* isolates

In this study, we first determined the MIC ranges of ciprofloxacin and colistin against *P. aeruginosa* strains using the broth microdilution assay. The MICs of antibiotics are shown in Fig. 1.

Fig. 1. Minimum Inhibitory Concentrations (mg/mL) of ciprofloxacin and colistin against *P. aeruginosa* PAO1 and the clinical isolate.

Persister formation assay in the exponential and stationary phases and viable bacterial counts

To assess the effects of ciprofloxacin and colistin on bacterial survival in the exponential and stationary phases, isolates were exposed to 5-fold MICs of ciprofloxacin and colistin antibiotics for 3.5 h. Most isolates were killed by ciprofloxacin and colistin, and only a small portion (w1%) survived the challenge of these antibiotics. Viable counts were measured for 1 h at 20 min intervals. As shown in Fig. 2 and Fig. 3, the growth of *P. aeruginosa* isolates was strongly prevented in the presence of 5-fold MIC of ciprofloxacin and colistin. In the exponential and stationary phases, bacterial count reduced for 1 CFU/ml compared to the control, after 3.5 h exposure to the tested antibiotics. Accordingly, 5-fold MIC of ciprofloxacin and colistin reduced the bacterial growth. These results revealed the variations in bacterial survival following antibiotic treatment owing to persister cells. According to the statistical analysis, at times 20, 40, 60 and 80 min, the counts of PAO1 and clinical isolates in the exponential and stationary phases treated with ciprofloxacin and colistin were significantly different from the control group (Fig. 2, Fig. 3).

Fig. 2. Time-dependent killing of PAO1 and clinical isolates exposed to ciprofloxacin. PAO1 isolate (A: exponential phase and B: stationary phase) and clinical isolate (C: exponential phase and D: stationary phase). The bacterial culture without any antibiotic treatment served as the control. The values are indicative of the means of three biological replicates and error bars indicate the standard deviation (independent samples T test, repeated measure, LSD).

Fig. 3. Time-dependent killing of PAO1 and clinical isolates exposed to colistin. PAO1 isolate (A: exponential phase and B: stationary phase) and clinical isolate (C: exponential phase and D: stationary phase). Bacterial culture without any antibiotic treatment served as the control. The values are indicative of the means of three biological replicates and error bars indicate the standard deviation (independent samples T test, repeated measure, LSD).

Fig. 4. Time-dependent killing of PAO1 and clinical isolates in the exponential and stationary phases exposed to ciprofloxacin (A: PAO1 isolate and B: clinical isolate) and colistin (C: PAO1 isolate and D: clinical isolate). Graph data are indicated as the means $\pm$ SD of three independent replicates. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. **** $P < 0.0001$ (by independent samples T test).

Fig. 5. Time-dependent killing of PAO1 and clinical isolates exposed to ciprofloxacin (A: exponential phase and B: stationary phase) and colistin (C: exponential phase and D: stationary phase). Graph data are indicated as the means $\pm$ SD of three independent replicates. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. **** $P < 0.0001$ (by independent samples T test).

Fig. 6. Time-dependent killing of ciprofloxacin and colistin in *P. aeruginosa* PAO1 (A: exponential phase and B: stationary phase) and the clinical *P. aeruginosa* isolate (C: exponential phase and D: stationary phase). Graph data are indicated as the means $\pm$ SD of three independent replicates. *P < 0.05. **P < 0.01. ***P < 0.001. ****P < 0.0001 (by independent samples T test).

Effects of ciprofloxacin and colistin on the expression of type II TA system genes in *P. aeruginosa* in the exponential and stationary phases.

Our results showed the different expression levels of TA systems (*relBE*, *Xre-COG5654*, *vapBC*, *Xre-GNAT*) in *P. aeruginosa* strains in the exponential and stationary phases. Furthermore, the results of the binary comparison showed a significant difference between the expression of TA system genes (*relBE*, *Xre-COG5654*, *vapBC*, *Xre-GNAT*) in the standard strain (PAO1) and clinical isolate of *P. aeruginosa*. Persister cells the PAO1 strain formed after ciprofloxacin exposure showed the significant increased expression of *relBE/RHH-RelE*, */Xre-COG5654*, */Xre-GNAT*, *vapBC* */*Xre-PIN* TA systems in the exponential phase. A similar trend was observed in the clinical isolate, except for the *vapBC* */*Xre-PIN* system. In the stationary phase, the expression of *relBE/RHH-RelE* was decreased in the clinical isolate, and in the PAO1 strain, the expression of the *relBE/RHH-RelE* and *vapBC* */*Xre-PIN* was decreased (Fig. 7).

During the exponential phase of persister cells caused by colistin exposure, the expression of *vapBC* */*Xre-PIN* and */Xre-COG5654* systems decreased in both clinical and PAO1 isolates. In the stationary phase, the expression of */Xre-GNAT* and */Xre-COG5654* systems decreased for both clinical and PAO1 isolates (Fig. 7).

Fig. 7. Expression levels of type II TA system genes in the exponential and stationary phases in the presence of 5-fold MIC of ciprofloxacin (A: PAO1 strain B: clinical isolate) and colistin (C: PAO1 strain D: clinical isolate). Graph data are indicated as the means $\pm$ SD of three independent replicates. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. **** $P < 0.0001$ (by independent samples T test).

Fig. 8. Expression levels of type II TA system genes in PAO1 and clinical isolates in the presence of 5-fold MIC of ciprofloxacin (A: exponential phase B: stationary phase) and colistin (C: exponential phase D: stationary phase). Graph data are indicated as the means $\pm$ SD of three independent replicates. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. **** $P < 0.0001$ (by independent samples T test).

Fig. 9. Expression levels of type II TA system genes in the presence of 5-fold MIC of ciprofloxacin and colistin (A: exponential phase B: stationary phase) in PAO1 and clinical strains. Graph data are indicated as the means $\pm$ SD of three independent replicates. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. **** $P < 0.0001$ (by independent samples T test).

Discussion

Persistent infections are generally arising from difficult-to-remove pathogens that have not developed classical resistance. *P. aeruginosa* has the ability to adapt to the host environment, causing a chronic infection that cannot totally be removed by antibiotic treatment. Failure of infection treatment has been associated with the evolution of antibiotic resistance and also the presence of antibiotic-tolerant persister cells [19]. Different mechanisms have been introduced as the cause of persister cell formation, among which is the TA systems. TA systems can be classified into five groups based on their gene products (type I, II, III, IV or V). TA toxins can interact with RNA, ribosomes, or DNA gyrase, leading to a bacteriostatical or bacteriocidal reaction within cells and causing the formation of persister cells [20, 21]. In this study, we investigated the role of type II TA systems in the induction of persister cells in clinical and standard (PAO1) *P. aeruginosa* isolates by evaluating the expression of type II TA system genes (*relBE/RHH-RelE*, *vapBC* */*Xre-PIN*, *Xre-COG5654*, and *Xre-GNAT*) in the exponential and stationary phases after exposure to ciprofloxacin and colistin. In this study, we showed that type II TA systems were involved in the formation of persister cells in *P. aeruginosa* strains. Different expression levels of these systems were observed in the exponential and stationary phases, indicating different functions of TA systems in the formation of persister cells in different phases of bacterial growth (Fig. 7). Similar alterations in the expressions of type II TA system genes was observed in both bacterial isolates in the exponential and stationary phases (Fig. 8). Also, there was no difference between the ability of the tested antibiotics in changing the expression of the systems except for the *vapBC* system. In fact, expression of the *vapBC* system was different in both clinical and PAO1 strains in the exponential and stationary phases after exposure to ciprofloxacin and colistin antibiotics (fig9). In 2014, Oksana I. Demidenok et al. reported that the *vapBC* system in *Mycobacterium smegmatis* causes a dormant mode [22]. In a follow-up study by Cooper et al. in 2009, the global transcriptional analysis of *S. solfataricus* was carried out after heat shock (temperature shift from

80°C to 90 °C) and according to their results, several *vapBC* genes were triggered by the thermal shift, suggesting a role for this system in heat-shock-response [23]. In this study, we also showed that the expression of the *vapBC* system was different in *P. aeruginosa* compared to other type II TA systems in this study. These results are consistent with the studies mentioned above and indicate the role of this system in the formation of persister cells. What this study adds to the previous data is the different behavior of this system in the strains. Amraei F et al. showed that the increased expression of type II TA systems in *Brucella* may lead to antibiotic resistance and persister cell formation [24]. The contribution of TA systems in bacterial persister cell formation was initially discovered in 1983 by Harris Moyed. He found that two mutations in the *hipA* gene increased bacterial tolerance to antibiotics [25]. In this study, according to statistical analysis, the expression of type II TA systems varied depending on the stage of bacterial growth. In conclusion, at each phase different systems play a role depending on their mechanism of action.

To evaluate the formation of persister cells in the presence of ciprofloxacin and colistin in each strain, colonies were counted in stationary and exponential phases. The results showed that the rate of persister cells was different in exponential and stationary phases, such that the rate of persister cells in stationary phase was higher than exponential phase in both strains (Fig. 4). However, there was no significant difference between the two antibiotics in terms of inducing persister cells formation in the two strains. Also, there was no significant difference between PAO1 and clinical strains in terms of persister cell formation. In the exponential phase, similar numbers of persister cells were formed in both strains, and in the stationary phase there was no difference in the formation of persister cells in both strains after exposure to ciprofloxacin and colistin (Fig. 4, 5, 6). In 2018, Ying Wang et al. found a difference between the rate of persister cell formation in *Staphylococcus aureus* in the stationary and exponential phases after exposure to ciprofloxacin. They showed that the number of persister cells formed in the stationary phase was higher. They also observed that in the exponential phase, the α -ketoglutarate dehydrogenase genes of the tricarboxylic acid cycle (TCA) were expressed in small amounts during persister cell formation, but in the stationary phase, TCA genes were suppressed during the formation of persister cells, and therefore, more persister cells were formed [26]. Also, in 2004, Keren et al. concluded that when *S. aureus* enters the stationary phase, persister cell formation increases dramatically [27]. In 2009, Ilana Kolodkin-Gal et al., found that in *Escherichia coli*, the sigma factor during the stationary phase is responsible for the resistance of dormant bacteria to mazEF-mediated cell death [28]. According to previous studies, suppressed expression of TCA genes or increased expression of sigma factor during the stationary phase may increase the formation of persister cells. Also, according to our study, difference in the expression of type II TA systems involved in the formation of persister cells in each phase may increase the formation of persister cells in the stationary phase compared to the exponential phase, which requires further studies.

Conclusion

The results of this study showed that the expression of type II TA system genes in persister cells of *P. aeruginosa* increased compared to the control in exponential and stationary phases. More studies are needed to identify the exact role of these genes in the formation of *P. aeruginosa* persister cells.

Declaration**Ethical approval and consent to participate**

The ethics committee of Iran University of Medical Sciences has approved the study protocol (code:IR.IUMS.FMD.REC.1398.161). All methods were performed in accordance with the relevant guidelines and regulations/declaration of Helsinki. The patient signed a written informed consent form.

Consent for publication

Not applicable

Availability of data and material

All the datasets supporting the conclusions of this article are available. Additional data of this paper can be obtained upon request. The corresponding author (Faramarz Masjedian Jazi: Masjedian.f@iums.ac.ir) should be contacted if someone wants to request the data from this study.

Competing interests

The authors declare that they have no competing interests.

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Authors 'contributions

This article was part of a study that contributed to an MSC thesis by Rezvan Golmoradi zadeh. Behrouz Sadeghi kalani and Faramarz Masjedian jazi designed the study. Rezvan Golmoradi zadeh and Maryam Mirshekar performed experiments of the research and drafted the manuscript and Mahmood Barati performed the statistical analysis. All authors read and approved the final manuscript.

Conflicts of interest

We have no conflicts of interest to disclose.

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Figures

Figure 1

Minimum Inhibitory Concentrations (mg/mL) of ciprofloxacin and colistin against *P. aeruginosa* PAO1 and the clinical isolate.

Figure 2

Time-dependent killing of PAO1 and clinical isolates exposed to ciprofloxacin. PAO1 isolate (A: exponential phase and B: stationary phase) and clinical isolate (C: exponential phase and D: stationary phase). The bacterial culture without any antibiotic treatment served as the control. The values are indicative of the means of three biological replicates and error bars indicate the standard deviation (independent samples T test, repeated measure, LSD).

Figure 3

Time-dependent killing of PAO1 and clinical isolates exposed to colistin. PAO1 isolate (A: exponential phase and B: stationary phase) and clinical isolate (C: exponential phase and D: stationary phase). Bacterial culture without any antibiotic treatment served as the control. The values are indicative of the means of three biological replicates and error bars indicate the standard deviation (independent samples T test, repeated measure, LSD).

Figure 4

Time-dependent killing of PAO1 and clinical isolates in the exponential and stationary phases exposed to ciprofloxacin (A: PAO1 isolate and B: clinical isolate) and colistin (C: PAO1 isolate and D: clinical isolate). Graph data are indicated as the means $\pm$ SD of three independent replicates. *P < 0.05. **P < 0.01. ***P < 0.001. ****P < 0.0001 (by independent samples T test).

Figure 5

Time-dependent killing of PAO1 and clinical isolates exposed to ciprofloxacin (A: exponential phase and B: stationary phase) and colistin (C: exponential phase and D: stationary phase). Graph data are indicated as the means $\pm$ SD of three independent replicates. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. **** $P < 0.0001$ (by independent samples T test).

Figure 6

Time-dependent killing of ciprofloxacin and colistin in *P. aeruginosa* PAO1 (A: exponential phase and B: stationary phase) and the clinical *P. aeruginosa* isolate (C: exponential phase and D: stationary phase). Graph data are indicated as the means $\pm$ SD of three independent replicates. * $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$. **** $P < 0.0001$ (by independent samples T test).

Figure 7

Expression levels of type II TA system genes in the exponential and stationary phases in the presence of 5-fold MIC of ciprofloxacin (A: PAO1 strain B: clinical isolate) and colistin (C: PAO1 strain D: clinical isolate). Graph data are indicated as the means $\pm$ SD of three independent replicates. *P < 0.05. **P < 0.01. ***P < 0.001. ****P < 0.0001 (by independent samples T test).

Figure 8

Expression levels of type II TA system genes in PAO1 and clinical isolates in the presence of 5-fold MIC of ciprofloxacin (A: exponential phase B: stationary phase) and colistin (C: exponential phase D: stationary phase). Graph data are indicated as the means $\pm$ SD of three independent replicates. *P < 0.05. **P < 0.01. ***P < 0.001. ****P < 0.0001 (by independent samples T test).

Figure 9

Expression levels of type II TA system genes in the presence of 5-fold MIC of ciprofloxacin and colistin (A: exponential phase B: stationary phase) in PAO1 and clinical strains. Graph data are indicated as the means $\pm$ SD of three independent replicates. *P < 0.05. **P < 0.01. ***P < 0.001. ****P < 0.0001 (by independent samples T test).