International Journal of Antimicrobial Agents 53 (2019) 467–473

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: www.elsevier.com/locate/ijantimicag

Impact of zinc uptake regulator Zur on the susceptibility and oxidative stress response of *Acinetobacter baumannii* to antibiotics

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ARTICLE INFO

Article history:
Received 20 September 2018
Accepted 24 November 2018

Editor: Jean-Marc Rolain

Keywords:
Acinetobacter baumannii
Reactive oxygen species
Zinc uptake regulator
Zur
Glutathione

ABSTRACT

Zinc is a highly coveted redox-inactive micronutrient required for the growth and virulence of *Acinetobacter baumannii.* In this study, the role of the zinc uptake regulator Zur in the susceptibility and oxidative stress response of *A. baumannii* to antibiotics was evaluated. Inactivation of *zur* increased the susceptibility of *A. baumannii* AB5075 to colistin, gentamicin, rifampicin and tigecycline. Furthermore, activities of superoxide dismutase and catalase decreased significantly in the Δ*zur* mutant compared with the parental strain. Colistin, gentamicin, rifampicin and tigecycline raised the superoxide anion radical ($\cdot O_2^-$) and hydrogen peroxide (H_2O_2) contents of the Δ*zur* mutant compared with the parental strain. In addition, the antibiotics lowered glutathione and concomitantly raised glutathione disulphide levels in the Δ*zur* mutant. All of the antibiotics, except tigecycline, significantly raised the NAD^+/NADH and ADP/ATP ratios in *A. baumannii*. We conclude that decreased capability of the Δ*zur* mutant to detoxify reactive oxygen species increased its susceptibility to antibiotics.

1. Introduction

Acinetobacter baumannii is a Gram-negative, opportunistic pathogen responsible for nosocomial infections including pneumonia, urinary tract infection, burn infection, secondary meningitis and systemic infections [1]. Ever-increasing rates of resistance to antibiotics [2–5] make *A. baumannii* a significant human pathogen with a critical need for new antibiotic development [6]. The ability of *A. baumannii* to tolerate desiccation and disinfection [7] and to form biofilms [8] contribute to morbidity and mortality [3]. The importance of micronutrients, including zinc, on the virulence of *A. baumannii* has been demonstrated [9,10].

Zinc is a redox-inactive metal that is a component of structural, catalytic and regulatory proteins [11,12]. At physiological concentration, Zn, being a cofactor of superoxide dismutase (SOD), protects against free radical formation [13,14]. However, it is toxic at high concentrations owing to mismetallation of non-zinc proteins [15]. Thus bacteria, including *A. baumannii*, have evolved regulatory mechanisms to control the intracellular zinc concentration [14,16].

In *A. baumannii*, the zinc uptake regulator Zur, a member of the ferric uptake regulator (Fur) family, regulates zinc homeostasis [17]. In Zn-replete conditions, Zur binds Zn^{2+} leading to repression of Zn acquisition proteins (ZnuABC), Zn transporters (ZnuD1 and D2) and the TonB system that provides energy for Zn transport [18]. Although zinc is redox-inactive in vivo, Eijkelkamp et al. reported compromised redox balance in favour of reactive oxygen species (ROS) production [19]. In *A. baumannii, zur* deletion increased susceptibility to β-lactam antibiotics [9]. Furthermore, expression of genes engaged in ROS detoxification as well as *soxR*, which is responsible for sensing superoxide stress, were grossly decreased [17]. Although it is expected that SOD activity decreases in response to lowered *soxR* expression, Sein-Echaluce et al. reported significantly increased SOD and catalase activities in a *zur* mutant of *Anabaena* sp. PCC 7120 [11].

The contributory role of oxidative stress to antibiotic-mediated killing of bacteria, including *A. baumannii*, has been widely reported [20–23]. We hypothesised that *zur* deletion would enhance susceptibility to antibiotics owing to a reduced ability to detoxify ROS. To this end, the oxidative response of a Δ*zur* mutant strain of the wild-type (WT) *A. baumannii* AB5075 to antibiotics was investigated.

2. Materials and methods

2.1. Strains and culture conditions

Acinetobacter baumannii WT strain AB5075 and its *zur* mutant (AB09851, designated Δ*zur*) were obtained from the

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https://doi.org/10.1016/j.ijantimicag.2018.11.022

A. baumannii transposon mutant library at http://tools.uwgenomics.org/tn_mutants/ [9]. All strains were grown at 37°C in Luria–Bertani (LB) broth or on LB agar supplemented with 5 µg/mL tetracycline to maintain the transposon insertion of the mutant.

2.2. Zinc concentration assay

The concentration of Zn in WT and Δ*zur A. baumannii* strains was determined using a Zinc Assay Kit (MAK032; Sigma-Aldrich). Briefly, *A. baumannii* cells were grown to exponential phase, were pelleted, washed and suspended in phosphate-buffered saline (PBS), and were homogenised using multiple freeze/thaw cycles. The extract was deproteinised by adding an equal volume of trichloroacetic acid (7%). The mixture was then centrifuged at 13 000 × *g* for 5 min to obtain the cell-free extract as the supernatant. Then, 50 µL of the cell-free extract was mixed with 200 µL of Zn reagent mix and the reaction was incubated at room temperature for 10 min. Absorbance of the reaction mixture was read at 560 nm.

2.3. Minimum inhibitory concentration (MIC) determination

MICs of colistin, gentamicin, rifampicin and tigecycline were determined using Etest strips (Liofilchem, Roseto degli Abruzzi, Italy). Briefly, LB agar plates were covered completely with appropriate cultures [optical density at 600 nm (OD_{600}) of ca. 0.5] and then the liquid was removed, Etest strips were deposited and the plates were incubated at 37°C for 18 h to determine the MIC. At least three independent MIC determinations were performed.

2.4. Time–kill assay

WT and Δ*zur* strains of *A. baumannii* were grown overnight at 37°C in LB broth supplemented with 5 µg/mL tetracycline to maintain the Δ*zur* cassette. Overnight cultures of WT and Δ*zur* were diluted to $OD_{600} = 1$ in LB medium without antibiotics. Then, 1 mL of the resulting culture was inoculated into 50 mL of LB medium in a 250-mL baffled flask and was grown aerobically in a shaking incubator (150 rpm) at 37°C. At exponential growth ($OD_{600} = 0.5$), colistin, gentamicin, rifampicin and tigecycline at 4 × MIC were added to the medium. The OD_{600} was read at 30-min intervals over a 3-h incubation period. Antibiotics were solubilised in dimethyl sulfoxide (DMSO) and were diluted with LB to achieve appropriate concentration of antibiotics in 0.04% DMSO and the desired concentrations of colistin, gentamicin, rifampicin and tigecycline (4 × MIC). All measurements were performed with these concentrations, unless otherwise stated.

2.5. Catalase

Catalase activity was determined using the procedure described by Shangari and O'Brien [24]. Briefly, WT and Δ*zur A. baumannii* strains in exponential phase were incubated with colistin, gentamicin, rifampicin or tigecycline (4 × MIC) at 37°C for 30 min. The cells were pelleted, washed, suspended in PBS and homogenised using multiple freeze/thaw cycles. The mixture was centrifuged at 13 000 × *g* for 5 min to obtain the cell-free extract as the supernatant. Then, 20 µL of cell-free extract was incubated with 65 µM H_2O_2 in 6.0 mM sodium phosphate buffer (pH 7.4) (100 µL). The reaction was stopped by adding 32.4 mM ammonium molybdate (100 µL) and then absorbance of the yellowish molybdate and H_2O_2 complex was read at 400 nm within 30–60 s using a VersaMax™ microplate reader (Molecular Devices, San Jose, CA).

2.6. Hydrogen peroxide (H_2O_2) assay

The H_2O_2 content of WT and Δ*zur A. baumannii* strains exposed to colistin, gentamicin, rifampicin or tigecycline was estimated as outlined in the Peroxide Assay Kit (MAK311; Sigma-Aldrich). Briefly, WT and Δ*zur* strains of *A. baumannii* at exponential phase were incubated with colistin, gentamicin, rifampicin or tigecycline (4 × MIC) at 37°C for 30 min. H_2O_2 in the cell-free extract (prepared as described for the catalase assay) oxidises Fe^{2+} and the absorbance of the purple Fe^{3+}–xylenol complex was read at 585 nm.

2.7. Superoxide dismutase (SOD)

The activity of SOD in *A. baumannii* was determined as described by Misra and Fridovich [25]. Cell-free extract of WT and Δ*zur A. baumannii* strains exposed to colistin, gentamicin, rifampicin or tigecycline (4 × MIC) was prepared as described for the catalase assay. Briefly, 15 µL of cell-free extract was added to 125 µL of 0.05 mol/L carbonate buffer (pH 10.2) and the reaction was started by adding 15 µL of freshly prepared epinephrine (0.3 mmol/L). The increase in absorbance at 480 nm (A_{480}) was read every 30 s for 150 s using a VersaMax™ microplate reader. One unit of enzyme activity was defined as 50% inhibition of the rate of autoxidation of epinephrine as determined by the change in A_{480}/min.

2.8. Superoxide anion radical ($\cdot O_2^-$) generation

WT and Δ*zur A. baumannii* cells were grown to exponential phase and were incubated with colistin, gentamicin, rifampicin or tigecycline (4 × MIC), followed by addition of nitroblue tetrazolium (0.5 mL; 1 mg/mL), and were incubated at 37°C for 30 min. Following incubation, 0.1 mL of HCl (0.1 M) was added and the resulting mixture was centrifuged at 1500 × *g* for 10 min. Reduced nitroblue tetrazolium in the pellets was extracted with DMSO (0.04%), was diluted with 0.8 mL of PBS (pH 7.5) and the absorbance was read at 575 nm. The superoxide anion content of the cells was calculated using the molar extinction coefficient of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) formazan (17 000 $M^{-1}cm^{-1}$ at pH 7.4–8.0) as described by Ajiboye et al. [26].

2.9. Glutathione (GSH) and glutathione disulfide (GSSG) assay

Reduced GSH in WT and Δ*zur* strains of *A. baumannii* exposed to colistin, gentamicin, rifampicin or tigecycline was as outlined in the Glutathione Assay Kit (CS0260; Sigma-Aldrich). WT and Δ*zur* cells of *A. baumannii* at exponential phase were incubated with colistin, gentamicin, rifampicin or tigecycline (4 × MIC) at 37°C for 30 min. The incubated cells were pelleted, were washed with PBS and were treated with 5-sulfosalicylic acid (5%). The resulting mixture was subjected to two freeze/thaw cycles, followed by centrifugation at 10 000 × *g* for 10 min to obtain the cell-free extract as the supernatant. Then, 10 µL of cell-free extract was mixed with working reagent [final concentrations, 95 mM potassium phosphate buffer (pH 7.0), 0.95 mM ethylene diamine tetra-acetic acid (EDTA), 0.115 U/mL glutathione reductase, 48 µM NADPH and 0.031 mg/mL DTNB]. The blank was constituted the same way except that the sample was replaced with 5% sulfosalicylic acid. Absorbance was read at 412 nm in a microplate reader. The concentration of GSH in the cell-free extract was obtained from the GSH standard curve. The glutathione disulfide contents of the cell free extract was estimated as described by Ajiboye et al. [26].

2.10. NAD^+/NADH ratio

The NAD^+/NADH ratio in cells treated with or without colistin, gentamicin, rifampicin or tigecycline was estimated using the procedure outlined in the NAD/NADH Quantification Kit (MAK037; Sigma-Aldrich). Exponential-phase *A. baumannii* cells were treated as detailed in the GSH assay. Following 30 min of incubation, cells were washed with cold PBS and were centrifuged at 2000 rpm for 5 min. The cells were treated with NADH/NAD extraction buffer through two freeze/thaw cycles of 20 min on dry ice, followed by 10 min at room temperature. The cell-free extract was separated by centrifuging the samples at 13 000 × *g* for 10 min. Then, 50 µL of cell-free extract was treated with 50 µL of NADH/NAD extraction buffer, followed by 100 µL of master reaction mix (NAD cycling buffer and NAD cycling enzyme). The reaction was mixed and was incubated for 5 min at room temperature, after which 10 µL of NADH developer was added. Samples were incubated at room temperature for 2 h and the absorbance was read at 450 nm.

2.11. ADP/ATP ratio

The ADP/ATP ratio was assessed using the procedure detailed in the ADP/ATP Ratio Assay Kit (MAK135; Sigma-Aldrich). Exponential-phase *A. baumannii* cells were treated as described in the GSH assay. Following 30 min of incubation, 90 µL of the cells was mixed with an equal volume of ATP reagent and was incubated for 1 min at room temperature. Luminescence [relative light units (RLU)] was read for ATP (RLU_A). The mixture was then incubated for an additional 10 min and the luminescence for ATP (RLU_B) was read to provide the background before ADP measurement. Immediately after RLU_B reading, 5 µL of ADP reagent was added and mixed and the luminescence was read after 1 min (RLU_C). ADP/ATP was estimated using the expression below:

$$= \frac{RLU_C - RLU_B}{RLU_A}$$

2.12. Protein concentration assay

The concentration of protein in the cell-free extracts of WT and Δ*zur* *A. baumannii* was determined as described by Bradford [27]. Briefly, 5 µL of cell-free extract was mixed with 250 µL of Coomassie reagent. The mixture was incubated at room temperature for 10 min and the absorbance was read at 595 nm.

2.13. Statistical analysis

The results were expressed as the mean ± standard error of the mean of three independent experiments. Differences between treatments were determined by analysis of variance (ANOVA) followed by Dunnett's post-hoc test using GraphPad Prism for Windows v.6.01 (GraphPad Software Inc., La Jolla, CA).

3. Results

Zur regulates Zn^{2+} homeostasis in bacteria, including in *A. baumannii* [17]. In this study, the level of Zn in a Δ*zur* mutant of *A. baumannii* AB5075 was assessed and was compared with the parental *A. baumannii* strain. As expected, the Δ*zur* mutant exhibited an increased Zn concentration compared with the WT (Fig. 1). Next, the MICs of colistin, gentamicin, rifampicin and tigecycline were determined both for the Δ*zur* mutant and its parental strain. Consistent with Gebhardt et al. [9], significant ($P < 0.05$) decreases in the MICs of colistin, gentamicin, rifampicin and tigecycline were observed in the Δ*zur* mutant compared with the WT (Fig. 2A). Furthermore, the Δ*zur* mutant showed a slight growth defect compared with the parental strain (Fig. 2B).

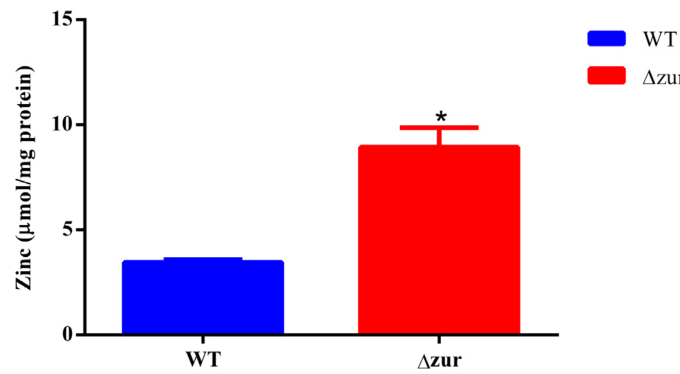

Fig. 1. Zinc concentration in the cell-free extract of wild-type (WT) *Acinetobacter baumannii* (AB5075) and the Δ*zur* mutant strain (AB09851). * Significantly different ($P < 0.05$). Results are expressed as the mean ± standard error of the mean of three independent experiments.

In addition, the susceptibility of the Δ*zur* mutant to colistin, gentamicin, rifampicin, tigecycline as well as to H_2O_2 was raised compared with the parental strain (Fig. 2C,D).

Deletion of *zur* was reported to lower the expression of catalase [17] and this could increase susceptibility to ROS-induced oxidative stress. Since ROS generation has been linked to antibiotic-induced bacterial death [20,22,28], we proposed that a reduced capability of the Δ*zur* mutant to detoxify H_2O_2 might be responsible for the increased antibiotic susceptibility. To validate this, first the activity of catalase in *A. baumannii* WT and Δ*zur* in the absence of antibiotics was assessed. Consistent with the findings of Mortensen et al. [17], the activity of catalase in the Δ*zur* mutant was significantly lowered compared with the WT strain (Fig. 3A). This activity was further lowered following exposure of WT and Δ*zur* cells to the antibiotics (Fig. 3A). Furthermore, colistin, gentamicin, rifampicin and tigecycline significantly raised the level of H_2O_2 in the Δ*zur* mutant compared with the WT strain (Fig. 3B).

The presence of Zn in Cu/Zn SOD explains its importance in ROS detoxification. Indeed, *zur* deletion in *Anabaena* spp. PCC 7120 raised SOD activity [11]. Thus, we evaluated the impact of *zur* deletion on the activity of SOD and, contrary to Sein-Echaluce et al. [11], we noted a significant decrease in SOD for the Δ*zur* mutant compared with the WT (Fig. 4A). Antibiotics further lowered SOD activity in the Δ*zur* mutant compared with the WT strain (Fig. 4A). Antibiotics can increase respiratory activity leading to enhanced $\cdot O_2^-$ generation [29–31]. Thus, it seems plausible that the diminished SOD activity will reduce the capability of the Δ*zur* mutant to detoxify superoxide leading to the increased susceptibility to antibiotics observed in this study. To test this, we assessed $\cdot O_2^-$ in *A. baumannii* WT and Δ*zur* following exposure to colistin, gentamicin, rifampicin or tigecycline. The level of $\cdot O_2^-$ in the Δ*zur* mutant increased significantly compared with the WT *A. baumannii* strain (Fig. 4B).

Catalase is not the only existing mechanism for H_2O_2 detoxification [32–34]; indeed, reduced GSH is also involved [35,36]. Thus, we sought to investigate the level of GSH in *A. baumannii* exposed to these antibiotics. The GSH content of the Δ*zur* mutant strain exposed to colistin, gentamicin, rifampicin and tigecycline decreased significantly compared with the WT strain (Fig. 5A). The connection between zinc metabolism and thiol proteins and peptides, including GSH, prompted us to ask what was responsible for the reduced GSH. To this end, the level of glutathione disulphide (GSSG) was assessed because it is the product of H_2O_2-mediated oxidation of GSH. The level of GSSG increased significantly in the Δ*zur* mutant of *A. baumannii* exposed to colistin, gentamicin, rifampicin and tigecycline compared with the WT strain (Fig. 5B). Indeed, the increased H_2O_2 observed in the Δ*zur* strain of *A. baumannii* (Fig. 3B) suggests that the GSH system is not sufficient for detoxification.

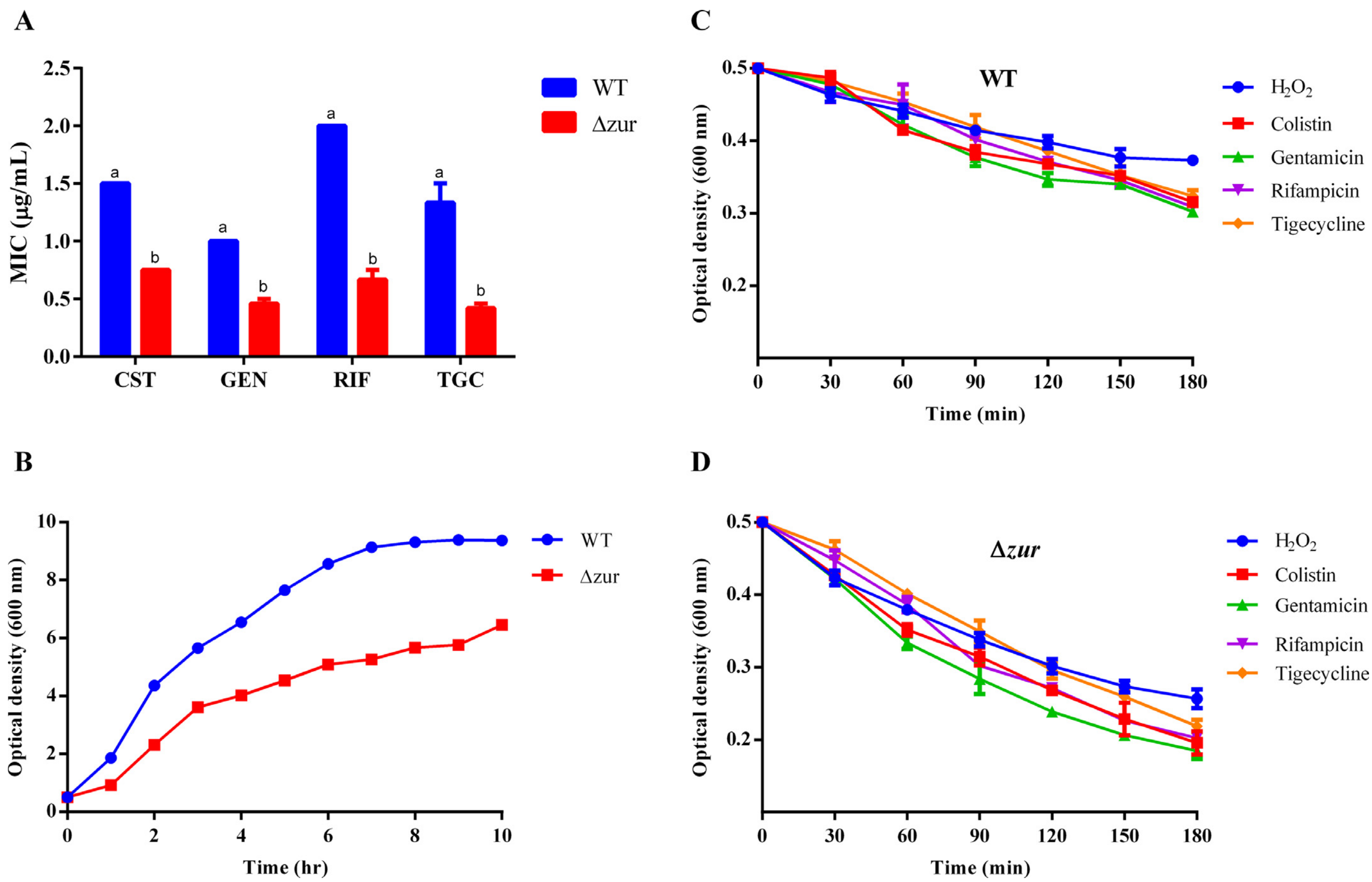

Fig. 2. Inactivation of *zur* increases the susceptibility to antibiotics. (A) Minimum inhibitory concentrations (MICs) of colistin (CST), gentamicin (GEN), rifampicin (RIF) and tigecycline (TGC) against wild-type (WT) *Acinetobacter baumannii* (AB5075) and the Δ*zur* mutant strain (AB09851). (B) Growth curve of WT and Δ*zur* mutant strains. (C,D) Time–kill assays of WT (C) and Δ*zur* mutant strains to H_2O_2 (5 mM), colistin, gentamicin, rifampicin and tigecycline (4 × MIC). Bars with different superscript letters are significantly different ($P < 0.05$).

Antibiotic-mediated fuelling of electron transport chain activity with NADH [37–39] is responsible for increased electron leakage and ROS generation. We assessed the influence of electron transport on ROS generation in colistin-, gentamicin-, rifampicin- and tigecycline-treated Δ*zur* mutant and WT strains of *A. baumannii*. The antibiotics used in this study, except tigecycline, significantly raised the NAD^+/NADH ratio both in WT and Δ*zur* mutant strains of *A. baumannii* compared with untreated strains (Fig. 6A). However, antibiotic-mediated increases in the NAD^+/NADH ratio were not significantly different in the WT and Δ*zur* mutant strains. Similarly, the ADP/ATP ratio, an index of redox status in bacteria [40], increased significantly in colistin-, gentamicin- and rifampicin-treated, but not tigecycline-treated, *A. baumannii* WT and Δ*zur* mutant strains (Fig. 6B).

4. Discussion

Transition metals, including zinc, are indispensable for the biochemistry of life owing to their involvement in enzyme catalysis and regulatory function [12]. Despite this, organisms including *A. baumannii* have evolved regulatory mechanisms to avoid toxicity resulting from overload [18]. In this study, we present the influence of the zinc uptake regulator Zur on the oxidative stress response and susceptibility of *A. baumannii* to antibiotics.

The susceptibility of a *zur* mutant compared with WT strains of *A. baumannii* to antibiotics including colistin, gentamicin and tigecycline has been reported [9]. In that study, deletion of *zur* had no significant effect on the susceptibility to colistin and gentamicin compared with the parental strain *A. baumannii* AB5075 [9]. Interestingly, Gebhardt et al. reported that the Δ*zur* mutant was more resistant to tigecycline, albeit without statistical significance [9]. In contrast, in the current study we noted increased susceptibility of the Δ*zur* mutant to colistin, gentamicin, rifampicin and tigecycline. The lowered MICs of colistin, gentamicin, rifampicin and tigecycline for the Δ*zur* mutant indicate increased susceptibility and the influence of Zur on antibiotic resistance.

Oxidative stress arising from increased ROS generation has been demonstrated and documented in antibiotic-induced bacterial lethality [20,26,39]. Consistent with these studies, the antibiotics (colistin, gentamicin, rifampicin and tigecycline) increased H_2O_2 and $\cdot O_2^-$ generation in *A. baumannii*. Interestingly, H_2O_2 and $\cdot O_2^-$ generation was more pronounced when the Δ*zur* mutant was treated with antibiotics, which could be due to lowered catalase activity in the mutant compared with the parental strain. Indeed, Mortensen et al. reported similarly decreased expression of catalase in a Δ*zur* mutant strain of *A. baumannii* [17]. The decreased SOD activity, which is contrary to previous studies [11], is expected because Mortensen et al. reported lowered *soxR* expression in the Δ*zur* mutant strain of *A. baumannii* [17]. It is plausible that the Δ*zur* mutant strain should have lowered SOD activity, as expression of its transcriptional regulator *soxR* is also lowered. Thus, increased $\cdot O_2^-$ content in the Δ*zur* mutant exposed to the antibiotics compared with the parental strain suggests reduced capability to detoxify ROS.

Enhanced ROS ($\cdot O_2^-$ and H_2O_2) generation, as noted in this study, has been reported to be a function of increased citric acid

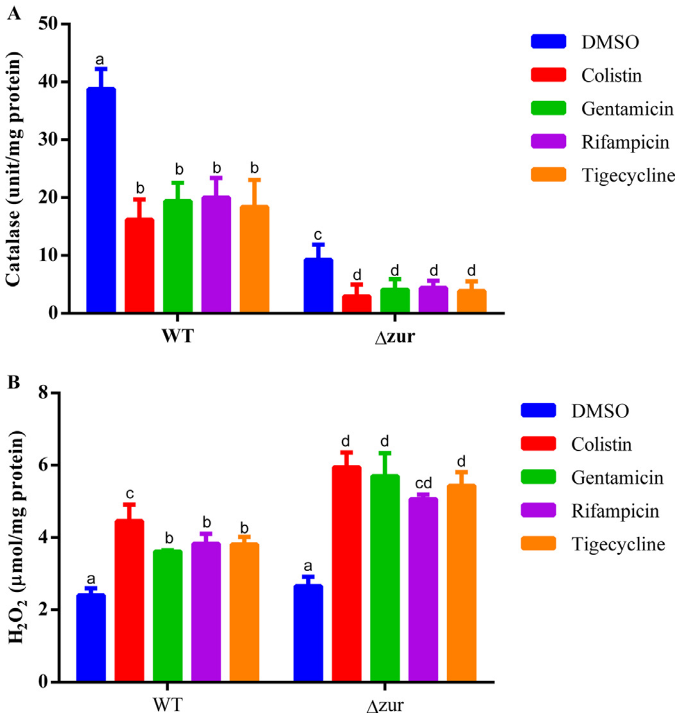

Fig. 3. (A) Activity of catalase in wild-type (WT) *Acinetobacter baumannii* (AB5075) and the Δ*zur* mutant strain (AB09851) exposed to colistin, gentamicin, rifampicin and tigecycline (4 × MIC); and (B) hydrogen peroxide (H_2O_2) generation in WT and Δ*zur* mutant strains exposed to colistin, gentamicin, rifampicin and tigecycline (4 × MIC). Bars with different superscript letters are significantly different ($P < 0.05$). Results are expressed as the mean ± standard error of the mean of three independent experiments. The MICs used for WT and Δ*zur* mutant strains are based on the values obtained in the current study. MIC, minimum inhibitory concentration.

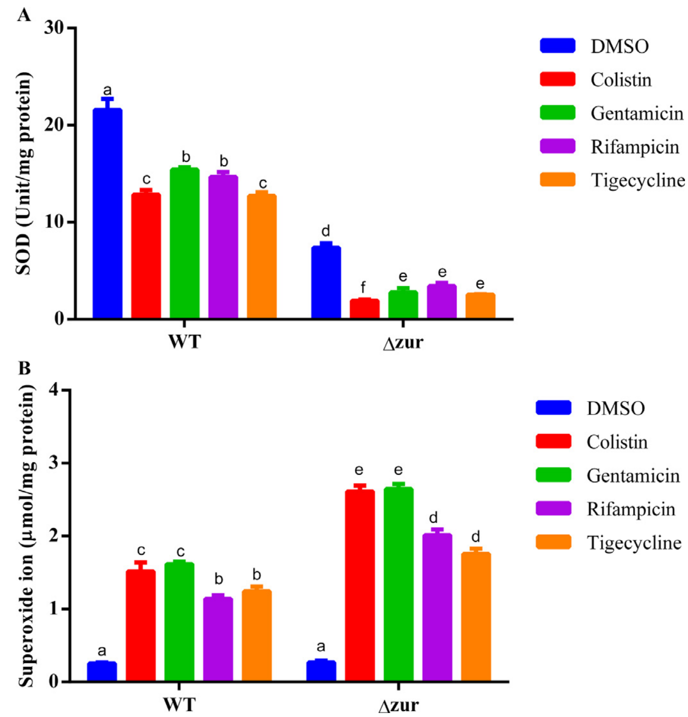

Fig. 4. (A) Activity of superoxide dismutase (SOD) in wild-type (WT) *Acinetobacter baumannii* (AB5075) and the Δ*zur* mutant strain (AB09851) exposed to colistin, gentamicin, rifampicin and tigecycline (4 × MIC); and (B) superoxide anion radical generation in WT and Δ*zur* mutant strains exposed to colistin, gentamicin, rifampicin and tigecycline (4 × MIC). Bars with different superscript letters are significantly different ($P < 0.05$). Results are expressed as the mean ± standard error of the mean of three independent experiments. The MICs used for WT and Δ*zur* mutant strains are based on the values obtained in this study. MIC, minimum inhibitory concentration.

cycle (TCA cycle) and electron transport chain activities [37]. All of the antibiotics, except tigecycline, increased the NAD^+/NADH ratio, an indicator of electron transport chain activity, suggesting the contribution of increased citric acid cycle and electron transport chain activities in antibiotic-mediated ROS increase. However, the reduced capability of the Δ*zur* mutant to detoxify ROS, as noted in the form of decreased SOD and catalase activities, potentiates the susceptibility to these antibiotics. Although $\cdot O_2^-$ and H_2O_2 generation increased significantly for tigecycline in the Δ*zur* mutant, there was no change in NAD^+/NADH and ADP/ATP ratios. This observation, which is in line with our previous findings [27,30], suggests that tigecycline-mediated ROS generation is not related to TCA cycle activity. ADP/ATP, an index of oxidative stress [40], is a reflection of respiratory intensity. The increase in the ADP/ATP ratio following exposure of *A. baumannii* to colistin, gentamicin or rifampicin suggests that ROS generation observed in this study is related to increased respiration [29]. However, the non-significant change in the ADP/ATP ratio of *A. baumannii* exposed to tigecycline further shows that the elevated ROS is independent of respiratory activity.

In this study, we have demonstrated that the increased susceptibility of a Δ*zur* strain of *A. baumannii* AB5075 to antibiotics is due to the decreased capability to detoxify ROS (Fig. 7). This is because SOD and catalase activities, the primary antioxidants re-

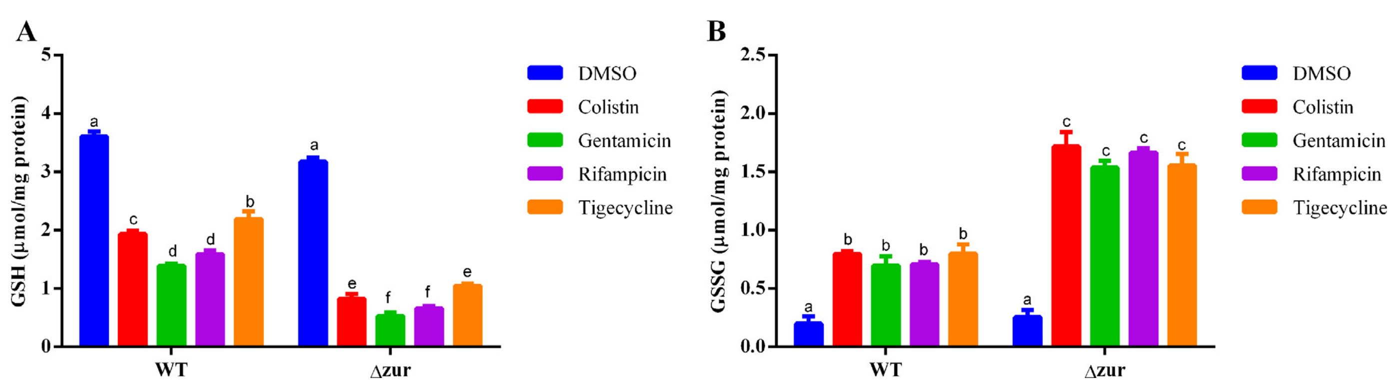

Fig. 5. Levels of (A) glutathione (GSH) and (B) glutathione disulphide (GSSG) in wild-type (WT) *Acinetobacter baumannii* (AB5075) and the Δ*zur* mutant strain (AB09851) exposed to colistin, gentamicin, rifampicin and tigecycline (4 × MIC). Bars with different superscript letters are significantly different ($P < 0.05$). Results are expressed as the mean ± standard error of the mean of three independent experiments. The MICs used for WT and Δ*zur* mutant strains are based on the values obtained in this study. MIC, minimum inhibitory concentration; DMSO, dimethyl sulfoxide.

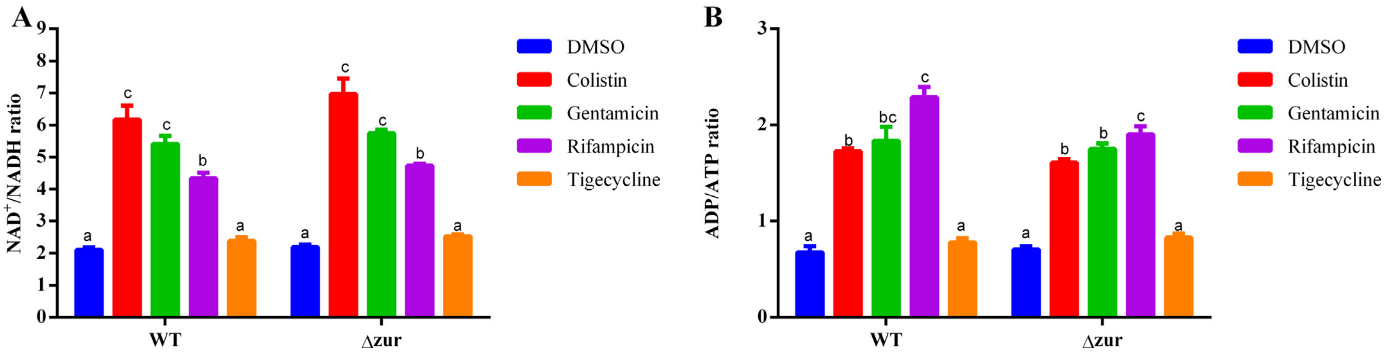

Fig. 6. (A) NAD^+/NADH and (B) ADP/ATP ratios in wild-type (WT) *Acinetobacter baumannii* (AB5075) and the Δ*zur* mutant strain (AB09851) exposed to colistin, gentamicin, rifampicin and tigecycline (4 × MIC). Bars with different superscript letters are significantly different ($P < 0.05$). Results are expressed as the mean ± standard error of the mean of three independent experiments. The MICs used for WT and Δ*zur* mutant strains are based on the values obtained in this study. MIC, minimum inhibitory concentration; DMSO, dimethyl sulfoxide.

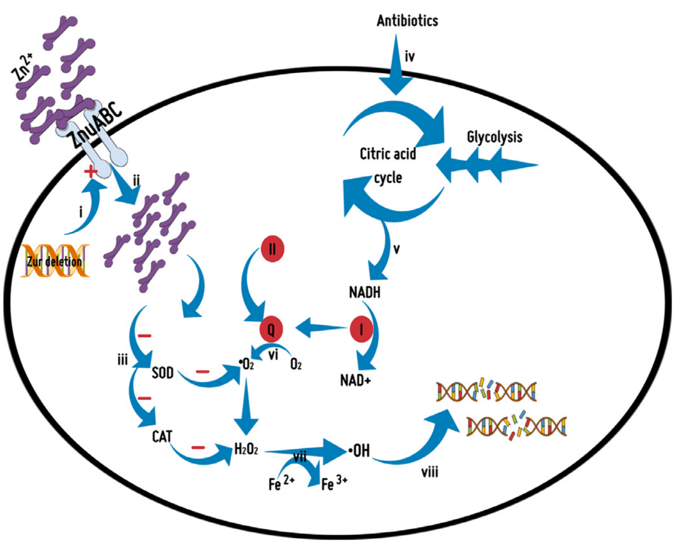

Fig. 7. Proposed mechanism of the impact of *zur* inactivation on the susceptibility of *Acinetobacter baumannii* to antibiotics. (i) Inactivation of *zur* prevents the regulation of Zn^{2+} transport by ZnuABC, (ii) leading to enhanced translocation of Zn^{2+} into *A. baumannii.* (iii) This event lowers the activities of superoxide dismutase (SOD) and catalase (CAT). (iv) Antibiotics stimulate the citric acid cycle (TCA cycle), (v) resulting in increased NADH generation via the TCA cycle and (vi) reactive oxygen species via electron transport following the transfer of an electron from NADH (at complex I) and $FADH_2$ (at complex II) to ubiquinone (Q). (vii) Since *zur* inactivation decreases the activities of SOD and CAT activities, hydrogen peroxide (H_2O_2) is available for the Fenton reaction. (viii) Hydroxyl radicals generated damage DNA and ultimately lead to *A. baumannii* death.

sponsible for the detoxification of ROS, decreased significantly in the antibiotic-treated Δ*zur* strain of *A. baumannii*.

Funding

TOA is the recipient of a TWAS-DFG Visiting Research Fellowship to Project Group P2, Robert Koch-Institut (Wernigerode, Germany) [WI 3272/4-1]. Work in this group is supported by the Deutsche Forschungsgemeinschaft (DFG) within FOR 2251.

Competing interests

None declared.

Ethical approval

Not required.

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