DnaK and GroEL are induced in response to antibiotic and heat shock in *Acinetobacter baumannii*

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We studied the expression of DnaK and GroEL in *Acinetobacter baumannii* cells (strains ATCC 19606 and RS4) under stress caused by heat shock or antibiotics. A Western blot assay showed that DnaK and GroEL levels increased transiently more than 2-fold after exposure of bacterial cells to heat shock for 20 min at 50 °C. Heat induction of DnaK and GroEL was blocked completely when an inhibitor of transcription, rifampicin, was added 1 min before a temperature upshift to 50 °C, suggesting that the induction of these chaperones depends on transcription. *A. baumannii* cells pretreated at 45 °C for 30 min were better able to survive at 50 °C for 60 min than cells pretreated at 37 °C, indicating that *A. baumannii* is able to acquire thermotolerance. DnaK and GroEL were successfully induced in cells pre-incubated with a subinhibitory concentration of streptomycin. Moreover, bacterial cells pretreated for 30 min at 45 °C were better able to survive streptomycin exposure than cells pretreated at physiological temperatures. DnaK expression was upregulated in a multidrug-resistant strain of *A. baumannii* (RS4) in the presence of different antimicrobials (ampicillin + sulbactam, cefepime, meropenem and sulphamethoxazole + trimethoprim). This study is to the best of our knowledge the first to show that *A. baumannii* DnaK and GroEL could play an important role in the stress response induced by antibiotics.

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INTRODUCTION

Acinetobacter baumannii is a non-fermentative, Gram-negative bacterium considered to be one of the most important opportunistic human pathogens. It can cause serious disease in compromised patients, including pneumonia, septicaemia, meningitis and urinary tract infections (Bergogne-Bérézin & Towner, 1996). Various mechanisms of antibiotic resistance have been recognized in this species, and combination therapy is usually required for effective treatment of *A. baumannii* nosocomial infections (Falagas *et al.*, 2006; Peleg *et al.*, 2008).

Analysis of the total DNA sequence of *A. baumannii* ATCC 17978 revealed 16 putative alien islands predicted to be involved in virulence (Smith *et al.*, 2007). In addition, a study by Fournier *et al.* (2006) typifies the genetic agility and broad resistance array of *A. baumannii*. The sequencing of the whole genome of a clinical epidemic *A. baumannii* strain in France (AYE) identified an 86 kb resistance island (AbaR1). Of the 88 predicted ORFs within

this genomic region, 82 were predicted to have originated from other bacteria, such as *Escherichia coli* and *Pseudomonas* and *Salmonella* species. Thus, the therapeutic challenges presented by *A. baumannii* infections are partially attributable to the fact that these bacteria exhibit significant genetic versatility in the acquisition of drug resistance and have the ability to survive in the hospital environment (Bergogne-Bérézin & Towner, 1996; Peleg *et al.*, 2008).

The heat-shock response is the mechanism by which cells react to increases in temperature to prevent damage, and it involves the expression of the almost universally conserved heat-shock genes. Many heat-shock proteins (HSPs) are molecular chaperones or proteases and function by facilitating refolding of damaged proteins or eliminating proteins that cannot be repaired (Gomes & Simão, 2009). In bacteria, the major molecular chaperones include the DnaK machine (DnaK, DnaJ and GrpE) and the GroE machine (GroES and GroEL), while the ATP-dependent proteases include Lon, FtsH, ClpAP and ClpXP. GroEL acts by providing a protective microenvironment for protein

Abbreviation: HSP, heat-shock protein.

folding, whereas DnaK acts by binding and protecting exposed regions on unfolded or partially folded protein chains from proteolysis and aggregation (Gomes & Simão, 2009).

Infection of a mammal by a bacterial parasite is a complex process involving a series of recognition events and phenotypic alterations, during which cells of the host and the bacteria undergo a process of mutual recognition and adaptation (Lathigra *et al.*, 1991). HSPs were assumed to play a role in infection, since microbial HSPs can stimulate immune responses in infected patients (Morrison *et al.*, 1989; Misra *et al.*, 1996; Mosier *et al.* 1998). However, temperature changes are not the only environmental trigger of HSP expression in bacteria: sublethal concentrations of ethanol, heavy metals, oxidative stress (Laport *et al.*, 2006; Yura *et al.*, 2000) and exposure to antibiotics (Neidhardt & VanBogelen, 1987; Yamaguchi *et al.*, 2003) can also induce HSP expression.

At the time of entrance in the new host, invading micro-organisms like *A. baumannii* are subjected to a range of stressful conditions, such as elevated temperatures, increased ionic strength and host defences, which induce the bacterial stress response. Indeed, some of the HSPs are themselves virulence factors, while others affect pathogenesis indirectly by increasing bacterial resistance to host defences or regulating virulence genes (Gophna & Ron, 2003).

Expression of the chaperones DnaK and GroEL was verified in *Acinetobacter calcoaceticus* using two-dimensional electrophoresis and N-terminal amino acid sequencing. DnaK was found to be amplified in response to ethanol, butanol, hexanol and heat shock, but GroEL was amplified only in response to heat shock and ethanol treatment (Benndorf *et al.*, 1999). The response to heat shock has greater similarity to the response to phenol treatment in *A. calcoaceticus* (Benndorf *et al.*, 2001). The predominant induction of HSPs (ClpB, HtpG and DnaK) by phenol in this bacterium could be caused by its higher lipophilicity, which may determine the ability of a compound to induce HSPs (Benndorf *et al.*, 1999, 2001). Despite these studies, the regulation of heat shock genes in members of the genus *Acinetobacter* remains poorly understood.

METHODS

Bacterial strains, culture conditions and susceptibility testing.

A. baumannii strains ATCC 19606 and RS4 were incubated in Luria-Bertani (LB) medium at 37 °C with constant aeration or on LB agar. *A. baumannii* RS4 was isolated by haemoculture in the clinical microbiology laboratory at West Paraná University Hospital (Cascavel, PR, Brazil) and identified by phenotypic markers according to Bouvet & Grimont (1986, 1987). Species identification was confirmed by multiplex PCR (Chen *et al.*, 2007). Susceptibilities to a variety of different antibiotics were determined by microbroth dilution in accordance with NCCLS performance and interpretive guidelines. Since *A. baumannii* RS4 is notoriously multidrug resistant

(Table 1), *A. baumannii* RS4 was grown at 37 °C in LB medium in both the presence and the absence of different antibiotics at a subinhibitory concentrations (ml^{-1} : 30 µg ampicillin plus 12 µg sulbactam, 30 µg cefepime, 18 µg meropenem and 120 µg sulphamethoxazole plus 8 µg trimethoprim) to perform RT-PCR and Western blot assays. Overnight culture was used to inoculate (1:100 dilution) 60 ml LB medium in a 250 ml flask. Cultures were incubated with aeration at 180 r.p.m. at 37 °C to mid-exponential phase (OD_{600} 0.7–0.8). Cells were then exposed to heat shock or antibiotics for different periods of time. Control cultures were grown for the same time in the absence of stress by temperature or antibiotics.

Western blot assays. *A. baumannii* ATCC 19606 was grown in LB medium to mid-exponential phase (OD_{600} 0.7–0.8) and exposed to different lengths of exposure (0 to 240 min) to heat shock at 45 and 50 °C or to antibiotics (10 µg rifampicin or 200 µg streptomycin ml^{-1}). *A. baumannii* RS4 was grown in LB medium to mid-exponential phase (OD_{600} 0.7–0.8) and exposed to different lengths of exposure (0 to 60 min) to antibiotics according to the protocol above. Cells were then collected and resuspended in Laemmli sample buffer. After SDS-PAGE (Laemmli, 1970), the proteins (equal amounts applied to each lane) were transferred to a nitrocellulose membrane, according to the method described by Towbin *et al.* (1979). The membranes were then treated as described by Avedissian & Gomes (1996) using anti-DnaK and anti-GroEL sera generated against the Gram-negative bacterium *Caulobacter crescentus* (kindly donated by S. L. Gomes, Universidade de São Paulo).

Survival assays. To evaluate sensitivity to various types of stress (heat shock and antibiotic), *A. baumannii* ATCC 19606 was grown exponentially to mid-exponential phase in LB medium at 37 °C and aliquots of culture were pretreated at 30 min with heat shock at 45 °C and then exposed to extreme heat shock at 50 °C or to stress caused by a subinhibitory concentration of 200 µg streptomycin ml^{-1} . After treatment, cultures were diluted and plated on LB agar to determine the number of viable cells.

RNA extraction and RT-PCR analysis. An overnight culture of *A. baumannii* RS4 was diluted into fresh LB medium. When the culture reached exponential growth, 15 ml culture was removed (as a negative control). Different antibiotic stocks were added separately (ml^{-1} : 30 µg ampicillin + 12 µg sulbactam, 30 µg cefepime, 18 µg meropenem and 120 µg sulphamethoxazole + 8 µg trimethoprim) and incubation was continued for another 60 min at 37 °C. During the antibiotic incubation, further 15 ml aliquots were removed at 20, 40 and 60 min. Immediately after the samples were collected, they were centrifuged at 12 000 r.p.m. for 10 min and the supernatant was removed. Cell pellets were frozen and stored at –40 °C. RNA extraction was carried out as described by Hottes *et al.* (2004). Total

Table 1. Antimicrobial susceptibility of *A. baumannii* strains

MICs are given as mg l^{-1} . ND, Not determined/no data available. Data for strain ATCC 19606 were obtained from the ATCC.

Antimicrobial(s)	ATCC 19606	RS4
Rifampicin	2	ND
Streptomycin	256	ND
Ampicillin + sulbactam	ND	34/18
Cefepime	ND	36
Meropenem	ND	22
Sulphamethoxazole + trimethoprim	ND	156/10

RNA extracted along with any contaminating DNA was digested with RNase-free DNase according to the manufacturer's instructions (Invitrogen) and the absence of residual DNA contamination was confirmed by PCR. RNA samples were further purified with acid phenol/chloroform/isoamyl alcohol (25:24:1, pH 4.5) (Invitrogen) extraction followed by salt/ethanol precipitation. RNA quantity was determined from the A_{260} , and quality was determined by 1.5 % (w/v) agarose gel electrophoresis and from the A_{260}/A_{280} ratio. Aliquots of each RNA sample were used for RT-PCR analysis: specifically, 0.4 μ g total RNA prepared from cells incubated with ampicillin + sulbactam and cefepime and 1 μ g total RNA extracted from cells incubated with meropenem and sulphamethoxazole + trimethoprim. Transcript detection analysis was carried out by SuperScript OneStep RT-PCR with Platinum Taq kit (Invitrogen). A 933 bp fragment corresponding to the coding region of the *A. baumannii* *dnaK* gene was amplified using the forward primer *dnaK*-Met (5'-TATGAATTCAGCGCTAAGC-GTCAGGCA-3') and the reverse primer *dnaK*-Half (5'-TATG-GATCCTTTACGTGGCTCTCTACC-3'), which are complementary to the *A. baumannii* *dnaK* gene (GenBank accession number CP000521.1). RNase inhibitor (Invitrogen) was added to all RT-PCRs. RT-PCR conditions were cDNA synthesis at 55 °C for 30 min, after initial denaturation for 2 min at 94 °C, then cycling as follows: 15 s at 94 °C, 30 s at 55 °C and elongation at 68 °C for 1 min. After 40 cycles, a final extension at 68 °C for 5 min was carried out. Negative controls were carried out with Taq DNA polymerase (Invitrogen) in the absence of SuperScript reverse transcriptase to confirm that amplified products were not derived from DNA. RT-PCR products were then resolved by electrophoresis on a 1 % (w/v) 1 $\times$ TBE agarose gel.

RESULTS AND DISCUSSION

Heat stress at 45 °C

To investigate the expression of chaperones by *A. baumannii* ATCC 19606 during heat shock, we incubated the strain at 45 °C. A Western blot assay was performed which showed that the level of GroEL and DnaK expression did not change in untreated cells during the 60 min incubation (not shown). The levels of HSPs at zero time represent the control for this experiment (Fig. 1). DnaK and GroEL levels doubled after exposure to heat shock at 45 °C for 10 min (Fig. 1). However, constitutively expressed chaperones have been verified for almost 60 min at 45 °C in *Acinetobacter*. In fact, when this bacterium is incubated at 44 °C, it can survive and grow normally over a 24–48 h period. This ability to tolerate moderate temperature (44 °C) is commonly used to differentiate *A. baumannii* from other bacteria of the same genus in clinical microbiology laboratories (Bouvet & Grimont, 1986, 1987). Similarly, at temperatures below 44 °C, the heat shock response in *E. coli* is superimposed on an essentially normal pattern of gene expression, suggesting that the synthesis of most proteins is not greatly affected by heat shock induction (Herendeen *et al.*, 1979; Neidhardt & VanBogelen, 1987).

Heat shock response at extreme temperature

In order to evaluate chaperone expression in *A. baumannii* ATCC 19606 at high temperatures, cells were grown to

Fig. 1. Western blot analyses of DnaK and GroEL during heat shock at 45 °C. (a) *A. baumannii* ATCC 19606 was grown at mid-exponential phase and exposed to heat shock at 45 °C for different times. Protein extracts were prepared and separated by SDS-PAGE. After transferring the proteins to nitrocellulose membranes, the blots were probed with anti-DnaK and anti-GroEL antisera, as described in Methods. Equal amounts of protein were applied to all lanes. (b) Relative amounts of DnaK (filled bars) and GroEL (open bars) determined by densitometry scanning of the membrane shown in (a). The highest value obtained was for GroEL expression at 45 min (100 %); the other values are expressed relative to this value. The data presented are representative of three independent biological experiments and standard deviations are shown as vertical bars.

mid-exponential phase and incubated at 50 °C for 2 h. A Western blot assay demonstrated that *A. baumannii* DnaK and GroEL were transiently expressed during heat shock at 50 °C (Fig. 2a, b). The heat shock response in bacteria is positively controlled at the transcriptional level by the product of the *rpoH* gene, the heat shock promoter-specific σ^{32} subunit of RNA polymerase. In *E. coli*, the shutting off of the response occurs as a consequence of declining σ^{32} levels and inhibition of σ^{32} activity. The DnaK chaperone machine interferes with efficient binding of σ^{32} to the RNA polymerase core, turning off the heat shock response (Blaszczak *et al.*, 1995). Curiously, in *C. crescentus*, it was shown that downregulation of the response is independent of DnaK levels, whereas the chaperone ClpB is necessary for normal turning off of HSP synthesis (da Silva *et al.*, 2003; Simão *et al.*, 2005). At present, little is known about the regulation of genes that encode HSPs and their role during the heat shock response in *A. baumannii*.

In *A. baumannii* ATCC 19606, the levels of the chaperones DnaK and GroEL decreased during the first 20 min of heat shock at 50 °C when rifampicin, a transcription inhibitor, was added 1 min before the temperature increase to 50 °C (Fig. 2a, b). Similar to the observed heat shock response in

Fig. 2. Induction of DnaK and GroEL in *A. baumannii* depends on transcription. (a) *A. baumannii* ATCC 19606 was grown at 37 °C until mid-exponential phase with (+) or without (–) 10 µg rifampicin ml^{–1} and shifted to 50 °C for the indicated times. Aliquots of the culture were collected and proteins were analysed by SDS-PAGE followed by Western blot using antisera against *C. crescentus* DnaK and GroEL. Equal amounts of protein were applied to all lanes. (b) Relative amounts of DnaK (filled bars) and GroEL (open bars) determined by densitometry scanning of the membrane shown in (a). The largest value obtained was for GroEL expression at 20 min in the absence of rifampicin (100 %); the other values are expressed relative to this value. The data presented are representative of three independent biological experiments and standard deviations are shown as vertical bars.

E. coli, our results suggest that heat shock induction in *A. baumannii* depends on transcription.

Thermotolerance

The increased expression of HSPs in *A. baumannii* ATCC 19606 with preconditioning of cells at 45 °C constituted thermoprotection. Pre-incubation of *A. baumannii* cells for 30 min at 45 °C induced good thermoprotection to heat shock at 50 °C; almost 80 % of cells survived high temperature exposure for 15 min (Fig. 3). However, after this period at 50 °C, only 48 % of cells preconditioned at physiological temperatures survived. Control cells or cells

not exposed to heat shock presented 100 % survival during the 60 min period (not shown).

Chaperone expression in response to antibiotics

DnaK and GroEL levels were also analysed by Western blot assay in cells pre-incubated with streptomycin. The relative DnaK and GroEL protein levels at time zero were 13 and 59 %, respectively (Fig. 4a, b), and these levels were maintained during the period of 60 min for untreated control cells (not shown). DnaK increased more than 4-fold after 60 min of exposure to a subinhibitory concentration of antibiotic, and GroEL levels doubled (Fig. 4a, b). Furthermore, *A. baumannii* cells pretreated for 30 min at 45 °C had an increased ability to survive antibiotic exposure compared with cells pretreated at physiological temperatures (Fig. 5). These results suggest that the chaperones DnaK and GroEL could play an important role in the stress response caused by streptomycin in *A. baumannii*.

Fig. 3. *A. baumannii* is able to acquire thermotolerance. *A. baumannii* ATCC 19606 was grown exponentially to mid-exponential phase. Half of the cultures were preconditioned for 30 min at the non-lethal temperature of 45 °C (filled bars), while the other half were pre-incubated at 37 °C (open bars). Cultures were then exposed to 50 °C for 60 min and cell survival was evaluated after dilution and plating on LB agar. The graph represents means of three experiments, and the bars indicate standard deviations.

DnaK is upregulated in a multidrug-resistant strain of *A. baumannii*, RS4

In order to analyse the role of HSP during the response to antibiotics in *A. baumannii*, DnaK expression was observed in the presence of subinhibitory concentrations of different antibiotics commonly used in the treatment of infectious diseases caused by this bacterium. Expression of *dnaK* was analysed at the transcriptional and translational levels using a multidrug-resistant strain of *A. baumannii*, RS4. The profile of *dnaK* transcripts and protein expression was analysed by RT-PCR and Western blot, respectively. No changes were observed in the pattern of *dnaK* mRNA or DnaK protein expression for untreated RS4 cells during the 60 min period (not shown). Untreated control cells

Fig. 4. HSPs are induced in the presence of streptomycin. (a) *A. baumannii* ATCC 19606 was grown at mid-exponential phase and incubated with 200 μg streptomycin ml^{-1} for different times at 37 °C. Protein extracts were prepared and separated by SDS-PAGE. After transferring the proteins to nitrocellulose membranes, the blots were probed with anti-DnaK and anti-GroEL antisera, as described in Methods. Equal amounts of total protein were applied to all lanes. (b) Relative amounts of DnaK (filled bars) and GroEL (open bars) determined by densitometry scanning of the membrane shown in (a). The largest value obtained was for GroEL expression at 60 min in the presence of streptomycin (100 %); the other values are expressed relative to this value. The data presented are representative of three independent biological experiments and standard deviations are shown as vertical bars.

Fig. 5. Preconditioning of *A. baumannii* at 45 °C makes cells more resistant to antibiotic effects. *A. baumannii* ATCC 19606 was grown to mid-exponential phase at 37 °C and pre-incubated at 45 °C (filled bars) or 37 °C (open bars) for 30 min and aliquots of the culture were exposed to 200 μg streptomycin ml^{-1} for different lengths of time. Cultures were then diluted, plated on LB agar and incubated at 37 °C to determine the number of viable cells. Values represent percentages of cell survival (means of three independent experiments) with respect to control cultures performed in the absence of stress. Standard deviations are shown as vertical bars.

displayed the same levels of transcripts and DnaK protein as visualized at time zero (Fig. 6) during the 60 min exposure. A susceptible strain was also tested under all of the study conditions. However, in contrast to the multi-drug-resistant strain, cells of the susceptible strain died in the presence of the different antibiotics. Furthermore, short incubations of the susceptible strain with different drugs produced degraded RNA and proteolysis, preventing us from obtaining reliable results.

To analyse *dnaK* mRNA synthesis in the presence of different antibiotics, an RT-PCR assay was carried out as described in Methods using total RNA isolated from *A. baumannii* RS4 cells incubated with ampicillin + sulbactam for 60 min at 37 °C. *dnaK* mRNA synthesis increased gradually during the 60 min incubation with these antibiotics (Fig. 6a). *dnaK* transcription increased 1.5-fold after 20 min in the presence of ampicillin and more than 5-fold after 60 min of exposure to the antibiotic. The amount of DnaK protein also increased in the presence of ampicillin + sulbactam: the levels at 40 min were twice those observed in the absence of the antibiotics (0 min). During ampicillin + sulbactam exposure for 20–60 min, *A. baumannii* exhibited variations in DnaK levels. These results indicate that the antibiotics ampicillin + sulbactam together induce expression of the *dnaK* gene in *A. baumannii* RS4 and suggest that the maintenance of DnaK levels depends on *dnaK* gene transcription. In contrast, transcription of *dnaK* increased drastically during cefepime exposure (about 5-fold), reaching maximum levels after 20 min of incubation with the antibiotic and decreasing to very low levels by 60 min (Fig. 6b).

Curiously, the exposure of RS4 cells to ampicillin or sulbactam alone did not induce *dnaK* mRNA synthesis as soon as DnaK protein (not shown). Sulbactam is a β -lactamase inhibitor that can bind to bacterial penicillin-binding proteins (PBP₂) that are involved in synthesizing and shaping the cell wall (Waxman & Strominger, 1983). The combination of sulbactam with penicillin or cephalosporin may therefore have a synergic effect (Fu *et al.*, 2003; Choi *et al.*, 2004).

In spite of the fact that *A. baumannii* exhibited transient transcription of the *dnaK* gene in the presence of cefepime, the levels of DnaK protein more than doubled after 20 min exposure to the antibiotic and thereafter produced near-constitutive levels of the protein up to 60 min (Fig. 6b). The amount of DnaK was actually maintained at the same level between 60 min and 2 h of exposure to cefepime (not shown). These results suggest that the initial levels of DnaK protein parallel the synthesis of *dnaK* mRNA; however, after 40 min of exposure to cefepime, a process that controls the translation of *dnaK* mRNA transcripts or low DnaK proteolysis could favour high levels of this protein in *A. baumannii*.

A similar profile of expression was observed for the *dnaK* gene at the transcriptional and translational levels in the presence of the antibiotics meropenem (Fig. 6c) and

Fig. 6. Different antibiotics induce the expression of the *dnaK* gene in *A. baumannii* RS4. From left to right, the panels show levels of RNA and protein and densitometric views of these data. RT-PCR and Western blot assays were carried out as described in Methods using equal amounts of RNA and protein, respectively, extracted from *A. baumannii* RS4 cells cultured in the absence (0 min, negative control) and presence of different antibiotics after 20, 40 and 60 min at 37 °C. As a negative control of the RT-PCR assay, all reactions were performed with *Taq* DNA polymerase in place of SuperScript reverse transcriptase and PCR products were visualized (not shown). Densitometry values were expressed relative to the highest levels of *dnaK* cDNA or DnaK protein. The data presented are representative of three independent biological experiments. Standard deviations are shown as vertical bars.

sulphamethoxazole + trimethoprim (Fig. 6d). In both assays, the antibiotics induced almost twice the mRNA synthesis of the *dnaK* gene as DnaK protein in *A. baumannii*. As shown in Fig. 6(c, d), the relative rate of DnaK synthesis increased progressively during incubation, not only with meropenem, but also with sulphamethoxazole + trimethoprim. Thus, changes in the rate of DnaK synthesis during exposure to meropenem and sulphamethoxazole + trimethoprim parallel the variations in the level of *dnaK* mRNA. *A. baumannii* has a high degree of resistance to sulphamethoxazole + trimethoprim, and little is known about the genetic basis of resistance to these compounds in this bacterium (Van Looveren *et al.*, 2004). Although mRNA *dnaK* presented a unique profile of expression in the presence of cefepime, the DnaK chaperone was highly induced by the three beta-lactam antibiotics after 60 min of incubation (Fig. 6a–c).

Although the amount of total RNA used in the RT-PCR assay was strictly identical for all incubation times with the same type of antibiotic, different amounts of RNA were

used in independent experiments (see Methods). Consequently, variations in *dnaK* mRNA levels can be seen at time zero when the four treatments are compared (Fig. 6a–d). The experimental strategy conducted in the RT-PCR did not allow us to compare the exact amounts of *dnaK* mRNA obtained for the same incubation time with two or more antibiotics. However, the expression profiles obtained with all antimicrobial drugs were reproducible and clearly showed transcription induction of the *dnaK* gene.

Resistance to antibiotics typically occurs as a result of drug inactivation or modification, target alteration or reduced accumulation associated with decreased permeability and/or increased efflux (Poole, 2002). The majority of these mechanisms of resistance depend on rapid overexpression of different kinds of protein (Peleg *et al.*, 2008). *A. baumannii* RS4 is resistant to many antibiotics. Thus, it seems obvious that this bacterium is able to cope with cell damage caused by these drugs because it has the ability to express components of the antibiotic-resistance machinery.

There is no evidence in the literature that HSPs could provide antibiotic resistance to bacteria, and this work does not intend to show that. However, our work showed that HSPs are upregulated at the transcriptional and translational levels by different kinds of antibiotic (Figs 4 and 6) and that this upregulation could improve the ability of the cell to cope with stress caused by antimicrobials (Fig. 5) and perhaps acts simultaneously with the antibiotic-resistance machinery to maintain cell survival.

Some studies have suggested a role for HSPs in the bacterial stress response caused by antibiotics. Yamaguchi *et al.* (2003) demonstrated that the chaperones DnaK and GroEL have an effect on the antimicrobial activity of the fluoroquinolone levofloxacin in *E. coli*. According to these authors, these chaperones might contribute to quinolone resistance because they sequester the aggregates that accumulate in cells exposed to fluoroquinolones. Furthermore, mutations in the *dnaK*, *groEL* and *lon* genes increased bacterial susceptibility to levofloxacin.

Recent studies have demonstrated that subinhibitory concentrations of the antimicrobials gentamicin and tobramycin induce a set of genes that affect the interaction of *Pseudomonas aeruginosa* with host cells, including the gene encoding Lon protease, which is known to play a role in protein quality control (Marr *et al.*, 2007). In *Staphylococcus aureus*, a mutation in the *dnaK* gene increased the susceptibility of the methicillin-resistant strain COL to the antibiotics oxacillin and methicillin (Singh *et al.*, 2007). In *S. aureus*, HSPs are thought to be involved in responses to antibiotics because they are induced when the cell wall is subjected to antibiotic stress (Singh *et al.*, 2001; Utaida *et al.*, 2003).

The rapid emergence of multidrug-resistant bacterial strains like *A. baumannii* RS4 calls for the development of novel antimicrobials. One possible alternative strategy in the development of these compounds is to identify molecular targets and design molecules with high specificity and affinity for these targets. DnaK has been used as a target in the development of a novel genre of biocides known as pyrrocoricin-based peptides. Pyrrocoricin binding prevents the frequent opening and closing of the multihelical lid over the peptide-binding pocket of DnaK, permanently closes the cavity and inhibits chaperone-assisted protein folding (Kragol *et al.*, 2001). Pyrrocoricin is able to kill tetracycline- and aminoglycoside-resistant strains of *E. coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* at low-micromolar concentrations (Cudic *et al.*, 2002).

Molecular chaperones clearly play important roles in bacterial stress tolerance, but their role in antibiotic tolerance is poorly understood in *A. baumannii*. In an effort to begin to elucidate these roles, the present study verified the expression of *dnaK* and *groEL* genes at the transcriptional and translational levels in the presence of antibiotics, and our results demonstrate that GroEL and

DnaK may have an important role during the stress response caused by antibiotics in *A. baumannii*. Further studies are under way in our laboratory to characterize the function of these chaperones in the antibiotic response.

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