RESEARCH ARTICLE

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Effects of meropenem exposure in persister cells of *Acinetobacter calcoaceticus-baumannii*

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Aim: To evaluate the influence of meropenem in the *Acinetobacter calcoaceticus-baumannii* (ACB) persister levels. **Methods:** Persister levels in planktonic and biofilm cultures from ACB isolates were evaluated after exposure to different meropenem concentrations. **Results:** A high variability of persister fractions was observed among the isolates cultured under planktonic and biofilm conditions. Meropenem concentration did not influence persister fractions, even when far above the MIC. No correlation was found between persister levels and biofilm biomass. **Conclusion:** The magnitude of persister levels from ACB planktonic and, particularly, biofilm cultures exposed to meropenem was independent of the antibiotic concentration, dosing regimen and biofilm biomass. These findings, in a context of meropenem failure to treat chronic infections, strengthen the importance of understanding persister behavior.

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KEYWORDS
• carbapenems • chronic infections • persistence • therapeutic failure

The *Acinetobacter calcoaceticus-baumannii* (ACB) complex has been considered an important public health concern responsible for several infections in intensive care unit patients [1]. Nowadays, ACB healthcare-associated infections can be even aggravated by the raising antimicrobial resistance levels that increased the difficulty imposed on their treatment, including resistance to carbapenems – which have been widely used against these infections [2]. However, the ineffectiveness of antimicrobial treatments, especially in the avoidance of recalcitrance of chronic infections, may also be due to the bacterial phenotype of tolerance to antimicrobials determined by the presence of persister cells [3].

Persisters comprise a small subpopulation of cells able to survive lethal doses of bactericidal antimicrobials to which they are susceptible, as well as to other stressors [4,5]. These cells can be stochastically formed in a bacterial population as a strategy for the adaptation to environmental variations that may result in persistence of bacterial infections [6]. On the other hand, the transient persistence phenotype may also be derived from the induction by stressor agents, which has been assigned to several molecular mechanisms and global regulators that can operate independently and in parallel, or even overlapped [7], including the SOS response and toxin–antitoxin modules [8–10].

Persister cells are especially important in biofilms [11], as even antimicrobials that are able to diffuse in biofilms do not act on these tolerant cells, hindering the treatment of the infection [5,12]. This scenario is additionally worsened since biofilm blocks the entry of large immune system components.

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The ability of ACB complex strains to adhere and form biofilm on epithelial cells as well as on medical devices, such as intravascular catheters and endotracheal tubes, enhances the colonization of mucosal surfaces by these bacteria and increases the risk of bloodstream and respiratory infections [13]. In addition, ACB biofilms may also be implied in the success of these pathogens to remain on surfaces and, consequently, to survive in the hospital environment [14].

Many pathogens have proven to be able to form persister cells [15–19], including *A. baumannii* [20,21]. Although, still very little is known about persistence in this bacteria, stationary-phase planktonic and biofilm cultures were evaluated after treatment with noncarbapenem antimicrobial agents showing differences in persister cell fractions [20,21]. In this context, the present study describes the characterization of persister levels in several clinical ACB isolates cultured under planktonic and biofilm conditions after exposure to different concentrations of meropenem.

Materials & methods

• Bacterial isolates

Twenty clinical ACB isolates from unique patients were obtained from the Department of Microbiology of the Clinical Pathology Laboratory of São Lucas Hospital, Porto Alegre, RS, Brazil, from August 2013 to April 2014. The isolates were previously identified as ACB using the Vitek System 2 (bioMérieux, Inc., MO, USA) and confirmed by polymerase chain reaction amplification assay targeting 16S rRNA gene specific for the *Acinetobacter* genus [22].

• Meropenem susceptibility testing

All isolates were evaluated for meropenem (Sigma Aldrich, MO, USA) susceptibility by the assessment of MIC using broth microdilution method according to the recommendations of the Clinical and Laboratory Standards Institute [23]. The values were interpreted according to the Clinical and Laboratory Standards Institute guidelines [24].

• Biofilm formation

The ability to form biofilm was evaluated in 96-well polystyrene plates using a method previously described [13] with some modifications. Briefly, all isolates were grown for 18 h at 37°C in lysogeny broth (LB; 10 g/l tryptone [Oxoid, Basingstoke, England], 5 g/l yeast extract [Himedia, Mumbai, India] and 5 g/l NaCl [Nuclear, Diadema, Brazil]), pH 7.0. A 1 µl-aliquot of each culture was inoculated in microplates containing 200 µl of fresh medium, in triplicate, and incubated at 37°C for 48 h without shaking. The wells were washed twice with phosphate-buffered saline and stained with 0.1% crystal violet (Newprov, Pinhais, Brazil) for 5 min. Subsequently, the stained biofilms were solubilized with 96% ethanol (Fmaia, São Francisco, Brazil) for 5–10 min and the adherent cells were measured using a SpectraMax 190 microplate reader (Molecular Devices, Sunnyvale, USA) at 570 nm. The isolates were categorized following Stepanovic *et al.* [25] classification as nonbiofilm producers (optical density [OD] $\leq OD_c$), weak biofilm producers ($OD_c < OD \leq 2OD_c$), moderate biofilm producers ($2OD_c < OD \leq 4OD_c$) and strong biofilm producers ($4OD_c < OD$). OD_c is the cut-off OD determined as the mean OD plus three SDs of the negative control.

• Determining the presence & level of persisters in planktonic & biofilm cultures

For the determination of the persister cell fractions in late exponential growth phase of planktonic cultures, all isolates were grown for 18 h at 37°C in LB, diluted 1:30 with 8 ml fresh medium, and incubated at 37°C for 3 h in static condition. The initial cell concentration was determined by decimal serial dilutions and drop plating onto nutrient agar (Oxoid). The cultures were exposed to meropenem (15 or 30 µg/ml, 15- and 30-fold the highest MIC found among the isolates, respectively, without medium replacement) for 48 h without shaking. In order to determine the number of colony-forming units (CFUs) per milliliter (CFU/ml) of the surviving fractions, a 1 ml-aliquot was removed after 24 and 48 h of antimicrobial exposure. These aliquots were centrifuged at 12,000 rpm for 7 min, and washed in 0.85% saline (Nuclear) to remove residues of the antimicrobial. Pellets were resuspended in 1 ml of 0.85% saline, diluted until 10^{-5} and spotted as 10 µl of each dilution on nutrient agar (Oxoid), in triplicate, and incubated at 37°C for 24 h.

To evaluate persister levels in biofilm, the isolates were submitted to this cultive condition as described above, in nine replicates. Afterward, the supernatant was removed and wells were washed twice with phosphate-buffered saline. Biofilms from three replicates were disrupted

to determine the initial 48-h biofilm cell densities. Adherent cells from other six replicates were exposed to 200 µl fresh LB containing meropenem (15 or 30 µg/ml), in static condition, and, at 24 and 48 h after antimicrobial exposure, the medium containing the antimicrobial was removed from three replicates for each period of time. The wells were washed twice with 0.85% saline to remove residues of the antimicrobial and tightly adherent cells were dissociated using a sonicating water bath Ultra Cleaner 1400A (Unique, Indaiatuba, Brazil) for 10 min. As in the planktonic culture, cells were diluted until 10^{-5} and drops of each dilution were plated onto nutrient agar to determine CFU counts.

The fractions of remaining cells were further evaluated for meropenem susceptibility after 48 h of antimicrobial exposure by MIC determination, as described above, and regrown overnight into sterile LB with meropenem (15 or 30 µg/ml) in order to confirm the persistence phenotype and exclude the selection of resistant cells.

In addition, persisters levels until 96 h were evaluated in five randomly chosen isolates (Acb-1, Acb-4, Acb-8, Acb-13 and Acb-20). Aliquots were removed after 6, 24, 36, 48, 72 and 96 h of meropenem exposure, in both culture conditions (planktonic and biofilm) and antimicrobial concentrations (15 and 30 µg/ml), to verify the maintenance of persister cells. The same five isolates, in late exponential growth phase, were exposed to 100 and 200 µg/ml of meropenem (100- and 200-fold the highest MIC found among the isolates, respectively) for 48 h in order to assess whether higher antimicrobial concentrations could influence persister levels.

• Meropenem pulse dosing

The isolate Acb-1 was cultured in 8 ml LB broth until late exponential growth phase and exposed to meropenem at pulse dosing regimen of 15 µg/ml (15-fold MIC) every 12 h until 48 h. Before each resuspension in 8 ml of fresh LB containing 15 µg/ml of meropenem, cultures were centrifuged at 12,000 rpm for 7 min and washed twice with 0.85% saline to remove residues of the previous antimicrobial exposure. Aliquots were removed after 12, 24, 36 and 48 h of antimicrobial exposure to determine persister cell counts, and verify the possible effect of this treatment condition in the persister fractions. Furthermore, an aliquot was removed after 72 h, comprising 24 h without meropenem replacement, in order to assess the persister fractions after completion of the pulse dosing regimen with meropenem.

• Heritability of persistence

To evaluate the heritability of persistence, persister cells from Acb-1 isolate formed after exposure to 15 µg/ml of meropenem were centrifuged at 12,000 rpm for 7 min, washed twice with 0.85% saline, and cultured for 18 h at 37°C in LB without antimicrobial. The culture was diluted 1:30 with fresh LB, incubated until late exponential phase, and then cells were submitted to a new exposure to 15 µg/ml of meropenem. This cycle was repeated three additional times; aliquots were removed at 24 and 48 h, subsequently washed in 0.85% saline to remove the antimicrobial residue, diluted until 10^{-5} and dropped on nutrient agar (Oxoid) to determine CFU counts.

• Statistical analysis

All persister fraction values were determined as the mean of three biological replicates. The longitudinal data of the persister cell fractions in 24 and 48 h after meropenem exposure were analyzed by ANOVA for repeated measures via mixed-effects model using SAS software version 9.4 (SAS Institute, Inc., NC, USA) in order to determine the influence of antimicrobial concentrations and growth conditions, and also verify correlation between biofilm categories and persister fractions, as well as among the MIC values and persister levels. Moreover, unpaired *t*-test was employed to assess the influence of meropenem pulse dosing treatment in the persister cell fractions, and compare the persister levels at 48 and 96 h in both culture conditions, using GraphPad Prism software version 7.00 (GraphPad, Inc., CA, USA). p-values <0.05 were considered significant.

Results

• Meropenem susceptibility & biofilm formation

All isolates were susceptible to meropenem, presenting MIC values ranging from 0.25 to 1.0 µg/ml. In total, 18 (90%) ACB isolates were able to produce biofilm with different biomass quantities: three isolates (16.7%) produced strong biofilms, whereas eight (44.4%)

Table 1. Characterization of *Acinetobacter calcoaceticus-baumannii* isolates regarding the clinical origin, MIC to meropenem, ability to form biofilm and biofilm cell density.

Isolate	Clinical specimen	MIC (μg/ml)	Biofilm biomass category	Biofilm cell density (CFU/ml)† ± SD‡
Acb-1	Tracheal aspirate	1.0	Weak	$1.20 \times 10^7 \pm 2.0 \times 10^6$
Acb-2	Skin lesion	1.0	Strong	$2.00 \times 10^8 \pm 4.36 \times 10^7$
Acb-3	Surgical wound	1.0	Weak	$8.87 \times 10^6 \pm 1.03 \times 10^6$
Acb-4	Urine	1.0	Weak	$8.97 \times 10^6 \pm 9.07 \times 10^5$
Acb-5	Blood culture	0.5	Strong	$1.53 \times 10^8 \pm 3.06 \times 10^7$
Acb-6	Wound secretion	0.5	Nonproducer	NA
Acb-7	Wound secretion	0.25	Nonproducer	NA
Acb-8	Sputum	0.25	Weak	$1.01 \times 10^7 \pm 1.15 \times 10^6$
Acb-9	Tracheal aspirate	0.5	Weak	$1.13 \times 10^7 \pm 1.15 \times 10^6$
Acb-10	Urine	1.0	Moderate	$2.47 \times 10^7 \pm 3.51 \times 10^6$
Acb-11	Oropharynx secretion	0.5	Moderate	$2.27 \times 10^7 \pm 1.53 \times 10^6$
Acb-12	Urine	0.5	Moderate	$2.40 \times 10^7 \pm 4.00 \times 10^6$
Acb-13	Blood culture	0.25	Moderate	$2.30 \times 10^7 \pm 2.00 \times 10^6$
Acb-14	Wound secretion	0.5	Weak	$1.23 \times 10^7 \pm 2.08 \times 10^6$
Acb-15	Oropharynx aspirate	1.0	Weak	$1.04 \times 10^7 \pm 1.40 \times 10^6$
Acb-16	Tracheal aspirate	0.5	Moderate	$2.27 \times 10^7 \pm 3.79 \times 10^6$
Acb-17	Bronchial lavage	0.5	Moderate	$2.50 \times 10^7 \pm 4.58 \times 10^6$
Acb-18	Bronchial lavage	1.0	Moderate	$2.40 \times 10^7 \pm 2.65 \times 10^6$
Acb-19	Blood culture	0.25	Moderate	$2.43 \times 10^7 \pm 3.06 \times 10^6$
Acb-20	Tracheal aspirate	0.5	Strong	$1.40 \times 10^8 \pm 1.53 \times 10^7$

†Values represent the mean of three replicates.
‡SD among replicates values.
CFU: Colony-forming unit; NA: Not applicable SD: Standard deviation.

and seven (38.9%) formed moderate and weak biofilms, respectively. Two isolates (10%) were classified as nonbiofilm producers. The MIC value, biofilm biomass category and its respective cell density for all isolates are indicated in **Table 1**.

• ACB persister cell levels after meropenem exposure

Persister cells were detected in all isolates cultured under planktonic and biofilm conditions after exposure to meropenem for 48 h. Assessing planktonic cells of all isolates exposed to both meropenem concentrations, a significant difference among the persister fractions was found ($p \leq 0.0001$), independently of concentration, ranging from 0.0009 to 0.7214% of the initial populations **(Figure 1A & Supplementary Table 1)**. As observed in planktonic culture, a very heterogeneous pattern of persister fractions was also verified among the isolates cultured as biofilm ($p \leq 0.0001$) **(Figure 1B)**. Persister levels found in the isolates cultured under biofilm condition were even higher than fractions obtained in planktonic cells ($p \leq 0.0001$), ranging from 0.1147 to 7.2292% **(Supplementary Table 1)**. Our data also demonstrated that the means of persister cell fractions were similar across the four categories of biofilm production in ACB complex isolates after 48 h of meropenem exposure ($p = 0.3892$). Furthermore, the isolates treated with 15 μg/ml of meropenem until 96 h, in both culture conditions, were able to present persister cells for long periods of antimicrobial exposure. However, persister fractions significantly dropped in two of five isolates in planktonic culture, as well as in three of five isolates cultured under biofilm condition, which also indicated variation among the isolates regarding the maintenance of persisters population over time.

Persister cell levels detected after 48 h of meropenem exposure in planktonic and biofilm cultures remained susceptible when regrown into LB supplemented with the antimicrobial. Moreover, the meropenem MIC evaluated after the treatment remained unchanged for all

isolates, demonstrating that resistance mechanisms were not acquired and confirming the persistence phenotype. No statistically significant correlation was found between MIC values and persister cell fractions (p = 0.1863).

• Meropenem concentrations do not influence persister fractions

The meropenem concentrations used to assess the persister cell levels in planktonic and biofilm cultures were determined from previous characterization of the ACB isolates as susceptible to meropenem. No statistically significant association was found between the antimicrobial concentrations tested (15 and 30 µg/ml) and the remaining cell fractions in late exponential phase (p = 0.9698) **(Figure 2A)**, as well as in biofilm condition (p = 0.4493) **(Figure 2B)**, after exposure for 48h. In addition, the five isolates treated with 100 and 200 µg/ml of meropenem in late exponential phase exhibited similar persister levels when compared with the fractions obtained after 15 or 30 µg/ml meropenem exposures, demonstrating that approximately 13-fold higher concentrations of this drug do not influence persister cell levels in the ACB complex isolates evaluated (p = 0.3138) **(Figure 2A)**.

• Pulse dosing do not eradicate ACB persisters

The meropenem pulse dosing treatment evaluated was not effective to eliminate Acb-1 persister cells in late exponential phase, remaining 0.0232% of the original population **(Figure 3)**. Indeed, no statistically significant difference was found between fractions obtained after 72 h from the pulse dosing regimen and a single exposure to antimicrobial (p = 0.4522).

• Persistence state is not inherited in ACB

Persister cells from the first exposure to 15-fold MIC of meropenem for 48 h were re-exposed to this antimicrobial at the same concentration for three consecutive cycles, and similar persister fractions were obtained in comparison with the initial population **(Figure 4)**. Thus, persistence after exposure to meropenem in the Acb-1 isolate showed to be a transient and nonheritable phenotype, as persister cell levels were not changed through the passages.

Discussion

Meropenem, as well as other carbapenems, have been considered the choice drugs to treat infections caused by ACB in many healthcare institutions, but high levels of resistance to these drugs have been associated with their decreased efficiency. However, our study highlights the ability of ACB isolates to present persister cells in the presence of meropenem as another probable important reason for the therapeutic failure using this antimicrobial.

Persister cells were found in both biofilm and planktonic cultures from all isolates after meropenem exposure, being detected until at least 96 h in the presence of the antimicrobial, which may be due to the presence of cells stochastically produced in the population, and/or induced by the exposure to the antimicrobial, as already described [6,21,26]. A higher level

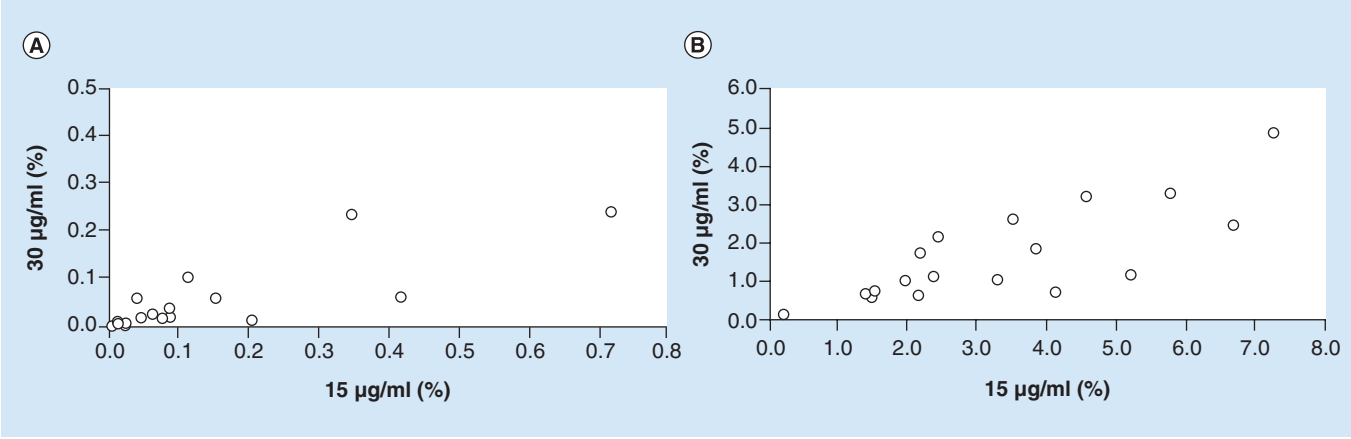

Figure 1. Correlation between persister cells formed by *Acinetobacter calcoaceticus-baumannii* after exposure to 15 and 30 µg/ml of meropenem for 48 h. Heterogeneous pattern of persister fractions found in planktonic **(A)** and biofilm **(B)** cultures. Each data point represents mean of three biological replicates.

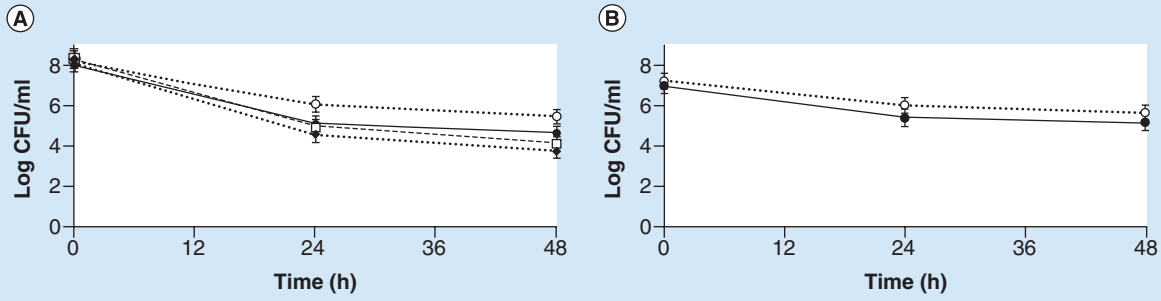

Figure 2. Killing curves of *Acinetobacter calcoaceticus-baumannii* after exposure to meropenem for 48 h. (A) Acb-1 isolate was cultured until late exponential phase and treated with 15-fold MIC (°), 30-fold MIC (•), 100-fold MIC (□) and 200-fold MIC of meropenem (◊). **(B)** Acb-1 isolate was cultured under biofilm condition and exposed to 15-fold MIC (°) and 30-fold MIC (•) of meropenem. Each data point represents mean and standard deviation (error bars) from three biological replicates.
CFU: Colony-forming unit.

of persister cells was found in biofilms when compared with planktonic counterparts in exponential phase, as has also been described in *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* [19,27]. Considering that bactericidal action of meropenem is due to inhibition of bacterial cell wall synthesis by binding to and inactivating penicillin-binding proteins [28], the killing efficiency of this antimicrobial depend strongly on the physiological state of the target bacterium, and cells in biofilms are predominantly in nongrowing or slow-growing state, hindering meropenem action. Conversely, in the exponential phase, the cellular metabolic activity is accelerated, and, therefore, the cellular growth is increased, favoring meropenem action, which, probably, results in a smaller fraction of persister cells. Likewise, high levels of persister cells verified in biofilms may also be linked to a cell-to-cell signaling, since some quorum-sensing messenger molecules that coordinate gene expression in a bacterial cell population were suggested as inducers of tolerance in biofilms [29–31]. However, we did not find correlation between persister levels and biofilm biomass, indicating that density of the population does not strongly influence the persister fractions, on the contrary to what have been previously described for *P. aeruginosa* [19]. Indeed, the strong biofilm producers (initial population around 1 log higher than others) presented some of the smallest persister

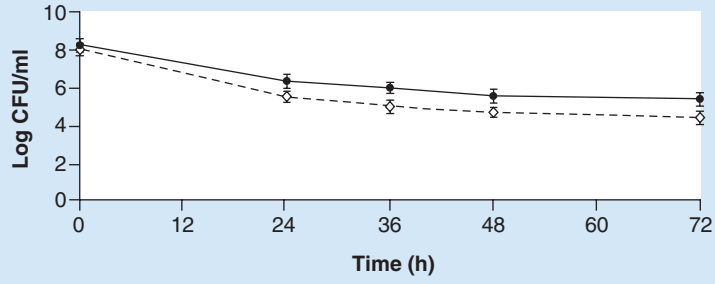

Figure 3. Meropenem pulse dosing regimen does not significantly reduce persister cells in *Acinetobacter calcoaceticus-baumannii*. Late exponential culture of Acb-1 isolate was exposed to 15-fold MIC of meropenem in a dosing regimen every 12 h until 48 h, and cultured until 72 h (comprising 24 h without meropenem replacement) (◊), or in a single antimicrobial exposure with the same meropenem concentration (•). Each data point represents mean and standard deviation (error bars) from three biological replicates.
CFU: Colony-forming unit.

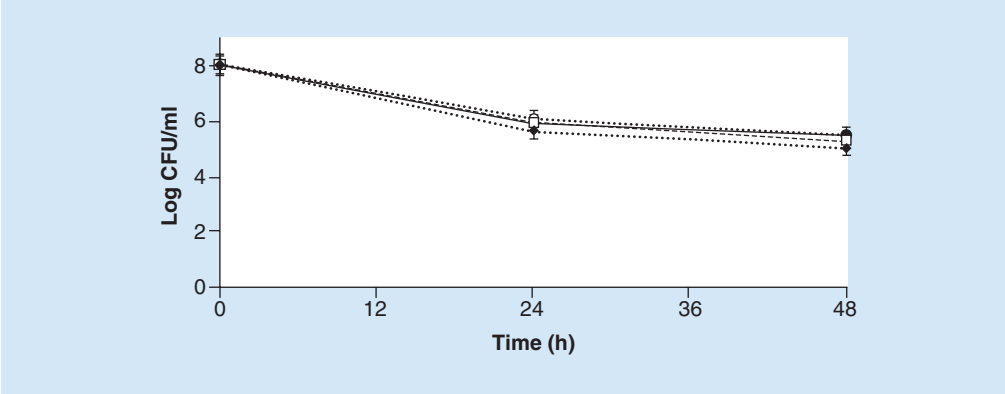

Figure 4. Nonheritability of *Acinetobacter calcoaceticus-baumannii* persistence phenotype. Acb-1 isolate was cultured in lysogeny broth until late exponential phase and exposed to 15-fold MIC of meropenem for 48 h in four consecutive cycles. (°) cycle 1; (•) cycle 2; (□) cycle 3; (◊) cycle 4. Each data point represents mean and standard deviaiton (error bars) from three biological replicates.

fractions found in all isolates, and fractions even 35-fold higher were formed by weak biofilm producers. These findings indicate that quorum-sensing factors may not necessarily be decisive for the control of persister levels, but other messenger molecules and/or extracellular polymeric substances could also be involved in the regulation of persisters in *Acinetobacter* biofilms. Biofilm biomass produced by *A. baumannii* also showed no influence on the enhancement in antimicrobial resistance in another study, but biofilm production by this microorganism was suggested as a possible mechanism of nonMDR isolates to get a better survival facing antimicrobial exposure [32], which allow us to speculate that biofilm can be used as a strategy to tolerate antimicrobials by induction of persister formation. Furthermore, the meropenem tolerance mediated by persister cells showed to be a noninherited transitional phenotype, as also described for other bacteria [29,33–34], constituting a possible adaptation to survive a stressful environment, like the hospital environment.

A high heterogeneity in the persister cell fractions was found among the isolates, as has already been reported in *A. baumannii* exposed to high concentrations of polymyxin B or tobramycin [20]. Considering that successive exposures to meropenem did not change persister cell levels, the heterogeneity encountered may be derived from four distinct nonexcluding origins: individual ability to stochastically produce persisters; particular regulation mechanisms; the metabolic scaffold of each isolate; and influence of other previous stresses provided by the hospital environment in a different magnitude to each isolate. A surprising heterogeneity was also found among the isolates that produced low-biofilm biomass, whose persister levels ranged from 0.205 to 7.2292%. Thus, the variability observed here points to the importance to study different isolates in order to accurately elucidate the mechanisms involved in the expression of persistence in the ACB complex, which has been ascribed to be derived from variation in cellular parameters generated by epigenetic and/or nonredundant genetic mechanisms [7,35]. In *Escherichia coli*, a combination of toxin–antitoxin (TA)systemscontributes significantly to the induction and maintenance of persistence [15,36], demonstrating that TA systems may be good candidates to modulate this phenotype in the ACB complex. Nevertheless, the occurrence of different TA systems has been described in *A. baumannii* [37,38], but their role on the persister cells formation has not already been established. Overall, the molecular mechanisms involved in the antimicrobial tolerance in ACB complex remain largely unknown. In this context, it should be emphasized that studies in other bacteria demonstrated that induction of persister cells do not rely on a single genetic mechanism, but, in fact, a global regulator could affect the expression of several genes simultaneously, resulting in a tolerant phenotype [7]. Evidence of this polygeny could be inferred from transposon mutagenesis analyses that failed to produce isolates completely tolerant and from the heterogeneous response of a single isolate against different antimicrobial classes [39,40].

Additionally, we found that meropenem concentration did not affect the persister cell fractions after 48 h of exposure, as well as pulse dosing regimen replacing meropenem every 12 h was not able to significantly alter persister levels when compared with a single antimicrobial exposure. These findings indicate that increasing meropenem concentrations do not eradicate ACB persisters and, therefore, may not enhance treatment efficacy, although prolonged infusion of high-dose meropenem has achieved the target cumulative fraction of response for several *Enterobacteriaceae* and *P. aeruginosa*, which can improve the therapy of infections caused by these bacteria [41]. On the other hand, time of meropenem exposure may influence ACB persister death, as persister fractions detected after 48 and 96 h of antimicrobial treatment were significantly different in some isolates.

Conclusion & future perspective

In conclusion, the ability of ACB complex isolates to present persisters after meropenem exposure, especially in biofilms, may contribute significantly to the failure to treat chronic and relapsing infections with this drug, since even extremely high concentrations did not decrease persister levels. Therefore, our data emphasize the need to develop drugs that are not only able to eradicate planktonic persisters, but also their biofilm counterparts, possibly in combination with compounds capable to disrupt extracellular polymeric substances of *Acinetobacter* biofilms. In this sense, the elucidation of the molecular mechanisms involved in the induction and maintenance of this transient phenotype in ACB complex isolates will be of paramount importance in the design of effective therapeutic alternatives.

Supplementary Data

To view the supplementary data that accompany this paper, please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/fmb-2016-0118

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Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

This study was approved by the Research Ethics Committee of the Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS) under protocol number 483469.

EXECUTIVE SUMMARY

- Therapeutic failure using meropenem may also happen due to persister cells.
- Extremely high concentrations of meropenem were unable to eradicate persisters from *Acinetobacter calcoaceticus-baumannii*.
- A great heterogeneity in persister levels may occur among different isolates exposed to the same antimicrobial.
- Biofilm biomass was not correlated with persister levels after meropenem exposure.
- Persistence state is not inherited in *A. calcoaceticus-baumannii*.

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