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Analysis of *Acinetobacter baumannii* survival in liquid media and on solid matrices as well as effect of disinfectants

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SUMMARY

Background: *Acinetobacter baumannii* is a cause of healthcare-associated infections and has considerable potential to survive on inanimate hospital surfaces under hostile conditions (e.g. disinfection or desiccation).
Aim: To learn more about its survival strategy and capacity to persist in liquid media and on surfaces mimicking hospital environments.
Methods: The effect of temperature, nutrient deprivation, permanence on inanimate surfaces, and exposure to disinfectants on the survival of four *A. baumannii* strains (ATCC 19606^T and three clinical isolates) was studied by monitoring the number of total and viable cells using fluorescent microscopy and of culturable cells by standard cultures.
Findings: Bacterial survival was differentially affected by temperature (cells maintained at 20°C remained culturable at least within 30 days) and physical environment (desiccation favoured cell resistance to stress at 37°C). Moreover, persistence was associated with two adaptation patterns: one linked to entry into the viable but non-culturable state, whereas the other apparently followed a bust-and-boom model. During a study on the effect of disinfectant (commercial bleach and quaternary ammonium compounds), it was found that treatment with these antibacterial compounds did not eliminate *A. baumannii* populations and provoked the reduction of culturable populations, although a fraction of cells remained culturable.
Conclusion: The ability to persist for long periods on different surfaces, mimicking those usually found in hospitals, along with *A. baumannii*'s capacity to survive after a disinfection process may account for the recurrent outbreaks in intensive care units.

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Introduction

Acinetobacter baumannii is a well-known pathogen that causes serious opportunistic infections in hospitals worldwide [1–3]. In one recent review article, Wieland *et al*. refer to 150 nosocomial outbreaks reported in 22 countries (e.g. USA, Italy, Spain, France, Greece, Turkey, Taiwan, and South Korea) representing all continents [4].

The healthcare environment is a potential source of microbial pathogens and therefore can promote their spread between individuals. There is evidence that contaminated surfaces play an important role in transmission of several opportunistic pathogens including *Acinetobacter* spp. [5–8].

The capacity for persistence and survival of bacteria in hospital environments even under hostile conditions is conferred by adaptation mechanisms associated with different survival strategies. In non-spore-forming bacteria such as *Acinetobacter* spp., two main strategies have been described: bust-and-boom and cellular quiescence/dormancy (i.e. acquisition of viable but non-culturable (VBNC) state) [9]. According to the first strategy (bust-and-boom), most cells die upon exposure to adverse conditions (mainly linked to starvation) and a few survivors subsist at the expense of dead cells. In the case of transition to the VBNC state, cells lose the ability to grow on standard laboratory media normally used to culture them, even though they retain metabolic activity and, more importantly, pathogenicity [10–14]. Moreover, when both strategies occur simultaneously, VBNC cells can serve as a source of nutrients and this 'altruistic' mechanism can sustain the survival of the persisting culturable cells [15]. Once environmental conditions become favourable again, the survivors (bust-and-boom model) or dormant cells (i.e. those in the VBNC state) can recover and resume growth, thus ensuring the persistence of populations.

The ability of *A. baumannii* to adapt and to survive in different environments is a key reason for its persistence and success as an opportunistic pathogen [16]. Due to the wide spread of nosocomial diseases caused by *Acinetobacter* spp., recent studies have been focused on understanding their pathogenesis and antibiotic resistance patterns. Nevertheless, little is known about the survival strategies that could explain the persistence and dissemination of *A. baumannii* in hospital environments. Farrow *et al*. found that some strains exhibited a profound resistance to desiccation and that the majority of cells could survive on a dry surface for a long time [17]. Likewise, other studies have shown that many clinical *A. baumannii* strains can survive for $\geq$20 days on dry surfaces [8,13,18]. Moreover, several authors have explained *Acinetobacter* spp. persistence by implicating the bust-and-boom model [18,19].

Dancer proposed that high-touch surfaces could represent important areas due to their high potential for cross-transmission of pathogens [20]. Likewise, Denton *et al*. reported a prolonged outbreak in a neurosurgical intensive care unit (ICU) attributable to the epidemic strain being frequently isolated from hand in touch with the areas in close proximity to patients, thus demonstrating a clear association between the levels of surface contamination and new patient infection [21].

In principle, there is evidence that environmental cleaning facilitates control of hospital-acquired infections [22,23]. The use of antiseptics and disinfectants that reduce or eliminate bacterial reservoirs, including those of *A. baumannii*, constitutes an important strategy for the control and prevention of pathogen spread [21,24,25]. Consequently, inadequate disinfection has been associated with infection outbreaks [26]. Although some general effects of disinfectants on microbial populations have been reported previously, the specific physiological and phenotypical responses induced in *A. baumannii* upon treatment with disinfectants remain poorly defined [27,28]. A better understanding of *A. baumannii* survival in the hospital environment, its persistence strategies, and its capacity to resist disinfection, could provide key information for control of *A. baumannii* nosocomial infections. The aim of this study was to assess how environmental conditions (starvation, temperature, and desiccation) affect survival and persistence of *A. baumannii* (ATCC 19606^T and three clinical isolates) in liquid media and on surfaces mimicking hospital environments. A second aim was to study the dynamics and responses of *A. baumannii* populations exposed to several disinfectants routinely used in the hospitals of the Basque Country.

Methods

Acinetobacter baumannii strains and inocula preparation

Four *A. baumannii* strains were used in this study: American Type Culture Collection (ATCC) strain 19606^T and three clinical isolates of *A. baumannii* (strains HUMV-2790, HUMV-2471 and HUMV-3743 obtained from skin ulcer, sputum and wound exudates of patients at the Hospital Universitario Marqués de Valdecilla (Santander, Spain) [29]. Strains were separately grown overnight at 37°C in Mueller–Hinton (MH) broth. The overnight culture was used to inoculate fresh MH broth and the cells were grown at 37°C with shaking (120 rpm) until the culture reached the stationary phase. The cells were collected by centrifugation (4500 *g*, 4°C, 15 min) and the cell pellets were washed three times with sterile saline solution (0.9% w/v NaCl). The pellets obtained after the last wash were suspended in sterile saline solution and immediately used as inocula in survival assays.

Survival assays

A. baumannii cells from stationary phase were incubated under nutrient deprivation at 37°C and/or 20°C in saline solutions or on solid surfaces. The latter included sterile cellulose acetate filters (Whatman, GE Healthcare Life Sciences, Marlborough, MA, USA), square glass coverslips (20 mm $\times$ 20 mm), stainless steel discs (1 cm in diameter), and square pieces of cotton tissue obtained from a laboratory coat (10 mm $\times$ 10 mm). Saline solutions, coverslips, stainless steel discs, and squares of cotton cloth were sterilized by autoclaving, whereas cellulose acetate filters were sterilized by a 20 min exposure to UV-C ($\sim$253.7 nm; 70 mW/cm^2).

Survival assays in the aqueous environment were carried out in Erlenmeyer flasks containing 300 mL of sterile saline solution by incubating cell suspensions at 20°C and 37°C with shaking (120 rpm). To avoid the presence of residual organic matter, the glass flasks were first cleaned with acid, rinsed with deionized water, and kept at 250°C for 24 h.

To perform survival assays on cellulose acetate filters, the filters were inoculated by filtering cellular suspensions (described above). Other solid surfaces were inoculated with drops of cellular suspensions and air-dried for 60 min inside a biologic safety cabinet (Labculture® Esco Class II, Type A2 BSC; Esco Micro Pte Ltd, Singapore). In all cases, final densities were of approximately 10^8 cells/mL or 10^8 cells/cm^2. The solid materials were incubated in sterile Petri dishes at 20°C and/or 37°C. Ambient humidity inside Petri dishes was measured with a Fisher Scientific™ Traceable™ Digital Hygrometer/Thermometer (Fisher Scientific, Hampton, NH, USA) and maintained at a relatively low level (21–27%). To recover *A. baumannii* from solid surfaces along the survival experiments, inoculated solid matrices randomly chosen in triplicate were individually placed in 10 mL of sterile saline solution and vigorously shaken for 2 min. Subsamples originated from the survival assays were periodically collected to enumerate total, viable, and culturable bacteria and the ability to form biofilms.

Disinfection assays

The bactericidal activity of four disinfectant solutions was tested on *A. baumannii* ATCC 19606^T populations. The selected disinfectant solutions were undiluted (40,000 ppm) and diluted (4000 ppm) domestic bleach (Garbe) and two commercially available disinfectants widely used in hospitals: Instrunet® Surfa'Safe (Laboratoires Anios, active ingredients: didecyldimethylammonium chloride and polyhexamethylene biguanide hydrochloride) and 0.5% (v/v) working solution of Instrunet® Aniosyme DD1 (Laboratoires Anios; active ingredients: lauryldimethyl amine oxide and polyhexamethylene biguanide hydrochloride and quaternary ammonium propionate, polyhexamethylene biguanide hydrochloride and enzyme mix (protease, amylase, lipase)).

Before carrying out disinfection assays, the effectiveness of different neutralizers was tested according to procedures UNE-EN 1040 and AFNOR NF T 72-150 by means of the dilution–neutralization method [30–32].

Based on the results of these tests, two neutralizers that were able to efficiently deactivate disinfection reagents and that showed no toxicity towards *A. baumanni* cells were selected for disinfection assays. Namely, Neutralizer A (1% dextrose, 0.7% lecithin, 0.25% sodium bisulfite, 0.1% sodium thioglycolate, 0.6% sodium thiosulfate, 0.2% L-cysteine, 0.5% tryptone, and 0.1% Tween® 80 in buffered saline, pH 7.2) was selected to perform experiments with diluted and undiluted bleach, whereas Neutralizer B (3% saponin, 0.3% lecithin, and 0.3% Tween 80 in buffered saline; pH 7.2) for those carried out with Instrunet Surfa'Safe and Instrunet Aniosyme DD1 [33].

Disinfection assays with *A. baumannii* ATCC 19606^T populations maintained in sterile saline solution were performed according to the approved standard procedures AFNOR NF T 72-150 [31]. The disinfectants were also tested on populations of four *A. baumannii* strains maintained on stainless steel discs, according the approved standards AFNOR NF T 72-190 [34]. The pilot assays were performed with *A. baumannii* ATCC 19606^T to select two appropriate disinfectants, diluted bleach (4000 ppm) and Instrunet® Surfa'Safe, for testing their effect on populations of *A. baumannii* clinical isolates. Briefly, 10 μL of bacterial suspensions were dropped on to sterile steel discs to obtain the final density 10^6–10^7 cells/cm^2) and further air-dried for 60 min as described previously. Fifty microlitres of selected disinfectants were then added and discs were incubated at 20°C for 0.5, 2, 5, and 15 min (the ultimate time-point was tested only for *A. baumannii* ATCC 19606^T). At the end of the contact time, discs were transferred to vials containing 10 mL of selected neutralizer and incubated for 2 min. The process was repeated twice more by using sterile saline solution and three obtained suspensions were mixed. The total, viable, and culturable bacteria were enumerated. The same procedure was performed with the control samples by using sterile saline solution instead of disinfectants.

Bacterial counts

The total bacterial counts (TBC) were directly determined by epifluorescence microscopy, according to the standard procedure that involves staining with Acridine Orange [35]. Viable bacteria were defined as those with intact cytoplasmic membranes (MEMB$^+$) and active electron transfer system (CTC$^+$). The MEMB$^+$ bacteria were counted by using the Live/Dead® BacLight™ kit (Thermo Fisher Scientific Inc.) according to the manufacturer's instructions [36]. Bacteria with active electron transport system (CTC$^+$) were enumerated as described by Rodriguez *et al.* [37]. To determine TBC, MEMB$^+$ and CTC$^+$ counts, filters with stained bacteria were examined with a Nikon epifluorescence microscope equipped with a filter block B-2A (EX450-490 excitation filter, DM505 dichroic mirror, and BA520 barrier filter) and with a DAPI filter block (EX340-380 excitation filter, DM400 dichroic mirror, and BA435-485 barrier filter). In general, at least 20 fields were checked. Culturability, expressed as colony-forming units (cfu), was determined by spreading aliquots of *A. baumannii* cells on MH agar followed by incubation at 37°C for 24 h.

Quantification of the ability to adhere to abiotic surfaces

The ability to adhere to surfaces was assessed by the Crystal Violet method with some modifications described by Bravo *et al.* [18,38]. During the survival experiments, cultures were classified by their ability to form biofilm according to Stepanović *et al.* [39]. The cut-off OD (ODc) was defined as a value equal to three standard deviations above the mean OD of the negative control, and the following categories were established: $OD \leq OD_C$, non-adherent; $ODc < OD \leq (2 \times ODc)$, weakly adherent; $(2 \times ODc) < OD \leq (4 \times ODc)$, moderately adherent; $(4 \times ODc) < OD$, strongly adherent.

Statistical data analysis

All the results presented were means of at least three experiments, and the coefficients of variation between replicate experiments were <12%. The differences between the means were calculated by one-way analysis of variance. $P \leq 0.05$ was considered significant. Logarithmic transformation for bacterial counts was used.

Results

Long-term survival of A. baumannii populations

The effects of temperature (20°C versus 37°C) and media (liquid versus solid matrix) on integrity, viability, and

culturability of *A. baumannii* strains under nutrient deprivation are summarized in Figure 1.

The total bacterial count (TBC) determined for one control strain and three *A. baumannii* clinical isolates during their permanence in aqueous environments or on membrane filters revealed no significant variations in cell number, suggesting that *A. baumannii* cells preserved their integrity for ≥30 days. Moreover, populations incubated at 20°C maintained their

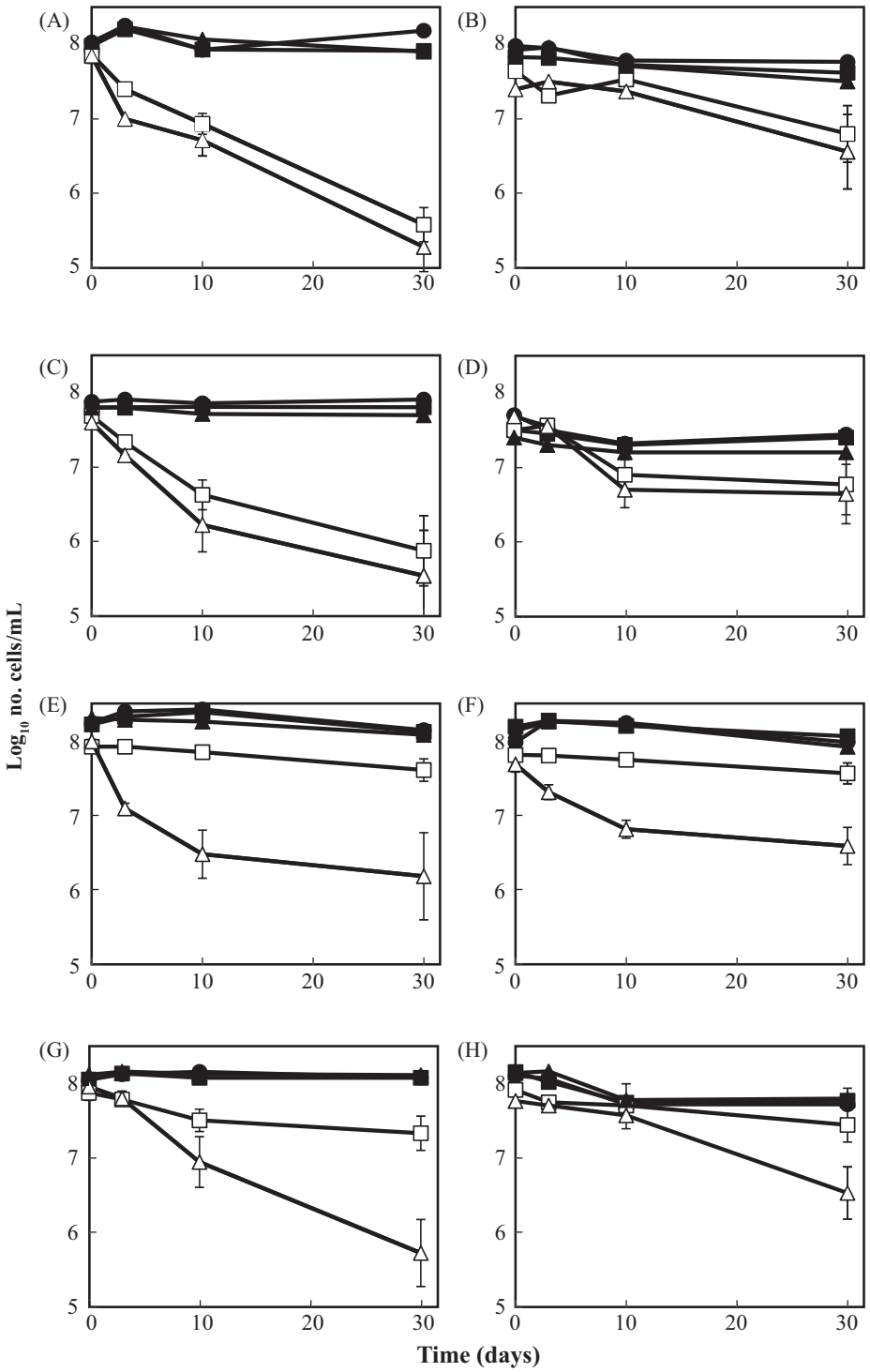

Figure 1. Survival of *Acinetobacter baumannii* ATCC 19606 (A, B) and clinical isolates HUMV-2790 (C, D), HUMV-2471 (E, F), and HUMV-3743 (G, H) maintained in aqueous environment (A, C, E, G) and on dry filters (B, D, F, H). Counts of total (●), viable (■, □) and culturable (▲, △) cells obtained for populations maintained at 20°C (closed symbols) and at 37°C (open symbols). As the total counts were nearly identical at 20°C and 37°C, the graphs were simplified by showing only values obtained at 20°C. Data are mean values from three independent experiments with bars representing the standard deviations.

culturability and viability both in saline solution and on membrane filters (Figure 1). By contrast, incubation at 37°C revealed two different behaviour patterns. The first was associated with *A. baumannii* strains HUMV-2471 and HUMV-3743. Whereas both strains maintained high viability, their culturability decreased significantly ($P \leq 0.05$) ($\log_{10}$ reduction factors of about 2 and 1.3 in liquid and solid environments, respectively), resulting in a VBNC subpopulation. By contrast, the counts of viable and culturable cells of the *A. baumannii* strains ATCC 19606^T and HUMV-2790 decreased by $\log_{10}$ reduction factors of ≥ 2 ($P \leq 0.05$) after 30 days of permanence in saline solution (Figure 1A and C) and of 0.8–1.2 on membrane filters during the same period of time (Figure 1B and D). The concurrent loss of membrane integrity and culturability suggests that the VBNC state was not induced under these conditions. However, when exposure of *A. baumannii* ATCC 19606^T to adverse conditions was extended, long filamentous cells fluorescing simultaneously in green (MEMB$^+$) and red (MEMB$^-$) were observed (Figure 2). To clarify the ambiguity of staining, the viability of cells was also assessed by an alternative method by counting cells with active electron transfer system (CTC$^+$). The numbers of CTC$^+$ cells estimated for stressed populations in saline solution were slightly higher than MEMB$^+$ counts (2.5–6.3-fold), which supports the idea that acquisition of VBNC phenotype was not a primary choice for these strains.

Additionally, survival experiments with *A. baumannii* strain ATCC 19606^T were extended to 90 days. TBC, MEMB$^+$ and cfu counts determined for populations exposed at 20°C for 90 days did not differ significantly from those obtained for 30-day-old populations. By contrast, cells exposed at 37°C for 90 days maintained integrity, whereas viability and culturability decreased slightly with respect to values obtained after 30 days of nutrient deprivation (data not shown).

The ability of populations maintained in saline solution or on membrane filters to adhere to abiotic surfaces was determined. The *A. baumannii* strains ATCC 19606^T, HUMV-2471, and HUMV-3743 behaved as moderately adherent strains, whereas HUMV-2790 was classified as a strongly adherent strain (Figure 3). Although the ability to adhere to solid surfaces

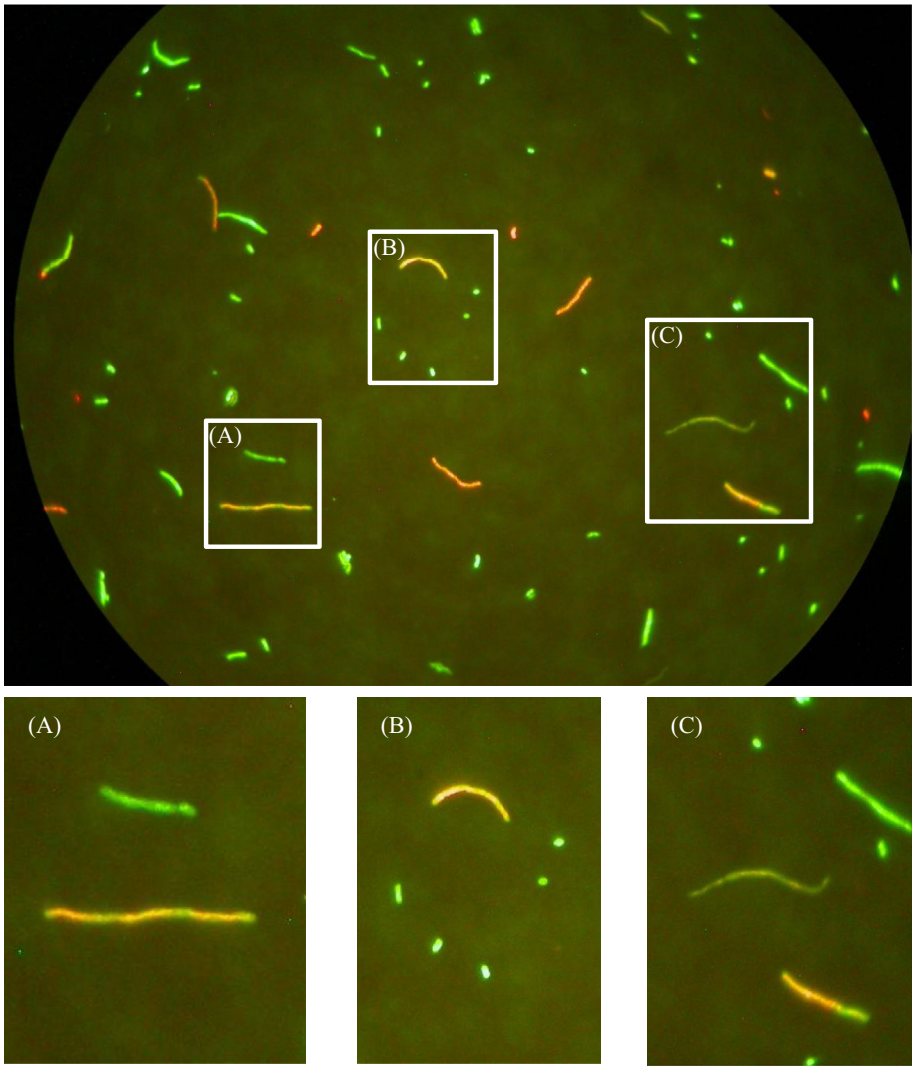

Figure 2. Epifluorescence microscopy image of *Acinetobacter baumannii* ATCC 19606^T incubated in saline solution at 37°C and stained with the Live/Dead®BacLight™ kit afterwards.

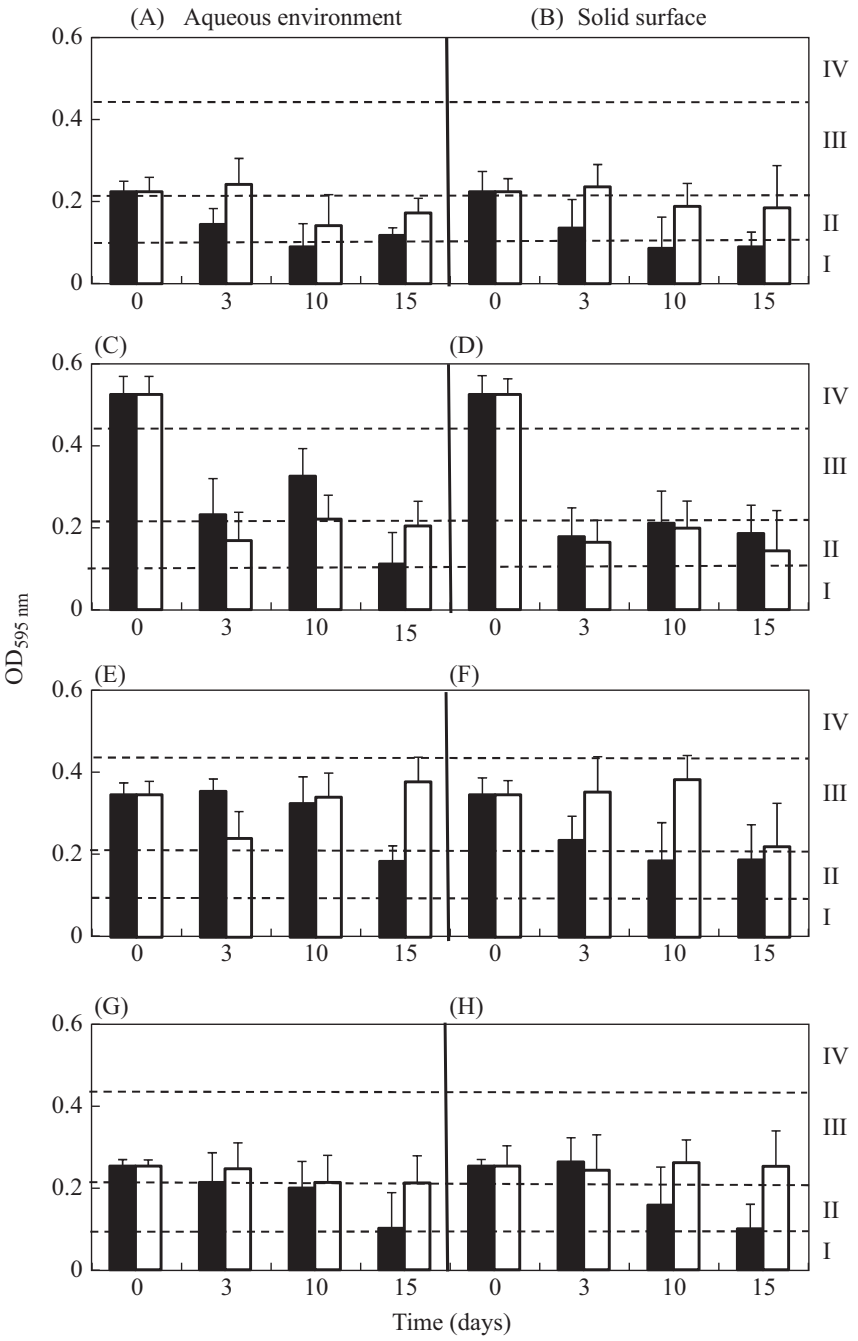

Figure 3. Changes in the ability of *Acinetobacter baumannii* populations to adhere to solid surfaces during its survival. *A. baumannii* ATCC 19606^T (A, B) and clinical isolates HUMV-2790 (C, D), HUMV-2471 (E, F) and HUMV-3743 (G, H) were maintained in aqueous environment or solid surface at 20°C (closed bars) and 37°C (open bars) for 15 days. The data for optical density, mean values from three independent experiments, are expressed as OD_{595nm} (±standard deviation). The dotted lines indicate the limits for OD_{595nm} values of non-adherent (I), weakly (II), moderately (III), and strongly (IV) adherent categories.

progressively decreased along the survival period, it was less affected (except for HUMV-2790) for populations maintained at 37°C than those at 20°C.

The survival patterns obtained for *A. baumannii* strains maintained under nutrient deprivation on diverse solid surfaces are summarized in Figure 4. *A. baumannii* populations adherent to glass coverslips and stainless steel discs preserved their integrity, viability, and cultivability during the time studied (30 days). However, all bacterial counts (TBC, MEMB$^+$ and cfu) obtained for populations adhered on fragments of cotton cloth gradually decreased during the course of the study.

Effect of disinfectants on A. baumannii populations

The effect of disinfectants on integrity, viability, and culturability of *A. baumannii* ATCC 19606^T populations maintained at 20°C on stainless steel discs are summarized in Figure 5. None of the disinfectants tested affected cellular integrity;

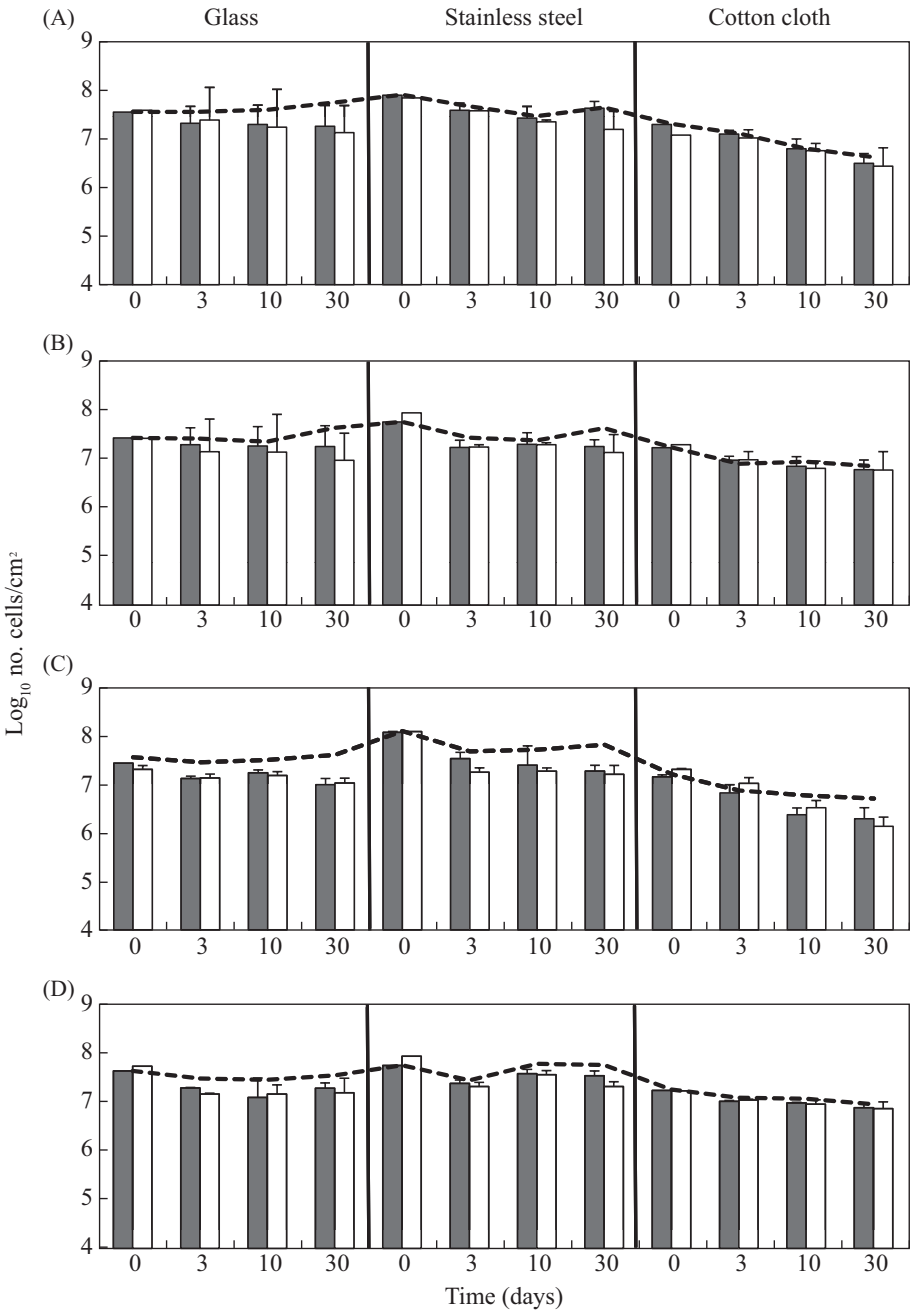

Figure 4. Survival of *Acinetobacter baumannii* ATCC 19606^T (A) and clinical isolates HUMV-2790 (B), HUMV-2471 (C), and HUMV-3743 (D) maintained on dry surfaces. Total (dotted lines), viable (closed bars), and culturable (open bars) cells were determined for *A. baumannii* populations maintained during 30 days on glass, stainless steel, and cotton cloth at 20°C. The data are mean values from three independent experiments with bars representing the standard deviations.

however, viability decreased ~10-fold after 15 min of exposure to undiluted or diluted (10%, v/v) (Figure 5A and B) bleach and 50-fold after 0.5 min exposure to Instrunet products (Figure 5C and D). The decreases in culturability occurred in the first 30 s, after which the number of cfu remained unchanged within 1.5×10^2 and 6.3×10^3 cells/cm^2 for populations exposed to a undiluted and diluted commercial bleach, or 7.2×10^2 and 4.3×10^3 cells/cm^2 for those exposed to Instrunet products, Surfa'Safe and 0.5% (v/v) solutions of Aniosyme DD1, respectively.

Due to effects of Instrunet products and the recommendation to use diluted bleach for the disinfection of facilities, we selected only diluted commercial bleach (10%, v/v) and Instrunet Surfa'Safe for further experiments to determine the effect of disinfectants on clinical strains. The $\log_{10}$ reduction in culturability factors obtained for *A. baumannii* populations exposed for 5 min to diluted commercial bleach (4000 ppm) and to Instrunet Surfa'Safe are presented in Table I. Except for HUMV-3743 strain, treatment with Instrunet Surfa'Safe was usually more efficient than that with commercial

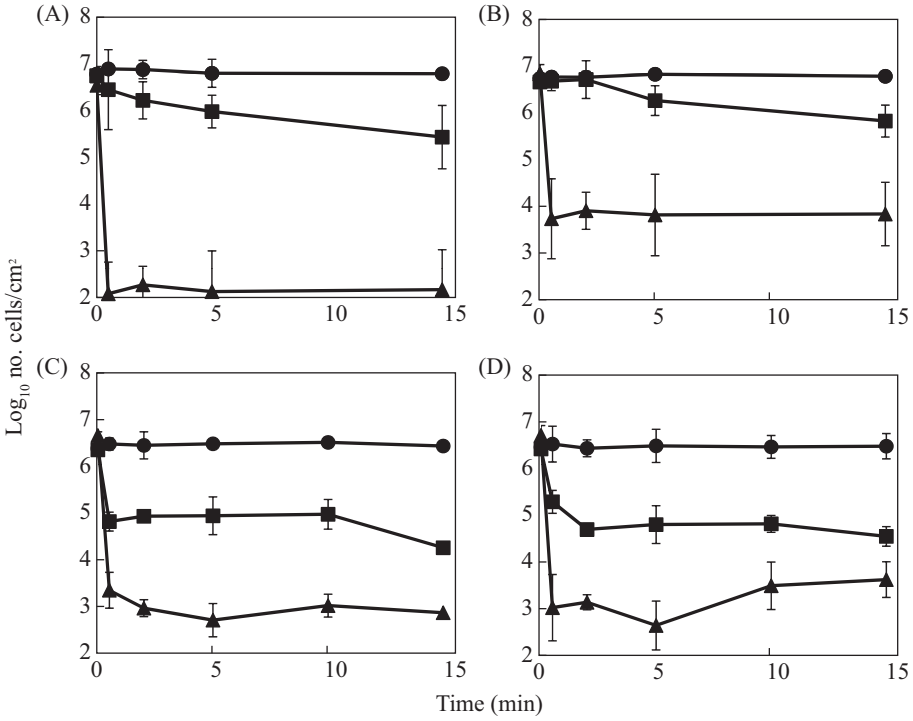

Figure 5. Bactericidal activity of undiluted bleach (40,000 ppm) (A), diluted bleach (4000 ppm) (B), Instrunet Surfa'Safe (C) and Instrunet Aniosyme DD1 (D) on *Acinetobacter baumannii* ATCC 19606^T populations maintained on stainless steel discs at 20°C. Total (●), viable (■) and culturable (▲) cells were enumerated at different exposure times. The data are mean values from three independent experiments with bars representing the standard deviations.

Table I
Mean $\log_{10}$ reduction in culturability (and standard deviation) calculated for *Acinetobacter baumannii* populations after contact time for disinfectants of 5 min at 20°C

A. baumannii strains	Diluted bleach (4000 ppm)	Instrunet® Surfa'Safe
ATCC 19606	2.71 (±0.44)	3.45 (±0.23)
HUMV-2790	3.14 (±0.19)	4.46 (±0.20)
HUMV-2471	2.90 (±0.47)	4.25 (±0.09)
HUMV-3743	4.90 (±0.17)	3.65 (±0.01)

bleach, resulting in a large reduction of cell culturability ($\log_{10}$ reduction factors of 3.45–4.46) but not in the total number of cells (data not shown). Moreover, the reference strain 19606^T was more resistant than the clinical isolates.

Discussion

Temperature is the key abiotic factor affecting bacterial survival and permanence in different environments [40–42]. Moreover, the optimum survival temperature of mesophilic bacteria is usually below the optimum growth temperature [40,43]. Consistent with this rule, the four *A. baumannii* strains studied here lost their culturability at 37°C. However, this negative effect was not observed for populations maintained both in liquid environment and on solid surfaces at 20°C. Besides, 30-day incubation under nutrient deprivation led to the appearance of two different types of survival pattern. The first was dominated by non-viable cells in populations of ATCC 19606^T and HUMV-2790 strains, whereas HUMV-2471 and HUMV-3743 strains responded by entering into the VBNC state (Figure 1). Differences in strain-dependent survival patterns, like those described in this study, have been previously reported for other Gram-negative bacteria such as *Escherichia coli*, *Campylobacter jejuni*, and even *A. baumannii* [44–47].

The decreases in the number of viable cells determined as cells with intact cytoplasmic membranes (MEMB$^+$) and the images of filamentous cells fluorescing simultaneously in green and red exhibiting altered cytoplasmic membrane permeability (Figure 2) suggested the formation of a fraction of non-viable bacteria. Stocks indicated that the apparent dual staining results are likely due to the possibility of propidium iodide to quench the SYTO9 emission, and Christiansen *et al.* considered dual-stained cells as only SYTO9 positive (i.e. alive); in our study the similar reduction in the number of cells with active electron transport system (CTC$^+$) (Figure 1) demonstrates a decrease in the fraction of viable cells, thus suggesting that these strains did not enter into the VBNC state as was previously described for an *A. baumannii* population exposed to nutrient deprivation [18,48,49].

Collectively, the above results indicate that both the bust-and-boom mechanism and entry into the VBNC state apparently confer the long-term survival of *A. baumannii* in different environments. Moreover, Li *et al.* have stated the possibility of its resuscitation from the VBNC state [50]. Therefore, these adaptation mechanisms are likely essential for *A. baumannii* persistence and survival during the recurrent outbreaks found in ICUs.

As adhesiveness could potentially affect *A. baumannii* persistence in hospital environments, experiments were done to assess *A. baumannii* attachment to abiotic surfaces and its dependence on environmental factors. When analysing the adhesiveness of the *A. baumannii* strains to abiotic surfaces, as previously described, strain-dependent differences were found, HUMV-2790 being the strain uniquely classified as strongly adherent [46,51].

Survival experiments revealed that starvation generally reduced the adhesion ability, especially at 20°C; strain-dependent differences were then found and HUMV-2790 strain could retain its strong capacity to adhere during the exposure to different temperatures. The loss of adhesiveness under nutrient deprivation might suggest that starved populations would find difficulty in developing biofilms and, therefore, in spreading and colonizing hospital surfaces. However, *A. baumannii* has been reported to resist desiccation beyond 30 days [52]. In agreement with the above notion, our results demonstrated that *A. baumannii* cells were able to persist several days without loss of viability and culturability on different solid surfaces. These results are consistent with the idea that hospital equipment and clothes could serve as a secondary reservoir for *A. baumannii* [8,21,53]. Nevertheless, the nature of materials may influence *A. baumannii* attachment and, as a result, its persistence on various surfaces [54]. In fact, we found that various solid matrices differed in their capacity to sustain *A. baumannii* survival and the cotton of the laboratory cloth appeared to be the only material that led to decline of *A. baumannii* populations. However, one needs to consider that, in this case, desiccation could affect the structure of the cotton cloth, subsequently trapping cells and hindering their recuperation (Figure 4) [13].

Finally, as disinfectants are widely used in hospitals worldwide, the last part of our study was assessing the effects of these antimicrobials on *A. baumannii* survival. Several studies have shown that widely used disinfectants, such as sodium hypochlorite or quaternary amine compounds (QACs), are 100% effective against *A. baumannii* isolates, especially if their populations are attached to surfaces. Thus, Orsinger-Jacobsen *et al.* described the complete inactivation of biofilms after 1 min of exposure to 3.125% hypochlorous acid (HOCl) [55]. The active ingredient of household bleach, HOCl, damages multiple cellular components including macromolecules by simultaneously reacting with proteins, nucleotides, and lipids. Inactivation of multiple targets, in turn, causes a rapid loss of respiration, oxidative damage of cell membranes, etc. [56]. As to QACs, they are known for their membrane-damaging properties and their damaging effect is also extended to intracellular targets [57]. Here we found that bleach and Instrunet products (QACs) quickly induced loss of *A. baumannii* ATCC 19606^T culturability and triggered cells' entry into the VBNC state (already after 30 s). According to these data, *A. baumannii* clinical isolates were found to be highly susceptible (even more than the control strain) to the disinfectants routinely used in hospitals. These findings are consistent with the results of previous studies demonstrating that environmental cleaning and treatment with disinfectants could greatly reduce the risk of disease transmission [20,58].

Although undiluted bleach and QACs caused the highest reduction of culturable population, they did not eliminate them completely and the other populations remained viable and existed as VBNC cells. Martró *et al.* could not confirm a correlation between resistance to antibiotics and a decreased susceptibility to antiseptics or disinfectants for clinical strains [59]. However, several authors have suggested a relationship between high multidrug resistance to antimicrobials and reduced susceptibility to disinfectants likely associated with the high prevalence of genes encoding resistance to QACs in *A. baumannii* hospital isolates [60—62]. Some antibiotic resistance mechanisms, such as those dependent on multidrug efflux pumps and permeability defects, were described for this micro-organism and were subsequently suggested to confer disinfectant resistance [61].

In conclusion, although starvation and physical environment differentially affect its survival, *A. baumannii* shows a great resistance to stress, being able to persist without loss of viability and culturability for long periods on different surfaces, mimicking those usually found in hospitals. Moreover, this study shows that the disinfectants tested cause a rapid loss of cultivability, but they do not effectively eliminate *A. baumannii* cells. This finding, along with the ability of this pathogen to survive on dry abiotic surfaces at room temperature, could explain the recurrent outbreaks found in ICUs.

Conflict of interest statement
None declared.

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