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Survival dynamics of cystic fibrosis-related Gram-negative bacterial pathogens (*Pseudomonas aeruginosa* and *Burkholderia cenocepacia*) in Dead Sea and Atlantic Ocean waters

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ABSTRACT

Clinical cystic fibrosis (CF) *Pseudomonas aeruginosa* ($n = 6$) and *Burkholderia cenocepacia* ($n = 4$) were inoculated in marine brines from the Dead Sea and the Atlantic Ocean and their survival was monitored over a 1 month duration. In Dead Sea samples, all *P. aeruginosa* and *B. cenocepacia* isolates were non-detectable by culture following 24 h incubation, including the non-selective enrichment samples. In the Atlantic Ocean brine, over a 1 month period, mean *P. aeruginosa* counts decreased by only 0.25 log10 units and mean *B. cenocepacia* counts decreased by approximately 4 log10 units (10,000 cfu/ml). This study demonstrated that Dead Sea brine exerted a lethal effect within 24 h on planktonic *P. aeruginosa* and *B. cenocepacia*. Thus, the Dead Sea effectively purges these organisms from its environment on a daily basis.

Key words | *Burkholderia cenocepacia*, cystic fibrosis, Dead Sea, environmental persistence, microbiology, *Pseudomonas aeruginosa*, survival

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INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) and *Burkholderia cenocepacia* (*B. cenocepacia*) are two important bacterial pathogens in patients with cystic fibrosis (CF), resulting in chronic lung infections with significant morbidity and mortality (LiPuma 2010). Both of these bacteria have been isolated from a wide variety of environmental sources, including waters (LiPuma 2010).

It has been reported previously that the high salinity of the Dead Sea of approximately 347 g/l of mainly divalent cations (Mg) is an inhospitable environment for cellular activity (Rhodes *et al.* 2012). An older study from a more hypersaline lake, Lake Assal near Djibouti, with a salinity of 400 g/l, showed a diverse population of euryhalines and strict halophiles (Brisou *et al.* 1974). Notwithstanding this, there have been several previous reports suggesting that the Dead Sea is inhabited primarily by unculturable bacteria (Brisou *et al.* 1974). Other recent reports indicate that freshwater springs within the Dead Sea are a rich source of bacteria, including *Epsilonproteobacteria* and *Gammaproteobacteria* (Ionescu *et al.* 2012), the latter class, to which the family Pseudomonadaceae and the genera *Pseudomonas* and *Burkholderia* belong.

More recently, there have been several reports on improved outcomes for CF patients spending time at the Dead Sea (Falk *et al.* 2006; Goldbart *et al.* 2007). Therefore, a combination of these factors could allow for a scenario of CF patients swimming and expectorating *P. aeruginosa* and *B. cenocepacia* in their sputum, at common bathing sites along its shoreline.

Therefore, the aim of this study was to compare the planktonic survival of *P. aeruginosa* and *B. cenocepacia* in Dead Sea brine with brine from the Atlantic Ocean.

MATERIALS AND METHODS

Bacterial isolates and water microcosm preparation

Clinical isolates ($n = 10$), consisting of *P. aeruginosa* ($n = 6$) and *B. cenocepacia* ($n = 4$) were obtained from the Northern Ireland Health and Social Care Microbiology Repository (MicroARK; www.microark.com). All these isolates had been isolated previously from adult CF patients and had been archived at −80 °C. All isolates were recovered on Columbia Blood agar (Oxoid CM0031, Oxoid Ltd, Basingstoke, UK), supplemented with 5% (v/v) defibrinated horse blood for 24 h at 37 °C, under aerobic conditions and passaged a further three times, prior to use.

Water (2 l) was extracted from a regular sampling point at the Dead Sea, with GPS co-ordinates 31°13.941′N, 35°23.858′E. Water (1 l) from the Atlantic Ocean was extracted at GPS co-ordinates 55°10′6.2724″N, 6°47′37.5828″W. Both water samples were transported to the laboratory at ambient temperature. On receipt of water samples, each was filter sterilised individually by passage through a 0.2 μm syringe filter and stored in the dark at ambient temperature. A microcosm of each water type was prepared by preparing 50 ml filter sterilised water into a sterile glass container.

Each isolate was grown aerobically overnight on nutrient agar (Oxoid CM1, Basingstoke, UK) (48 h incubation for the *B. cenocepacia* isolates) and an homogenous inoculum was prepared in ¼ Ringer's diluent (Oxoid BR0052), equating to approximately 10^{10} colony forming units (cfu)/ml, after which 0.1 ml of this inoculum was introduced into each prepared water microcosm of both water types. All 20 permutations of organism/water type were stored in the dark for 1 month (30 days), at ambient room temperature (constant 20 °C). Sampling was performed daily for the first week, by the enumeration of organisms employing serial dilutions on nutrient agar for all organisms, except for *B. cenocepacia* which were enumerated on Columbia Blood Agar, as described above. When direct counts became non-detectable, 0.1 ml water microcosm was non-selectively enriched, following inoculation into nutrient broth (Oxoid CM1; 25 ml). This enrichment was incubated for 48 h at 37 °C and examined/plated at 24 and 48 h for growth.

RESULTS AND DISCUSSION

Survival of organisms in the Dead Sea microcosm is shown in Figure 1(a). All clinical *P. aeruginosa* and *B. cenocepacia* isolates were non-detectable following 24 h incubation, including the non-selective enrichment samples, indicating

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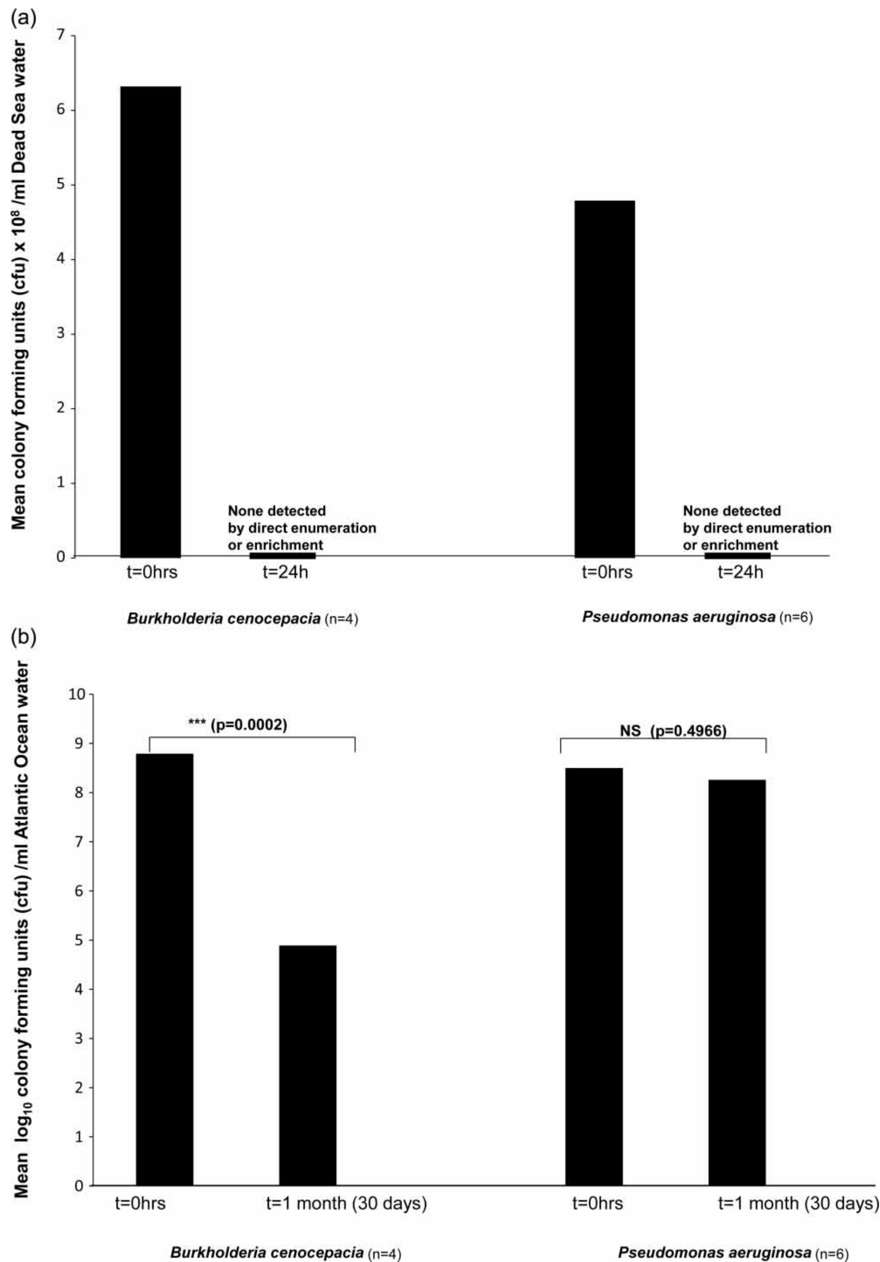

Figure 1 | Mean survival of clinical *P. aeruginosa* and *B. cenocepacia* at 20 °C: (a) in Dead Sea water and (b) in Atlantic Ocean water.

that no culturable organisms could be recovered after 24 h incubation in this water.

Survival of organisms in the Atlantic Ocean microcosm is shown in Figure 1(b). Over the 1 month period, mean *P. aeruginosa* counts decreased by 0.25 log10 units, which was not statistically significant ($p = 0.4966$). In contrast, mean counts of *B. cenocepacia* decreased by approximately 4 log10 units (10,000 cfu/ml), which was statistically significant ($p = 0.0002$).

This study demonstrated that Dead Sea brine exerted a lethal effect within 24 h on planktonic *P. aeruginosa* and *B. cenocepacia*. Thus, the Dead Sea effectively purges these organisms from its environment on a daily basis. In contrast, brine from the Atlantic Ocean, sampled

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just off the north coast in Northern Ireland, did not have a significant effect on the persistence of clinical *P. aeruginosa*, with numbers being maintained for 1 month, without any significant decrease in culturable bacterial counts. The relative survival of large numbers of both organisms in Atlantic Ocean water presents several important questions in terms of risk assessment for infection prevention purposes in the CF patient population. What is not known is the infective dose of these pathogens required to establish initial persistent colonisation of the upper airways in CF patients, leading to chronic colonisation and infection of the lower airways. Previous quantitative studies have demonstrated that *P. aeruginosa* is present in open ocean environments at a cell density of 0 to 8.3 cells/l (Khan *et al.* 2007), although its apparent marine distribution is with river outlets and shorelines, often associated with fresh water and sewage (Khan *et al.* 2007).

In conclusion, this study showed that Dead Sea water has a lethal effect on planktonic *P. aeruginosa* and *B. cenocepacia* CF clinical isolates, where all cells from both organisms were non-culturable within 24 h. This is in contrast to water from the Atlantic Ocean, where *P. aeruginosa* culturable cell density was relatively unaltered over the period of 1 month, whereas *B. cenocepacia* counts were reduced by approximately 4 log cycles.

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REFERENCES

Brisou, J., Courtois, D. & Denis, F. 1974 Microbiological study of a hypersaline lake in French Somaliland. *Appl. Microbiol.* **27**, 819–822.

Falk, B., Nini, A., Zigel, L., Yahav, Y., Aviram, M., Rivlin, J., Bentur, L., Avital, A., Dotan, R. & Blau, H. 2006 Effect of low altitude at the Dead Sea on exercise capacity and cardiopulmonary response to exercise in cystic fibrosis patients with moderate to severe lung disease. *Pediatr. Pulmonol.* **41**, 234–241.

Goldbart, A. D., Cohen, A. D., Weitzman, D. & Tal, A. 2007 Effects of rehabilitation winter camps at the Dead Sea on European cystic fibrosis patients. *Israel Med. Assoc. J.* **9**, 806–809.

Ionescu, D., Siebert, C., Polerecky, L., Munwes, Y. Y., Lott, C., Häusler, S., Bižić-Ionescu, M., Quast, C., Peplies, Glöckner, F. O., Ramette, A., Rödiger, T., Dittmar, T., Oren, A., Geyer, S., Stärk, H.-J., Sauter, M., Licha, T., Laronne, J. B. & de Beer, D. 2012 Microbial and chemical characterization of underwater fresh water springs in the Dead Sea. *PLoS One* **7**, e38319.

Khan, N. H., Ishii, Y., Kimata-Kino, N., Esaki, H., Nishino, T., Nishimura, M. & Kogure, K. 2007 Isolation of *Pseudomonas aeruginosa* from open ocean and comparison with freshwater, clinical, and animal isolates. *Microbiol. Ecol.* **53**, 173–186.

Lipuma, J. J. 2010 The changing microbial epidemiology in cystic fibrosis. *Clin. Microbiol. Rev.* **23**, 299–323.

Rhodes, M. E., Oren, A. & House, C. H. 2012 Dynamics and persistence of Dead Sea microbial populations as shown by high-throughput sequencing of rRNA. *Appl. Environ. Microbiol.* **78**, 2489–2492.

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