Efficacy of the biocide Steri-7 against the common Gram-negative bacterial pathogens (*Burkholderia cenocepacia*, *Burkholderia gladioli*, *Burkholderia multivorans*, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*) associated with cystic fibrosis (CF)

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Cystic fibrosis (CF) is the most commonly inherited disease in persons of a white and European background and has a genetic carriage rate of one person in 20 and an incidence of one live birth in 2500.[1] It is an autosomal recessive condition whereby two alleles carrying a polymorphism in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene phenotypically manifests the disease state through various multi-organ problems associated with a pharmacological dysfunction to regulate chloride-ion secretion across cell membranes.

The most common complication of CF is the recurrence of chronic chest infections usually caused by bacterial pathogens.[2] Patients continue to suffer from recurrent and chronic respiratory tract infections and most morbidity and mortality is due to such infections throughout their life.[3]

These infections are usually dominated by Gram-negative organisms, especially by the pseudomonads, including *Pseudomonas aeruginosa*, *Burkholderia cepacia* complex organisms and *Stenotrophomonas maltophilia*. In addition, some of these organisms are highly transmissible from patient to patient and many are highly resistant to most major classes of antibiotic agent, as well as to many biocide agents employed in the sanitising of healthcare environments.

Overall, the occurrence of multi-resistant and often pan-resistant transmissible strains leads to major concern for the efficacy of stringent infection control procedures, which are practised by CF centres globally, in an attempt to prevent the person-to-person spread of such transmissible strains.

Therefore, the aim of this study is to examine the biocidal action of the commercially available sanitising agent Steri-7 (www.steri-7.com) against common Gram-negative pathogens in patients with cystic fibrosis.

The wild-type bacterial isolates employed in this study were *B. multivorans*, *B. cenocepacia*, *S. maltophilia* and *P. aeruginosa*. In addition, the reference strain *B. gladioli* ATCC10854 was examined. With the exception of the reference strain, all other isolates were obtained from freshly expectorated sputum from adult CF patients (aged >16 years) following in-patient physiotherapy. All isolates were archived at –80°C within the Northern Ireland HSC Microbiological Culture Repository (MicroARK) housed within the Northern Ireland CF Bacterial Strain Repository at the Department of Bacteriology, Belfast City Hospital. Isolates were recovered by plating on Columbia blood agar (Oxoid CM0331) supplemented with 5% (v/v) defibrinated horse blood at 37°C for 48 h, followed by a further two passages prior to experimental use.

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Three formulations of Steri-7 were assessed, including i) Steri-7 HD concentrated formula, ii) Steri-7 surface cleaner, and iii) Steri-7 hand sanitiser. The biocidal activity of Steri-7 was assessed in two assays.

In the first assay, a semiconfluent lawn of each organism was prepared on individual Columbia blood agar (Oxoid CM0331) supplemented with 5% (v/v) defibrinated horse blood. Steri-7 formulation (50 µL) was added separately to each bacterial lawn and allowed to be absorbed and dried on each plate prior to incubation at 37°C for 48 h. Control plates were also set up without the addition of biocide agent. A 10-fold dilution of each of the three formulations was also examined in a similar manner.

In the second assay, 1 mL each of the three Steri-7 formulations was placed in a sterile glass tube (5 mL). Bacterial organisms were cultured overnight on Columbia blood agar (Oxoid CM0331) supplemented with 5% (v/v) defibrinated horse blood prior to the preparation of a bacterial suspension in 0.1% (w/v) peptone saline diluent (Oxoid) of approximately 10^6 ($\log_{10}6$) per mL. The actual number of each organism was determined quantitatively by the spread plate technique. Briefly, serial dilutions of each bacterial inoculum were prepared individually in quarter-strength Ringer's solution diluent (Oxoid BR52). From the 10^{-4}, 10^{-5} and 10^{-6} dilutions in duplicate, 100 µL inoculum was spread accurately on the surface of Columbia agar base (Oxoid CM331) supplemented with 5% (v/v) defibrinated horse blood (E&O Laboratories, Bonnybridge, Scotland) and incubated at 37°C for 48 h prior to counting. All cultured colonies were enumerated and the total viable count (TVC) was expressed as $\log_{10}$ colony-forming units (cfu) per mL of the original inoculum.

Bacterial inocula of each, equating to 1.25 x 10^6 cfu/mL *B. multivorans*, 1.85 x 10^6 cfu/mL *B. cenocepacia*, 4.15 x 10^5 cfu/mL *S. maltophilia*, 1.02 x 10^7 cfu/mL *P. aeruginosa* and 2.50 x 10^4 cfu/mL *B. gladioli* ATCC10854 were added separately to each tube containing biocide and exposed for a total of 5 min. After this period, 100 µL biocide plus organism was plated on Columbia agar base (Oxoid CM331) supplemented with 5% (v/v) defibrinated horse blood (E&O Laboratories) and incubated at 37°C for 48 h prior to counting. In addition, the remaining volume of each tube was transferred to 10 mL freshly prepared nutrient broth (Oxoid CM1) and enriched for 24 h at 37°C, after which time 20-µL volumes were plated on Columbia agar base (Oxoid CM331) supplemented with 5% (v/v) defibrinated horse blood (E&O Laboratories) and incubated at 37°C for 48 h.

Results showed that all three formulations of Steri-7 killed all five CF bacterial pathogens at undiluted concentrations. When these products were diluted 10-fold with 0.1% (w/v) peptone saline no growth was observed except for *B. multivorans*, *B. cenocepacia* and *P. aeruginosa* in the surface spray cleaner, the undiluted use of which is recommended. In relation to the second immersion assay, none of the

bacterial organisms survived 5-min contact time with any Steri-7 formulation, thus equating to a complete kill of all organisms present (log 4 – log 7). In order to determine if this was a bacteriocidal effect or a bacteriostatic effect, treated organisms were placed in non-selective enrichment broth to allow recovery of any culturable organism present. None were subsequently recovered so these formulations were shown to be bactericidal in nature.

Chronic chest infections with bacterial respiratory pathogens, mainly *P. aeruginosa* and the *B. cepacia* complex, are significant causes of morbidity and mortality in patients with CF, generally resulting in premature death, compared to CF patients with no history of significant chest infection.[2,3] Thus, it is important that stringent measures be taken in an attempt to prevent colonisation of the lung with these and other organisms, both by the patient and the healthcare professional in conjunction with infection control guidelines.[4] Although combinational antibiotic therapy is the cornerstone of the treatment of such chronic infections, high levels of resistance have been described for Gram-negative CF organisms once they have been acquired by the CF patient.[5] Therefore, other approaches are being sought, such as promoting the disruption of biofilm formation in *B. cepacia* and *P. aeruginosa* through alteration of quorum sensing mechanisms, in order to assist in the control of bacterial infection.[6]

There has been substantial evidence to demonstrate the efficacy of the Steri-7 biocide against several genera of organisms and multiple species (www.steri-7.com/pdf/Steri-7_Techinical_manual_updated_280907.pdf). However, the present small study aimed to evaluate the efficacy of this biocide against several highly resistant Gram-negative pathogens found in the sputa of patients with CF. Overall, it showed that this biocide worked as an effective bactericidal agent against the CF pathogens tested. □

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References

1 Devaney J, Glennon M, Farrell G *et al*. Cystic fibrosis mutation frequencies in an Irish population. *Clin Genet* 2003; **63** (2): 121–5.
2 Lyczak JB, Cannon CL, Pier GB. Lung infections associated with cystic fibrosis. *Clin Microbiol Rev* 2002; **15** (2): 194–222.
3 Rajan S, Saiman, L. Pulmonary infections in patients with cystic fibrosis. *Semin Respir Infect* 2002; **17** (1): 47–56.
4 Littlewood J. Pseudomonas aeruginosa *infection in people with cystic fibrosis. Suggestions for prevention and infection control*. Bromley, Kent: Cystic Fibrosis Trust, 1–24.
5 Moore JE, Crowe M, Shaw A, McCaughan J, Redmond AO, Elborn JS. Antibiotic resistance in *Burkholderia cepacia* at two regional cystic fibrosis centres in Northern Ireland: is there a need for synergy testing? *J Antimicrob Chemother* 2001; **48** (2): 319–21.
6 Huber B, Riedel K, Hentzer M *et al*. The cep quorum-sensing system of *Burkholderia cepacia* H111 controls biofilm formation and swarming motility. *Microbiology* 2001; **147** (Pt 9): 2517–28.
7 Osset J, Bartolome RM, Garcia E, Andreu A. Assessment of the capacity of *Lactobacillus* to inhibit the growth of uropathogens and block their adhesion to vaginal epithelial cells. *J Infect Dis* 2001; **183** (3): 485–91.

Antibiotic resistance reversal (ARR) in Gram-negative and Gram-positive pathogens employing electric fields

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Antibiotic resistance in clinical bacterial pathogens has now become a major global public health problem. In certain infections (e.g., chronic pulmonary infections in patients with cystic fibrosis), antibiotic resistance has become critical in the treatment of *Pseudomonas aeruginosa*, due to widespread resistance, which causes a major treatment dilemma. In addition, bacteria are developing resistance more quickly than new efficacious antibiotics are being produced, leading to an eventual drain on the effect of antibiotics. Therefore, several antibiotic stewardship policies have recently been introduced into hospitals in order to promote the judicious use of remaining antibiotics in an attempt to prolong the efficacy of antibiotics for treatment in human infections.[1,2]

An alternative strategy to prolong the efficacious life of antibiotics would be to seek a mechanism of resistance reversal, thereby allowing older and less-efficacious antibiotics to be reintroduced with great clinical success, and to avoid the use of new and very expensive antibiotics, whereby the latter classes of newer agents could be reserved for the complicated cases of infection.

Therefore, it is the aim of the current study to examine antibiotic susceptibility of Gram-negative and Gram-positive pathogens in the presence of an electric field, in order to explore the potential application of electric fields to reducing the burden of antibiotic resistance in clinical pathogens.

Three clinical bacterial isolates were used in this study, including the Gram-positive organisms *Enterococcus faecalis* NCTC775 and a wild-type clinical isolate of methicillin-resistant *Staphylococcus aureus* (MRSA), as well as the Gram-negative organism *Escherichia coli* NCTC9001. These isolates are part of the Northern Ireland Public Health Laboratory (NIPHL) Strain Repository (MicroARK) and were recovered from storage at –80°C.

All isolates were subcultured at least three times onto Columbia blood agar (CM0331; Oxoid, Basingstoke, UK), supplemented with 5% (v/v) defibrinated horse blood for 24 h at 37°C under aerobic conditions. Careful attention was given to purifying the isolates from single colony picks on at least three occasions, to ensure use of a single clonal type of each organism in downstream analyses.

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