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. □

This work was performed under an Innovation Voucher awarded by Enterprise Ireland to Sentinel Ireland. The authors do not have any interest/conflict of interest with this manuscript.

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

S. NAKANISHI[*†], J. R. RAO[‡§], M. MATSUDA[†], C. E. GOLDSMITH[*], W. A. COULTER[¥] and J. E. MOORE[*§]

[*]Northern Ireland Public Health Laboratory, Department of Bacteriology, Belfast City Hospital, Belfast, Northern Ireland, UK; [†]Laboratory for Molecular Biology, School of Environmental Health Sciences, Azabu University, Sagamihara, Japan; [‡]Applied Plant Science Division, Agri-Food and Biosciences Institute (AFBI), Newforge Lane, Belfast; [§]School of Biomedical Sciences, University of Ulster, Cromore Road, Coleraine; and [¥]School of Dentistry, Queen's University of Belfast, Royal Group of Hospitals, Belfast, Northern Ireland, UK

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.

Correspondence to: Professor John E. Moore,
Northern Ireland Public Health Laboratory, Department of Bacteriology
Belfast City Hospital, Belfast BT9 7AD, Northern Ireland, UK
Email: jemoore@niphl.dnet.co.uk

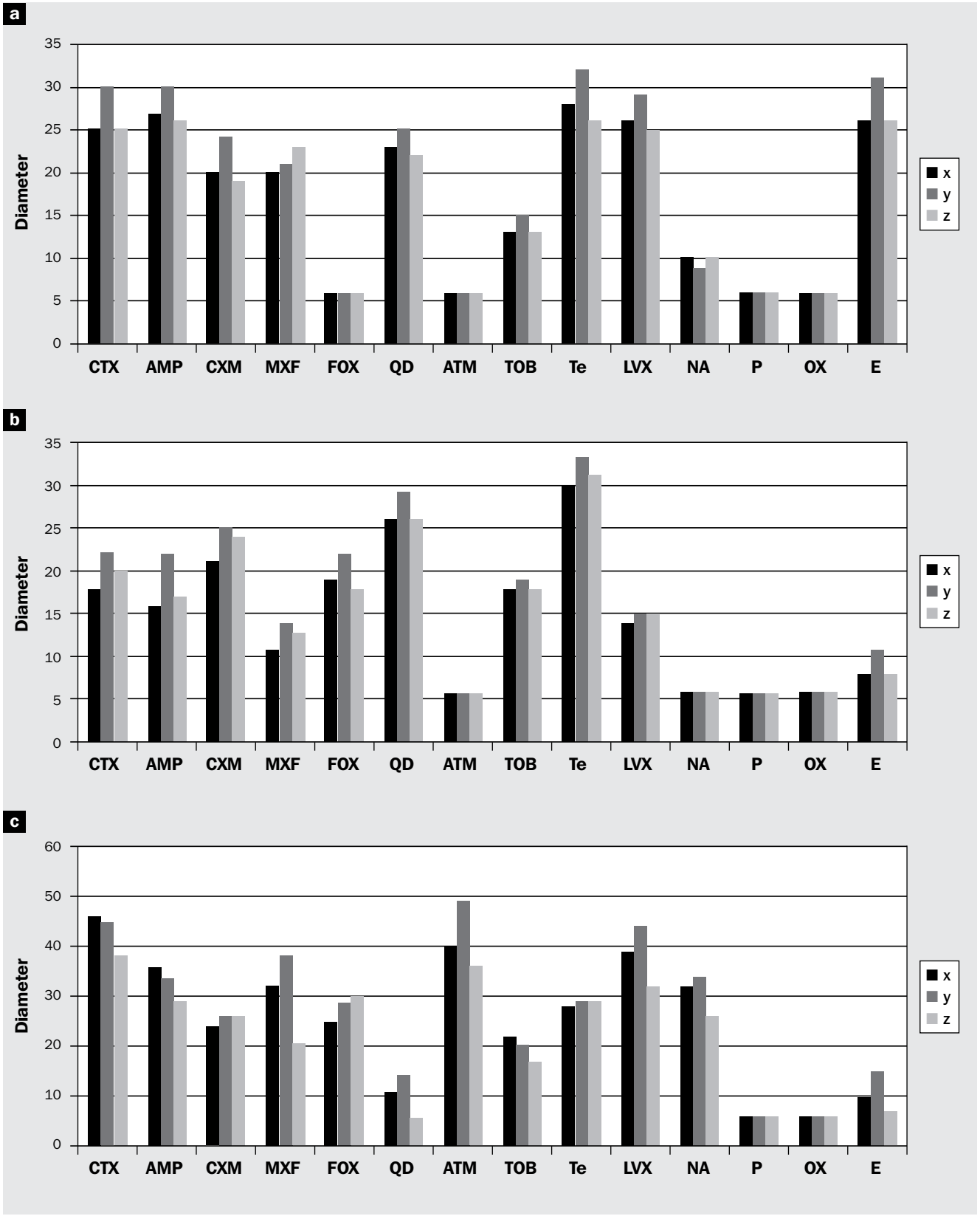

Fig. 1. Comparison of antibiotic susceptibility measured by disk-diffusion assay with 14 antibiotic agents i) before electrophoresis, ii) immediately on completion of electrophoresis, and iii) after the first passage of electrophoresised cells in **a)** *Enterococcus faecalis* NCTC775, **b)** methicillin-resistant *Staphylococcus aureus* (MRSA), and **c)** *Escherichia coli* NCTC9001.
y axis: zone of inhibition (mm). x: t0 (before electrophoresis). y: t15 (after 15-min electrophoresis). z: t1 (after 1 passage).
AMP: ampicillin (10 μg), MXF: moxifloxacin (1 μg), QD: quinupristin-dalfopristin (15 μg), ATM: aztreonam (30 μg), TOB: tobramycin (10 μg), FOX: cefoxitin (30 μg), Te: tetracycline (30 μg), LVX: levofloxacin (5 μg), P: penicillin (2 μg), OX: oxacillin (1 μg), E: erythromycin (5 μg), CTX: cefotaxime (5 μg), CXM: cefuroxime (30 μg) and NA: nalidixic acid (30 μg).

Fresh (24 h) cultures of each organism were subcultured separately on fresh Columbia blood agar (Oxoid CM0331) supplemented with 5% (v/v) defibrinated horse blood. A horizontal electrophoresis tank (Biorad) was cleaned and sterilised by immersion in absolute ethanol (Sigma, USA). After drying, the tank was filled with sterile electrophoresis buffer (0.9% [w/v] saline solution). A fresh inoculum of suspended bacterial cells was prepared by harvesting several colonies into 0.1% (w/v) peptone saline diluent (9 mL; Oxoid CM0733). Each organism was prepared and tested separately.

Inoculum was added to the electrophoresis tank to make a 0.5 McFarland standard, which was mixed thoroughly prior to commencement of electrophoresis. Electrophoresis was performed using direct current (DC) at a mean voltage of 275 V for a mean time of 18.4 min, which was determined by the temperature of the electrolysis buffer, and the experiment was terminated when the buffer reached 37°C.

Antibiotic susceptibility testing was performed in accordance with CLSI guidelines[3] using standard disk susceptibility testing on all isolates, measuring inhibition zone sizes (mm) to the following 14 antibiotics: ampicillin (10 µg), aztreonam (30 µg), cefotaxime (5 µg), cefoxitin (30 µg), cefuroxime (30 µg), erythromycin (5 µg), levofloxacin (5 µg), moxifloxacin (1 µg), nalidixic acid (30 µg), oxacillin (1 µg), penicillin (2 µg), quinupristin-dalfopristin (15 µg), tetracycline (30 µg) and tobramycin (10 µg). Susceptibility testing was performed at three points: i) before electrophoresis, ii). immediately after electrophoresis (no recovery time) and iii) one passage after electrophoresis.

Briefly, a cotton swab was charged with inoculum equivalent to a 0.5 McFarland standard, which was inoculated on the surface of plate count agar (PCA; CM0325, Oxoid). On drying, a standard disk-diffusion assay was performed with a susceptibility disk. Plates were incubated aerobically at 37°C for 24 h prior to reading, and the diameter of the zone of inhibition was measured (mm) manually. In addition, quantitative counts were performed on all isolates before and after electrophoresis, as described previously.[4]

Comparison of antibiotic susceptibility zone sizes across the 14 antibiotic agents tested before and after electrophoresis under a direct electric field is shown in Figure 1. Overall, electrophoresis increased antibiotic susceptibility in all three organisms tested, where it increased mean disk zone sizes by 11%, 15% and 9% in *Enterococcus faecalis* NCTC775, MRSA and *Escherichia coli* NCTC9001, respectively. These increases were statistically significant, giving probability values of $P=0.0028$, $P=0.0007$ and $P=0.0205$, respectively. Conversely, antibiotic susceptibility was reduced when examining zone sizes after first passage of electrophoresed cells compared to baseline (non-electrophoresed) cells (Fig. 1), particularly for the Gram-negative organism tested (*E. coli* NCTC9001). Antibiotic susceptibility did not alter significantly for the *Enterococcus faecalis* isolate (–1.2% mean zone size; $P=0.0693$); however, the *Escherichia coli* isolate showed a 13.4% reduction in mean zone sizes, which was statistically significant ($P=0.01$).

Electrophoresis had a marginal effect on quantitative counts of Gram-positive organisms, where there was no change in the *Enterococcus faecalis* count and a 1 $\log_{10}$ reduction in the MRSA, pre- and post-electrophoresis. However, there was a dramatic reduction in the Gram-negative count of 2 log for the *Escherichia coli* isolate tested. In addition, a wild-type isolate of *P. aeruginosa*, which had been isolated from the sputum of a patient with cystic fibrosis (CF), was also employed in this experiment, but was not recovered after electrophoresis, equating to a 5 $\log_{10}$ reduction.

This study demonstrates that the application of a DC electric field of approximately 275 V for 15 min at 37°C had a statistically significant effect on the antibiotic susceptibility of the target pathogens. The study was designed to terminate when the temperature of the electrophoresis tank reached 37°C, so that additional variables from the generation of heat shock proteins were not introduced as factors inducing increased susceptibility. A lethal effect was observed with the *P. aeruginosa* isolate, and also a marked reduction in numbers of the other Gram-negative *E. coli* NCTC9001 pathogen studied. Such effects were less marked in the Gram-positives organisms examined.

Lethal activity may have resulted in electrochemical changes, as opposed to direct electrical effects on the bacterial cells, and these would have included the *in situ* generation of chlorine and subsequently hypochlorite ions (i.e., $2\ NaCl + 2\ H_2O \rightarrow Cl_2 + H_2 + 2\ NaOH$ and $Cl_2 + H_2O \rightarrow HClO + HCl$), leading to the killing of the Gram-negative pathogens present. This *in situ* generation of hypochlorite via electrophoresis may represent a novel approach to generating a disinfectant potentially in tissues that are difficult to access topically.

The present study was not able to detail the exact mechanism for this action. However, the effect of the direct electrical field may alter cell surface polarity of membranes, thereby allowing increased uptake of antibiotic or the knock-out of certain efflux resistance mechanisms.

In conclusion, these data indicate that antibiotic susceptibility may be increased temporarily when a direct electric field is applied to a target pathogen, which may revert to a more resistant phenotype when the field is withdrawn. Such a phenomenon could be exploited in certain clinical scenarios, thereby reducing the reliance on expensive, newly developed antibiotics through employment of less-expensive generic antibiotics. Further work is required to elucidate the mechanism by which susceptibility is increased, as well as practical time/voltage combinations that may be applied to gain beneficial clinical effects without adverse side effects. □

References

1 Kaki R, Elligsen M, Walker S, Simor A, Palmay L, Daneman N. Impact of antimicrobial stewardship in critical care: a systematic review. *J Antimicrob Chemother* 2011; **66** (6): 1223–30.

2 Tamma PD, Cosgrove SE. Antimicrobial stewardship. *Infect Dis Clin North Am* 2011; **25** (1): 245–60.

3 Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*. Document M100-S15. Wayne, PA: CLSI, 2005.

4 Moore JE, Moore PJA, Millar BC *et al*. The presence of antibiotic resistant bacteria along the River Lagan. *Agric Water Manage* 2010; **98**: 217–21.