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INFECTION

The biofilm eradication activity of acetic acid in the management of periprosthetic joint infection

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Objectives

Periprosthetic joint infection following joint arthroplasty surgery is one of the most feared complications. The key to successful revision surgery for periprosthetic joint infections, regardless of treatment strategy, is a thorough deep debridement. In an attempt to limit antimicrobial and disinfectant use, there has been increasing interest in the use of acetic acid as an adjunct to debridement in the management of periprosthetic joint infections. However, its effectiveness in the eradication of established biofilms following clinically relevant treatment times has not been established. Using an *in vitro* biofilm model, this study aimed to establish the minimum biofilm eradication concentration (MBEC) of acetic acid following a clinically relevant treatment time.

Materials and Methods

Using a methicillin-sensitive *Staphylococcus aureus* (MSSA) reference strain and the dissolvable bead assay, biofilms were challenged by 0% to 20% acetic acid (pH 4.7) for ten minutes, 20 minutes, 180 minutes, and 24 hours.

Results

The MBEC of acetic acid was found to be: 15%, 11%, 3.2%, and 0.8% following a ten-minute, 20-minute, 180-minute, and 24-hour treatment, respectively.

Conclusion

This study found that the MBEC of acetic acid following a 10- or 20-minute treatment time exceeded its safety threshold, making these concentrations unsuitable as a topical debridement adjunct. However, a clinically acceptable concentration (5%) was still found to eliminate 96.1% of biofilm-associated MSSA following a 20-minute treatment time.

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Keywords: Revision surgery, Infection, Acetic acid

Article focus

- This study aimed to establish the minimum biofilm eradication concentration of acetic acid in the context of debridement surgery for periprosthetic joint infection.

Key messages

- The minimum biofilm eradication concentration of acetic acid with a ten- or 20-minute treatment time exceeded its safety threshold.
- A clinically acceptable concentration (5%) was still found to eliminate 96.1% of biofilm-associated methicillin-sensitive *Staphylococcus aureus* (MSSA) following a 20-minute treatment time.

Strengths and limitations

- This study provides an evaluation of the of the biofilm eradication potential of acetic acid using a clinically relevant outcome.
- The main limitation of this study was that acetic acid was only evaluated against MSSA biofilms.

Introduction

Periprosthetic joint infection (PJI) following joint arthroplasty surgery is one of the most feared complications due to its resistance to conservative treatment with standard antibiotic therapy,[1] the implications on patient health-related quality of life and function, and associated healthcare costs (£20 000 to

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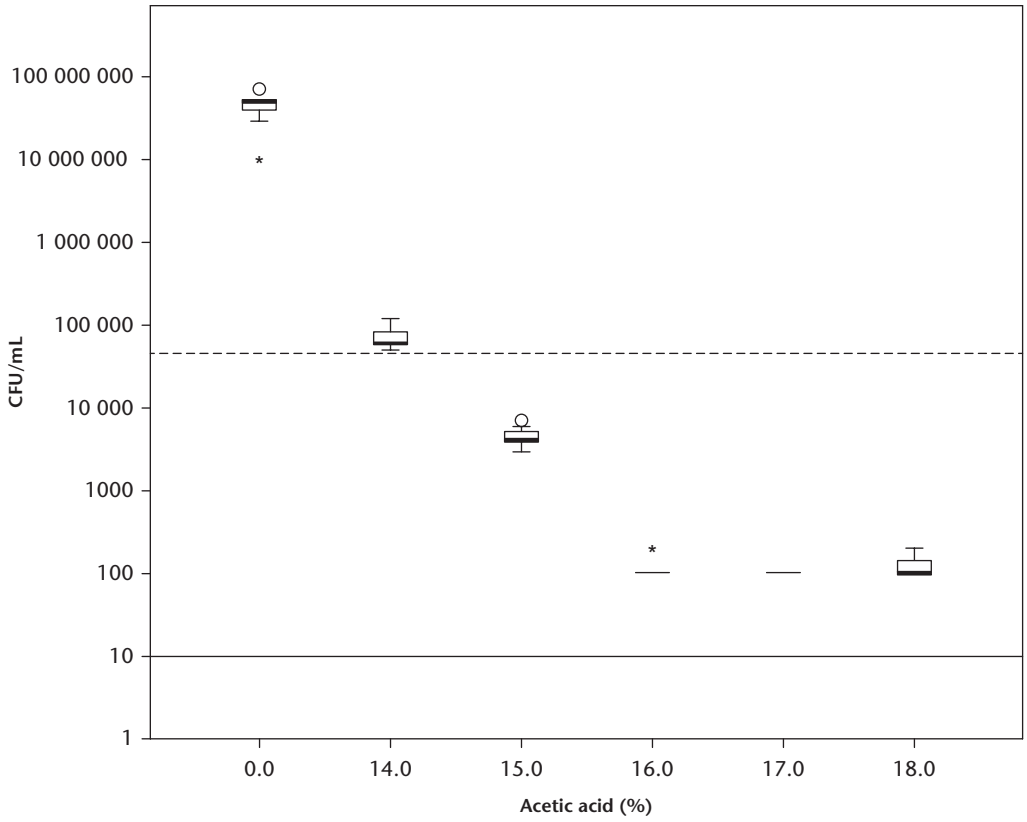

Fig. 1

Box plot of biofilm *S. aureus* CFU/mL detected following a ten-minute exposure to acetic acid. Dashed line represents a 99.9% reduction. Solid line represents detection limit of assay.

£100 000[2,3] per patient).[4] National surveillance data in the United Kingdom, based on inpatient, readmission episodes, and the number of revision procedures performed,[5-9] estimate the incidence of PJI to be between 0.5% and 2%, although this may under-report the true value.[10] The key to successful revision surgery for PJIs is thorough deep debridement.[11] In an attempt to rationalize antimicrobial and disinfectant use, there has been increasing interest in the use of acetic acid as an adjunct to debridement in the management of PJI.[12,13] Other commonly used topical adjuvant treatments for PJIs such as Betadine (Povidone-iodine), Dakin's solution (sodium hypochlorite) or hydrogen peroxide (H_2O_2) have been shown to be only partially effective in the eradication of bacterial biofilms.[14]

Acetic acid, commonly found in vinegar and produced by the oxidation of ethanol, has been used in the treatment of infection since the time of Hippocrates.[15] It is a weak organic acid that is active against Gram-positive and -negative organisms.[16-19] Previous studies have demonstrated its inhibitory and eradication action against bacteria in both planktonic and biofilm states.[18,19] It has also been used in the treatment of ear infections,[20] burn wounds,[21] and catheter-associated urinary tract infections.[16] It has United States Food and Drug Administration approval for the therapeutic use of a 0.25% solution in bladder irrigation and a 2% solution for treating otitis externa.[18] A recent study demonstrated that it had an acceptable safety profile and patient tolerance when used as an adjunct to debridement in PJIs.[13] The minimum biofilm eradication concentration is now widely recognized and is defined as the lowest concentration of an antimicrobial that eradicates 99.9% of the colony-forming units (CFU), (i.e. a three-log reduction) in a bacterial biofilm, compared with growth controls in the same conditions.[22] This reduction provides a much more robust approximation to the expected *in vivo* effect. The minimum biofilm eradication concentration (MBEC) is generally 100- to 1000-times greater than the minimum inhibitory concentration (MIC).[23,24] However the effectiveness of acetic acid in the eradication of established biofilms following clinically relevant treatment times has not been established. Using an *in vitro* biofilm model, this study aimed to establish the MBEC of acetic acid following the clinically relevant treatment times of 10 and 20 minutes. The MBEC of acetic acid was also established for exposure times of 180 minutes and 24 hours to allow comparison with previous investigations using alternative *in vitro* biofilm models.

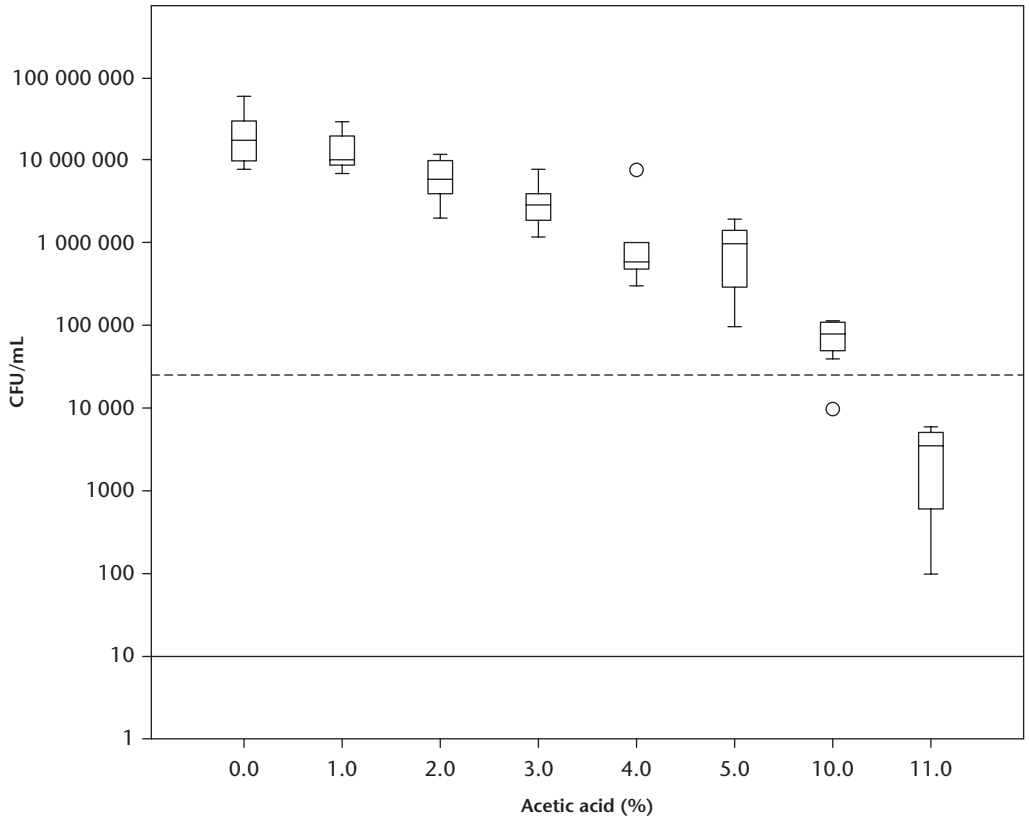

Fig. 2

Box plot of biofilm *S. aureus* CFU/mL detected following a 20-minute exposure to acetic acid. Dashed line represents a 99.9% reduction. Solid line represents detection limit of assay.

Materials and Methods

A methicillin-sensitive *Staphylococcus aureus* (MSSA) reference strain (American Type Culture Collection 29213), was used, which has been extensively studied in its biofilm state.[25-27] For culture, nutrient broth, with the pH adjusted to 7.5 prior to autoclaving, was applied. For plating, nutrient was solidified by adding 15 g/L Difco agar (Fisher Scientific, Loughborough, United Kingdom), prior to autoclaving. Using the dissolvable bead biofilm assay, as described by Dall et al,[28] sodium alginate beads (4% weight/volume, (w/v), Fisher Scientific) were incubated in a broth culture at 37°C in an orbital shaking incubator, (Gallenkamp Weiss Technik, Loughborough, United Kingdom), set at 200 rpm for 24 hours. Unchallenged control beads were washed twice in sterile phosphate buffered saline (PBS) and housed in 2 ml PBS for ten minutes, 20 minutes, 180 minutes, or 24 hours, under sterile conditions. The remaining beads were washed twice in sterile PBS and transferred under sterile conditions to treatment wells (Acetic acid 0-20% (pH 4.7)) at a desired concentration in 2 ml PBS for ten minutes, 20 minutes, 180 minutes, or 24 hours. Concentrations from 0% to 20% at 1% intervals were initially examined for all exposure times. Further rationalization was performed if a greater than two-log reduction occurred between 1% increments. The beads were washed for a third time with 2 ml sterile PBS before being dissolved, serially diluted, and plated per the dissolvable bead assay protocol.[28] Biological replicates were performed in triplicate with three further technical replicates for each biological replicate. Data were analyzed using SPSS statistical software version 22.0 (IBM Corp., Armonk, New York). Comparisons between continuous data were performed using a univariate analysis of variance.

Results

The control beads were found to have a mean 3.06×10^7 CFU/mL (95% confidence interval (CI) 2.75×10^7 to 3.36×10^7) within the biofilms attached to their surface. A three-log (99.9%) reduction was achieved by 15%, 11%, 3.2%, and 0.8% acetic acid following ten minutes, 20 minutes, 180 minutes, and 24-hour treatment times, respectively. Further rationalization of the MBEC was performed at 0.1% intervals, between 0% and 1% for 24-hour exposures and between 3% and 4% for 180-minute exposures. A summary of bacterial eradication for each treatment time is shown in Figures 1, 2, 3, and 4. A solution of 5% acetic acid, with a 20-minute treatment time, was found to have eradicated 96.1% (95% CI 69.3 to 123.1) of biofilm-associated *MSSA*, 3% acetic acid was found to eradicate 85.9% (95% CI 5.9 to 113.0) of viable bacteria (Fig. 2).

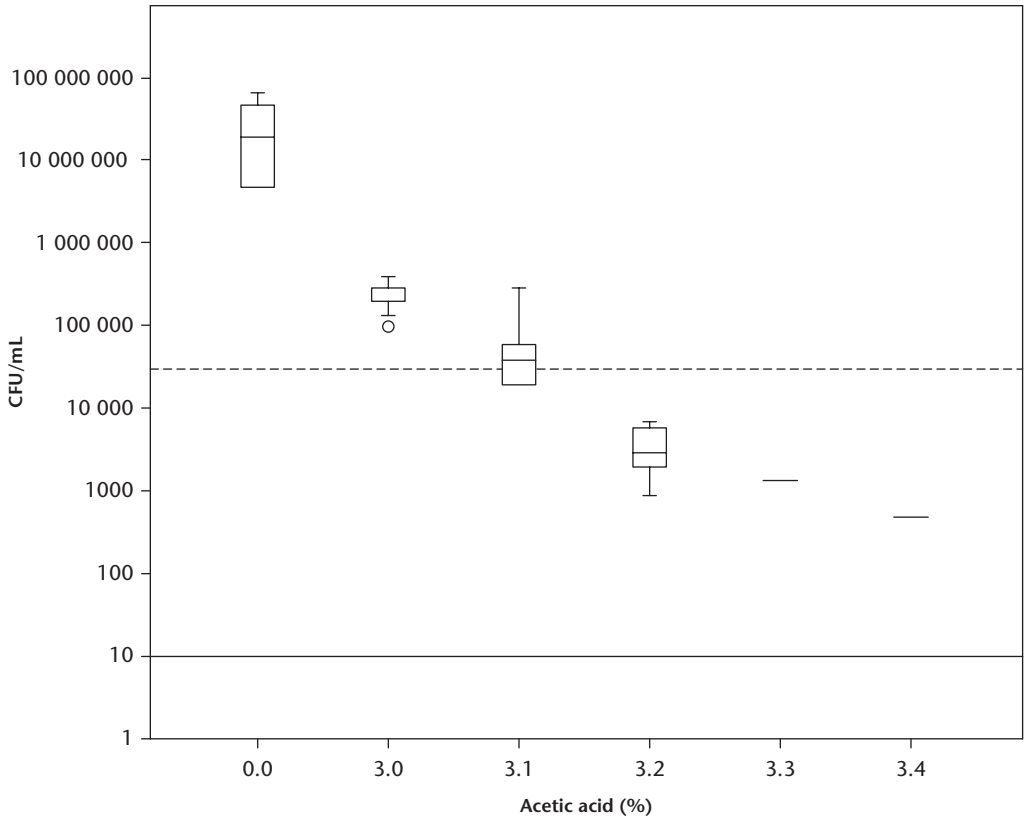

Fig. 3

Box plot of biofilm *S. aureus* CFU/mL detected following a 180-minute exposure to acetic acid. Dashed line represents a 99.9% reduction. Solid line represents detection limit of assay.

Discussion

This study has found that the MBEC of acetic acid, when used for clinically-relevant treatment times of ten minutes and 20 minutes, were 15% and 11%. Acetic acid is considered to be harmless below concentrations of 5%, as in vinegar, but in concentrations between 10% and 30% acetic acid has been found to be corrosive to human tissue.[18] Acetic acid is known to be mildly corrosive to metals such as iron, magnesium, and zinc.[29] However, for the concentrations and exposure times examined in this study, commonly used metals for orthopaedic implants and prostheses, such as stainless steel 316 and titanium, have been shown to be resistant to the corrosive effects of acetic acid in the environmental conditions encountered during debridement surgery.[30,31]

Williams et al[13] have reported that a 20-minute soak of 3% acetic acid was a safe and effective adjunct to surgical debridement in the management of PJIs following knee arthroplasty. This recommendation was based on 3/8 planktonic clinical isolates demonstrating zones of inhibition to 3% acetic acid on disc susceptibility testing and 0.19% acetic acid inhibiting growth in liquid cultures of clinical isolates that is the MIC. However, the MIC is recognized to be a poor predictor for microbial biofilm eradication, commonly resulting in treatment failure.[23,24] In our study, the MBEC of acetic acid following a 20-minute treatment time was found to exceed the concentration considered to be harmless (5%). However, although a microbiologically significant reduction of the biofilm could not be achieved with a clinically acceptable concentration and relevant treatment time, there may still be a role for acetic acid. We found that a solution of 5% acetic acid eradicated 96.1% of biofilm-associated MSSA and even 3% acetic acid eradicated 85.9% of viable bacteria. This near two-log reduction in bacterial load may still be useful in assisting debridement and the host immune system in clearing the remaining infection.

In this study, the 24-hour MBEC of acetic acid previously estimated by Bjarnsholt et al[18] has been further rationalized. Using a continuous flow assay, as described by Christiansen et al,[32] of 72-hour *S. aureus* biofilms Bjarnsholt et al[18] estimated the MBEC of acetic acid with a 24-hour treatment time to be between 0.5% and 1%. In our study, we used a dissolvable bead biofilm and the MBEC for acetic acid following a 24-hour treatment time was estimated to be 0.8%. However, it should be noted that Bjarnsholt et al[18] tested acetic acid against more mature (72- *vs* 24-hour) *S. aureus* biofilms. The maturity of biofilms, and more importantly, their mass, have both been shown to influence bacterial resistance to antimicrobials.[33-35]

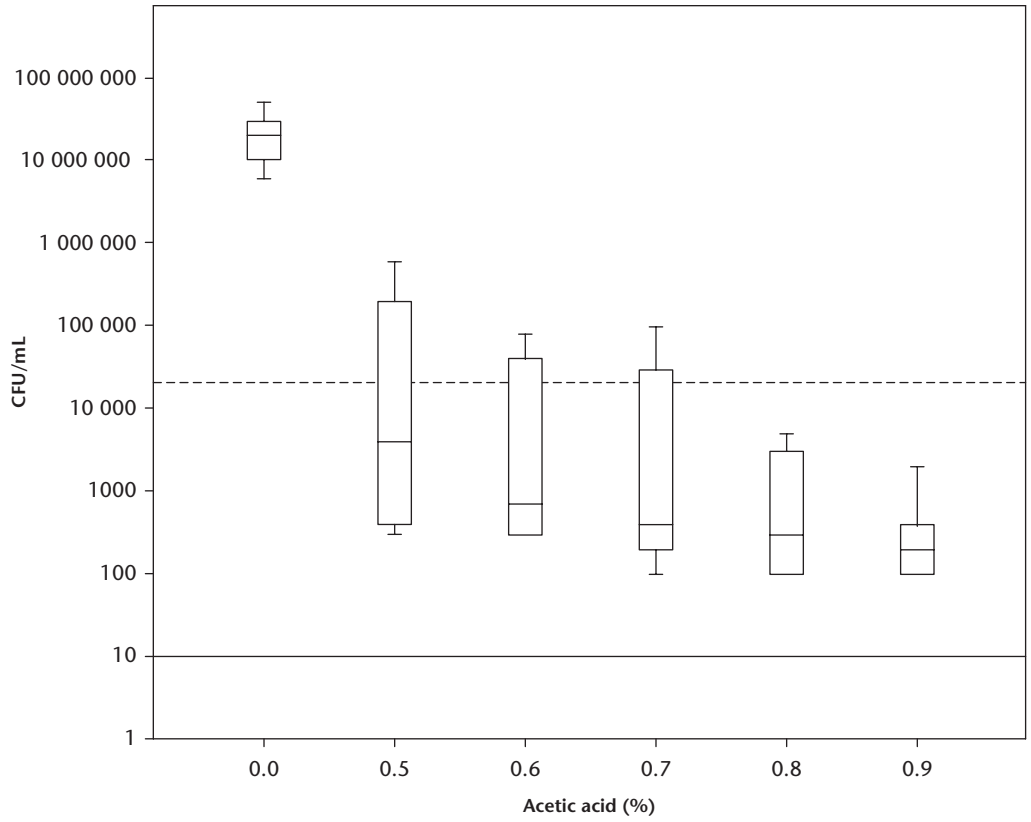

Fig. 4

Box plot of biofilm *S. aureus* CFU/mL detected following a 24-hour exposure to acetic acid. Dashed line represents a 99.9% reduction. Solid line represents detection limit of assay.

Halstead et al[19] estimated the minimum biofilm inhibitory concentration (MBIC) of acetic acid to range from $<$ 0.10% to 0.31% against clinical isolates, including methicillin-resistant *Staphylococcus aureus* (MRSA), taken from burns wounds using the crystal violet assay.[36-38] The MRSA isolate had a minimum biofilm inhibitory concentration (MBIC) 0.31%. The MBEC for acetic acid against these same clinical isolates was found to range from 0.10% to 2.5%, with the MBEC against the MRSA isolate being 1.25%. In general, Halstead et al[19] found that Gram-negative organisms were more susceptible to acetic acid than Gram-positive species. The MBEC was determined using an indirect count of viable cells using a modified version of the Calgary model, as described by Ceri et al,[25] rather than direct enumeration of viable cells. In a study performed by Halstead et al,[19] biofilms of clinical isolates were grown on polystyrene pegs for 72 hours, as per the Calgary model. After a three-hour treatment with acetic acid, the pegs underwent overnight culture in broth. Optical density of the broth was performed the next day to estimate the number of cells that had seeded into the broth from the biofilm on the polystyrene peg. The indirect method of viable cell enumeration following treatment used by Halstead et al[19] may explain why the MBEC was found to be lower than the concentration (3.2%) found in our study. It is possible that not all viable cells were liberated from the polystyrene pegs thus overestimating the bactericidal effect of acetic acid for a given concentration.

Previous studies also have postulated that the antibacterial effect of acetic acid was due to the non-dissociated form of acetic acid (CH_3COOH), rather than dissociated protons. Weak acids exist in an equilibrium of ionized and non-ionized forms in solution, unlike strong acids such as hydrochloric acid, which completely dissociate in solution into their ionized form. The non-ionized forms of weak acids can freely diffuse across hydrophobic phospholipid bacterial membranes.[39,40] Cytotoxicity caused by weak acids is due to disruption of the proton gradients that are necessary for ATP synthesis.[17,41] Intracellular acetic acid dissociates to form acetate, which combines with periplasmic protons pumped out by the electron transport chain and carries them back across the membrane by-passing transmembrane ATP synthase, thereby disrupting ATP formation.[17,41,42] The intracellular dissociation of acetic acid also reduces the pH of the cytoplasm, which in turn leads to protein unfolding with subsequent membrane and DNA damage.[17,42] Furthermore, the acetate released during acetic acid dissociation, induces osmotic stress to the cell. The extent of this effect is known to be anion-specific but the mechanism of action has not been fully described. It is known that different

weak acids at the same pH can have very different cytotoxic effects.[17,39,41] Bjarnsholt et al[18] observed that the maximal antibacterial effect of acetic acid was at pH 4.76, which was also the dissociation equilibrium point of acetic acid. Thus, it was recommended that the pH of the solution be kept at this level or lower to maximize availability of the non-dissociated acetic acid and its antibacterial effect.[18] This may limit its effectiveness as debridement adjunct *in vivo*. The pH of blood and synovial fluid has previously been shown to be 7.38 (standard error of the mean (SEM) 0.013) and 7.77 (SEM 0.04), respectively.[43] The pH of synovial fluid is mildly reduced in the presence of trauma (pH 7.56 SEM 0.03) and degeneration (pH 7.38 SEM 0.01) but within the surgical site the equilibrium point would be driven unfavourably towards the dissociated form of acetic acid,[18] limiting its effectiveness.

Our study is the first *in vitro* evaluation of acetic acid against bacterial biofilms in the context of PJIs. The treatment times, of 180 minutes[19] and 24 hours,[18] used in previous studies are not relevant to the evaluation of acetic acid as a debridement adjunct in PJIs, nor is the *in vitro* estimation of the MIC of acetic acid using planktonic cultures of clinical isolates.[13] The dissolving bead biofilm model used in this study has been shown to reproducibly form staphylococcal biofilms on the substrate surface and reliably quantify biofilm eradication.[28] Our study was limited by the fact that acetic acid was only evaluated against MSSA biofilms. However, from national surveillance data it has been found that for the majority of PJIs staphylococci are implicated. For mono-microbial PJIs, which amount to around 75%, 30% to 38% were attributed to MSSA, 4% to 5% MRSA, and 25% to 28% coagulase-negative staphylococci.[9] In poly-microbial cases, Gram-positive organisms were implicated in 70% to 80%.[9] Areas of future research should include further evaluation of the antibiofilm effect of acetic acid using other clinically relevant bacterial species, further elucidation of the antibiofilm mechanism of acetic acid and other weak acids, and the clinical evaluation of 5% acetic acid including the safety profile, patient tolerance, and surgical outcome when used in PJIs.

In conclusion, this study found that the MBEC of acetic acid following a ten- or 20-minute treatment time exceeded its safety threshold, making these concentrations unsuitable as a topical debridement adjunct. However, a clinically acceptable concentration (5%) was still found to eliminate 96.1% of biofilm-associated MSSA following a 20-minute treatment.

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Author Contributions

- S. T. J. Tsang: Study conception, Collecting and analyzing the data, Writing and editing the manuscript.
- P. J. Gwynne: Study conception, Analyzing the data, Writing and editing the manuscript.
- M. P. Gallagher: Study conception, Analyzing the data, Writing and editing the manuscript.
- A. H. R. W. Simpson: Study conception, Analyzing the data, Writing and editing the manuscript.

Conflict of interest

- The authors declare that there are no conflicts of interest.

Ethical statement

- Research Ethics Committee approval was not required for this *in vitro* study.