Low pH nasal rinse solution enhances mupirocin antimicrobial efficacy*

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Abstract

Background: Chronic rhinosinusitis (CRS) is a common condition negatively impacting a patient's quality of life. It has been hypothesized that bacterial biofilms are involved in the pathogenesis of CRS due to their persistence and difficulty to eradicate with conventional antibiotic therapy. Hence, the topical delivery of antibiotics via nasal rinse solution has gained a lot of attention due to the ability to deliver higher local concentrations, with less systemic absorption and side effects. This study investigates the efficacy of mupirocin dissolved in the 3 most commonly used sinus rinses in Australia Neilmed (isotonic saline), Flo Sinus Care (sodium chloride, sodium bicarbonate, potassium chloride, glucose anhydrous and calcium lactate and Pentahydrate) and FloCRS (sodium chloride, potassium chloride and xylitol).

Methods: Planktonic and biofilm cultures of *S. aureus* (ATCC25923, 2 methicillin-resistant *S. aureus* (MRSA) (C222 & C263), and 2 methicillin-susceptible *S. aureus* (MSSS) (C311 & C349) clinical isolates) were treated with mupirocin dissolved in three sinus rinses (Neilmed, Flo Sinus Care and FloCRS with different pH). To establish whether pH was a significant factor in determining antibiotic activity, experiments with Flo CRS were performed both at pH 5.64 and elevated pH 7.7. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined for planktonic cells. The biofilm biomass and metabolic activity were assessed by using crystal violet assay and alamarBlue assay respectively.

Results: The combination of mupirocin in low pH (pH 5.64) sinus rinse (FloCRS) had the highest efficacy in reducing the growth of *S. aureus* in both the planktonic and biofilm forms. Mupirocin diluted in FloCRS (pH 5.64) showed a significantly higher reduction in both biomass and metabolic activity than that was observed when mupirocin was diluted in Neilmed, Flo Sinus Care or FloCRS (pH 7.7).

Conclusion: The choice of irrigant solution for topical mupirocin delivery appears to be important for antimicrobial activity. The delivery of mupirocin via low pH FloCRS could be useful in eliminating *S. aureus* biofilms present on the sinus mucosa of patients with CRS.

Key words: CRS, *S. aureus*, antimicrobial, biofilm

Introduction

Chronic rhinosinusitis (CRS) has an estimated prevalence of 1.9 million in Australia, with a significant socio-economic burden on health care systems and the individual [1, 2]. It has a comparable functional burden to that of other chronic disorders such as back pain, chronic obstructive pulmonary disease, angina pectoris and chronic heart failure [3].

The etiology of CRS remains unclear but is believed to be multifactorial with chronic bacterial infection and biofilms, suggested as one of the possible contributing factors. In fact, a polymicrobial flora is commonly described in patients affected with CRS[4]. Among the micro-organisms present, *Staphylococcus aureus* is one of the most common bacterial pathogens cultured from the sinuses of CRS patients [5]. Interestingly, patients with polyps (CRSwNP) have a higher *S. aureus* colonization rate and this

associates to the percentage of eosinophils in the sinus mucosa and in the peripheral blood [6, 7]. It has been proposed that the escalation of *S. aureus* colonization in the chronically infected state, may promote immune dysregulation, barrier dysfunction, and bacterial dysbiosis, culminating in the formation of biofilms and persistence/recurrence of disease [7].

Despite, appropriate medical and surgical treatment a proportion of CRS patients will continue to have ongoing sinus disease, with infective exacerbations and significant crusting, with *S. aureus* the most frequently isolated bacterium. In a study by Drilling et al. with multiple cultures of *S. aureus* over a 12-month period, genomic analysis demonstrated the exact same strain of *S. aureus* on repeat culture in 79% of patients. This persistence is thought to be secondary to bacterial biofilms, small colony variants and intracellular bacterial residence, with all these forms inherently resistant to conventional oral antibiotic therapy [8].

Although all bacteria can form biofilms, *S. aureus* is particularly adept in doing so. Zajmi et al. observed that *S. aureus* biofilms are more organized and robust than other types of biofilms based on their ultrastructural study [9]. Furthermore, *S. aureus* is also known to secrete several toxins that have a direct effect on the innate and acquired immune system [10]. It is for these and other factors that *S. aureus* biofilms are thought to play a significant role in CRS, particularly recalcitrant cases. This is supported by clinical studies demonstrating an association between *S. aureus* biofilms with more severe disease, than other biofilm forms and worse post-operative outcomes in patients with CRS [11,12]. In recent times, the use of topical antibiotics has gained favour in the treatment of post-operative infective exacerbations of CRS. Topical delivery allows much higher concentrations of antibiotic to be delivered directly to the site of infection, with significantly less systemic side-effects [13, 14]. For both methicillin-susceptible (MSSA) and -resistant *Staphylococcus aureus* (MRSA) strains, mupirocin has been shown to have good bactericidal activity [15] and molecular studies show that it remains 100% stable in nasal secretions [16]. Furthermore, several clinical studies have also shown it to be an effective short-term treatment for recalcitrant staphylococcal CRS [17, 18] with a randomized double blind placebo controlled trial also showing it to have superior efficacy over amoxycillin and clavulanic acid in microbiological clearance of *S. aureus* (19). Despite its common use, however, little research has been undertaken to determine the optimal delivery solution for mupirocin.

In this study, we compare the effectiveness of commonly used sinus irrigation products with different pH as carriers of mupirocin at the same concentration against in-vitro *S. aureus* planktonic cells and biofilms.

Table 1. Constituents of each sinus irrigation product.

Sinus irrigation product	Contents
NeilMed	Sodium chloride & sodium bicarbonate
Flo Sinus Care	Sodium chloride, sodium bicarbonate, potassium chloride, glucose anhydrous and calcium lactate pentahydrate
FloCRS	Sodium chloride (0.75mg/ml when reconstituted), potassium chloride and xylitol

Materials and methods

Preparation of diluent carriers

Three of the most commonly used sinus irrigation products including NeilMed (NeilMed® Pharmaceuticals inc, NSW, Australia), Flo Sinus Care (ENT Technologies Pty LTD, Victoria, Australia) and FloCRS (ENT Technologies Pty LTD, Victoria, Australia) were selected. The ingredients for each sinus irrigation product are listed in Table 1.

The sinus irrigation products were dissolved in MilliQ water as instructed by the manufacturer and the pH was measured using a pH meter (sensIONTM, HacH Company, CO, USA). For the experiments, the sinus rinse irrigation products were dissolved in nutrient broth (NB; Oxoid Ltd., Basingstoke, United Kingdom) and the pH values were adjusted to be equivalent to the pH of the water-based solution using hydrochloric acid (Sigma-Aldrich, NSW, Australia) and sodium hydroxide solution (Sigma-Aldrich, NSW, Australia) with values shown in Table 2. For FloCRS, the pH of the solution was lower (pH 5.64) than those of the other two diluents. Therefore, to assess whether the low pH was an independent factor, a second nutrient broth solution of FloCRS was prepared, and the pH adjusted to 7.7. Nutrient broth was used rather than distilled water as bacteria cannot survive and grow in distilled water.

Bacteria preparation

The *S. aureus* strains used in this experiment were ATCC 25923 (American Type Culture Collection, Manassas, USA) and 4 clinical isolates: 2 methicillin-resistant *S. aureus* (MSSA) (C222 & C263) and 2 methicillin-susceptible *S. aureus* (MRSA) (C311 & C349). Clinical isolates were obtained from the sinonasal cavities of CRS patients in accordance with guidelines approved by Central Adelaide Local Health Network Human Ethics Committee (CALHN HREC) (Reference HREC/15/TQEH/132). *S. aureus* (MSSA and MRSA) strains were identified by an independent diagnostic laboratory (Adelaide Pathology Partners, Mile End, Australia) and kept in glycerol stocks at -80˚C for future use.

Minimal inhibitory concentration

The minimal inhibitory concentration (MIC) of mupirocin (Sigma

Table 2. pH value of the diluent carriers in nutrient broth and water.

Diluent Carrier	pH in Diluent Carrier	
	Water	Nutrient broth
Neil Med	7.53	7.53
Flo Sinus Care	7.85	7.85
FloCRS	5.64	5.64 and 7.70

Alrich, St. Louis, MO, USA) diluted in FloCRS, Flo Sinus Care and NeilMed was assessed using the broth microdilution method as described with modifications [20]. Modifications to this protocol were made with respect to the medium and incubation conditions, as required by the growth characteristics of *S. aureus*. In brief, single colonies of *S. aureus* were suspended in 0.9% saline (Sigma Aldrich, NSW, Australia), adjusted to 0.5 McFarland unit and diluted 1:100 in nutrient broth (Oxoid, Basingstoke, England) in serially diluted antibiotics in 96 well microtitre plates. A range from 16 to 0.015 µg/ml mupirocin diluted in different diluents was used. Before incubating the microtitre plates, triplicate 20 µl droplets from control wells for each bacterial test isolate were spotted onto nutrient agar plates and incubated overnight, as confirmation of inoculum colony forming unit (CFU) number. After incubation of the microtitre plates for 16 to 20 hours at 37˚C, the MIC values (OD 595nm) were read by FLUOstar OPTIMA plate reader (BMG Labtech, Ortenberg, Germany). The lowest concentration inhibiting the growth of bacterial isolates was recorded as MIC.

Minimal bactericidal concentration

To determine the minimal bactericidal concentration (MBC), 20 µl droplets from all wells in microtitre plates that showed no visible growth from above were spotted onto nutrient agar plates in the same manner as the control wells described above and incubated overnight. The following day, the MBC was determined as the lowest concentration of mupirocin required to kill the bacteria, as observed by the absence of growth on the nutrient agar plates.

Antibiofilm efficacy

Biofilm formation and treatment

Biofilms were established according to a published protocol [21]. In brief, single colonies of *S. aureus* were suspended in 0.9% saline (Sigma Aldrich), adjusted to 1.0 McFarland and diluted 1:15 in nutrient broth. 150 µl of diluted bacterial suspension was added into each well of a black 96-well microtiter plate (Thermo Fisher, Roskilde, Denmark). The microtiter plate was wrapped in aluminium foil and incubated for 48 hours at 37˚C on a rotating plate set at 70 rpm (3D Gyratory Mixer, Ratek Instruments, Australia).

Biofilms were washed twice with 1X phosphate buffered saline (PBS; Sigma Aldrich, Steinheim, Germany) to remove planktonic cells. This was followed by the addition of mupirocin diluted to a range of concentrations in FloCRS (pH 5.64 and pH 7.7), Flo Sinus Care and NeilMed and incubated for 20 hours at 37˚C on a rotating plate set at 70 rpm. The set up for the treatment included control wells with bacterial suspension (positive control), wells with nutrient broth alone (negative control), wells with bacterial suspension in diluent carrier only and wells with 5 different concentrations of mupirocin (ranging from 128-8 µg/ml). All experiments were performed as three independent replicates.

Biofilm biomass assessment

The treatment efficacy was quantified using the crystal violet assay to determine the biomass of bacterial biofilms as described [22]. Briefly, the biofilms were washed twice with 1X PBS. After drying, the plate was stained with 200 µl of 0.1% crystal violet (Sigma-Aldrich, Castle Hill, NSW, Australia) per well for 15 minutes. The stained plates were rinsed by two rounds of gentle immersion into distilled water and left to dry overnight. The crystal violet stain was solubilised by application of 200 µl per well of acetic acid (ThermoFisher Scientific, Auckland, New Zealand) and the plate was incubated on an orbital shaker at room temperature for an hour. Absorbance at 595 nm was measured for each well using FLUOstar OPTIMA plate reader (BMG Labtech, Ortenberg, Germany).

Bacterial biofilm metabolic activity assay

The bacterial biofilms were assessed using the alamarBlue cell viability assay as described [21, 23]. In short, the treated biofilms were washed twice with PBS followed by addition of 200 µl of a freshly prepared 10% alamarBlue solution (Life Technologies, Scoresby, Australia) in nutrient broth into each well. Plates were protected from light and incubated at 37˚C on a rotating plate for 3 hours. The fluorescence intensity was measured every 30 minutes using excitation 530 nm and emission 590 nm on a FLUOstar OPTIMA plate reader (BMG LABTECH, Offenburg, Germany).

Statistical analysis

Each experiment was performed using two technical replicates per condition with three biological replicates. Data were analysed using GraphPad Prism version 9.00 (GraphPad Software, La Jolla, CA, USA). All values were expressed as SD. Statistical significance for all results were analysed using one-way analysis of variance (ANOVA) and Dunnett's multiple comparisons test. Significance was determined at p-value <0.05.

Results

The pH did not affect *S. aureus* biofilm biomass or metabolic activity

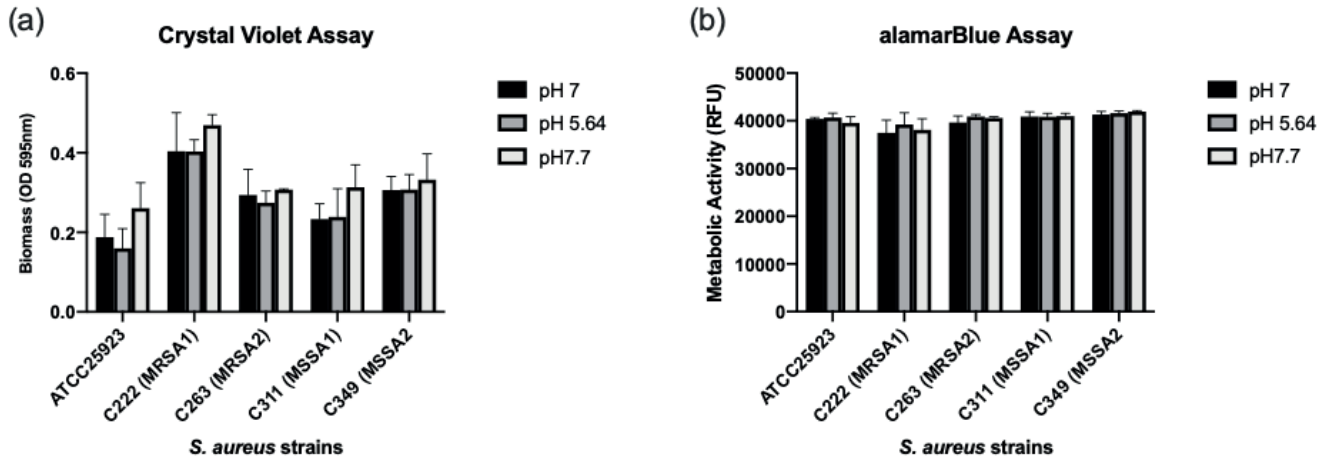

Figure 1. (a) Biofilm biomass and (b) metabolic activity of *S. aureus* stains. Biofilm biomass analysis by crystal violet staining (OD 595nm) and metabolic activity assessment (RFU) of five *S. aureus* bacterial strains: ATCC 25923, C222 (MRSA), C263 (MRSA), C311 (MSSA) and C349 (MSSA) at pH 5.64, pH 7 and pH 7.7. The significant difference was determined by One-way analysis of variance (ANOVA) with Dunnett's multiple comparisons test with pH7.

Figure 1 (a) and (b) show there was no significant difference in either the biofilm biomass or biofilm metabolic activity of all the strains at the 3 different pH (7, 5.64 and 7.7) tested. This indicated that pH did not appear to affect *S. aureus* biofilm formation and metabolic activity.

MIC and MBC for *S. aureus* planktonic growth

Mupirocin diluted in FloCRS at pH 5.64 required the lowest concentration of mupirocin needed to inhibit the planktonic growth of all *S. aureus* strains. However, the minimum bactericidal concentration (MBC) for mupirocin diluted in FloCRS at pH 5.64 was similar to mupirocin diluted in NeilMed and Flo Sinus Care in all strains except for ATCC 25923. For this strain, a lower MBC value was found for mupirocin diluted in Flo CRS at pH 5.64 than for mupirocin diluted in NeilMed and Flo Sinus Care. We then adjusted the pH of FloCRS to pH 7.7, a value that is within the range of Neilmed (pH 7.53) and Flo Sinus Care (pH 7.85). MIC values for mupirocin diluted in FloCRS (pH 7.7) were higher than those for mupirocin diluted in FloCRS (pH 5.64) for all *S. aureus* strains tested but were equal or lower than for mupirocin diluted in NeilMed and Flo Sinus Care. MBC values for mupirocin diluted in FloCRS (pH 7.7) were higher than those for mupirocin diluted in FloCRS (pH 5.64), NeilMed and Flo Sinus Care in 3/5 *S. aureus* strains tested but were lower than mupirocin diluted in FloCRS (pH 5.64), NeilMed and Flo Sinus Care in 2/5 strains tested. Results for the antimicrobial effect of mupirocin dissolved in NeilMed, Flo Sinus Care, FloCRS (pH 5.64) and FloCRS (pH 7.7) against *S. aureus* planktonic cells are shown in Table 3.

Antibiofilm property of mupirocin diluted in sinus rinse

The minimum biofilm eradication concentration (MBEC) for mupirocin in all carrier solutions measured using alamarBlue and crystal violet assays was >128 µg/ml (data not shown). Overall, among the three diluent carriers, mupirocin diluted in FloCRS (pH 5.64) showed the highest antibiofilm efficacy against all five *S. aureus* strain as determined by alamarBlue (metabolic activity) and crystal violet assay (biomass). This reduction in both metabolic activity and biomass was significantly higher for mupirocin diluted in FloCRS (pH 5.64) than that was observed when mupirocin was diluted in Neilmed., Flo Sinus Care or FloCRS (pH 7.7) ($p<0.05$). The graphical break down for biofilm biomass and metabolic activity in each sinus irrigation product is shown in figures below (Figure 2-5).

Meanwhile, Table 4 shows the result of treatment efficacy of NeilMed, Flo Sinus Care and FloCRS (pH 5.64 and 7.7) against *S. aureus* biofilm by showing percentage killing. The formula for the percentage of killing is shown below:

Percentage of killing = 100 - ((Average treatment value)/(Untreated value)) x 100.

Discussion

S. aureus is the most common pathogen cultured in post-operative CRS patients. The ability of *S. aureus* to persist through intracellular localization, small colony variants and biofilm formation is thought to explain its resistance to oral antibiotic therapy and its association with recalcitrant CRS. Topical mupirocin is frequently prescribed with reports of mixed success by the prescribing clinicians. This study suggests that mupirocin activity against *S. aureus* planktonic cells and biofilms may be enhanced when dissolved in FloCRS at pH of 5.64.

The antibiotic mupirocin is naturally produced by *Pseudomonas*

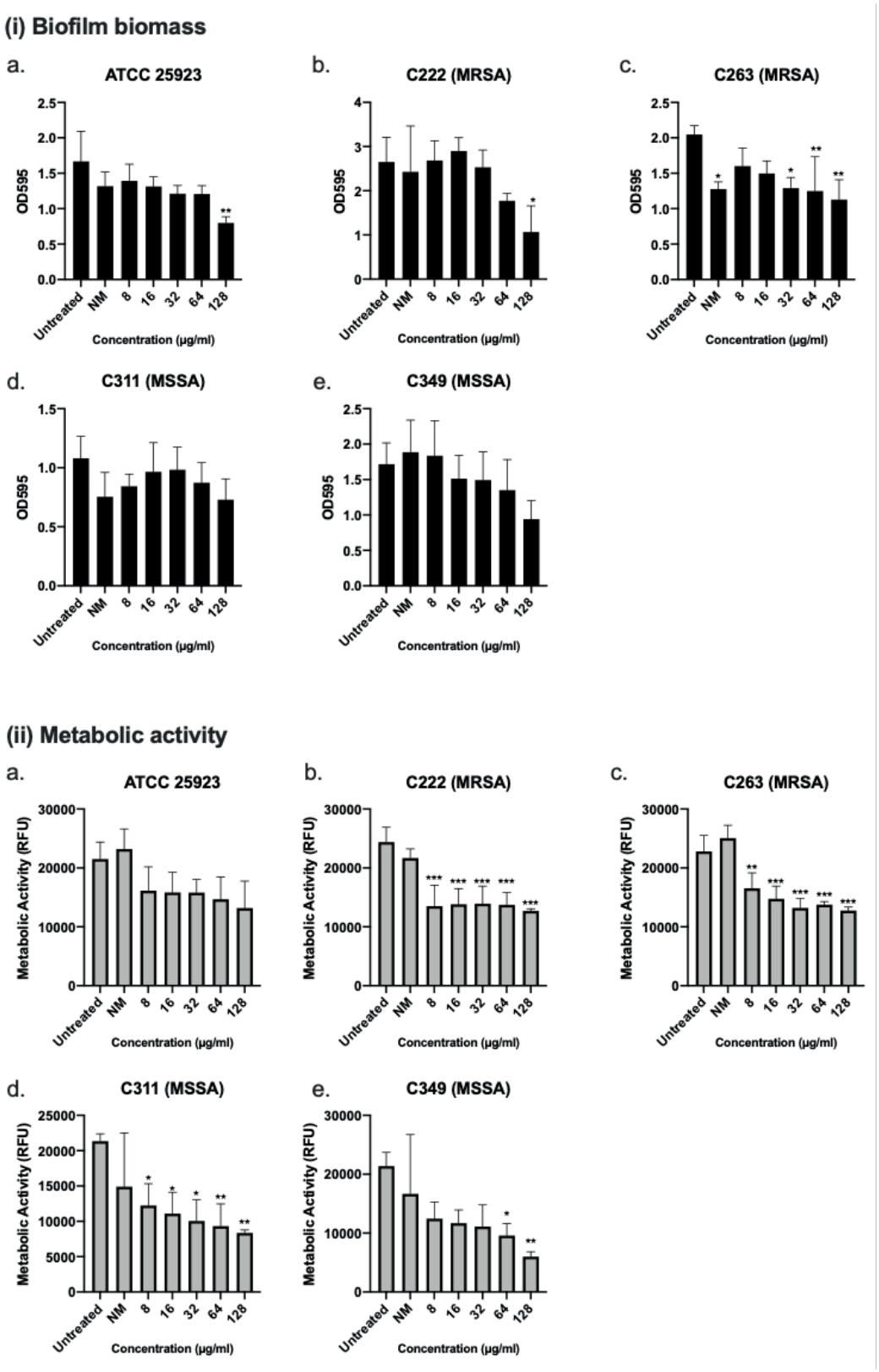

Figure 2. Assessment of (i) biofilm biomass and (ii) metabolic activity for NeilMed diluent with and without mupirocin. Biofilm biomass analysis through crystal violet staining (OD 595nm, black bars) and metabolic activity (RFU, grey bars) of five *S. aureus* bacterial strains (a) ATCC 25923, (b) C222 (MRSA), (c) C263 (MRSA), (d) C311 (MSSA) and (e) C349 (MSSA) after 24 hours treatment with NeilMed diluent only and NeilMed with five different concentrations of mupirocin. Error bars represent standard deviation of triplicate experiments. NM = NeilMed; One-way analysis of variance (ANOVA): ***, p-value < 0.001; **, p-value < 0.01; *, p-value < 0.05.

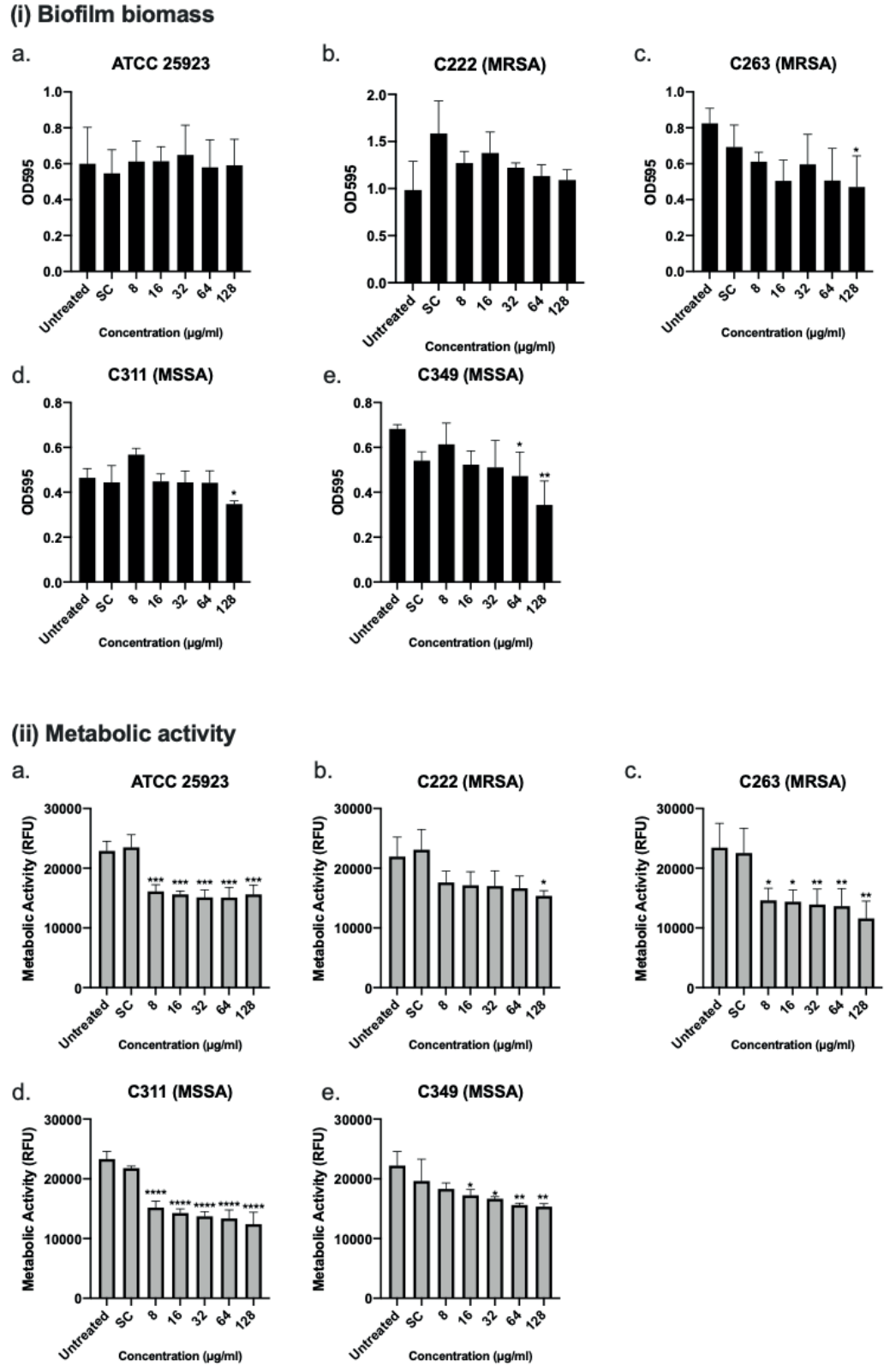

Figure 3. Assessment of (i) biofilm biomass and (ii) metabolic activity for Flo Sinus Care diluent with and without mupirocin. Biofilm biomass analysis through crystal violet staining (OD 595nm, black bars) and metabolic activity (RFU, grey bars) of five *S. aureus* bacterial strains (a) ATCC 25923, (b) C222 (MRSA), (c) C263 (MRSA), (d) C311 (MSSA) and (e) C349 (MSSA) after 24 hours treatment with Flo Sinus Care diluent only and Flo Sinus Care with five different concentrations of mupirocin. Error bars represent standard deviation of triplicate experiments. SC = Flo Sinus Care; One-way analysis of variance (ANOVA) with Dunnett's multiple comparisons test with untreated: ****, p-value < 0.0001; ***, p-value < 0.001; **, p-value < 0.01; *, p-value < 0.05.

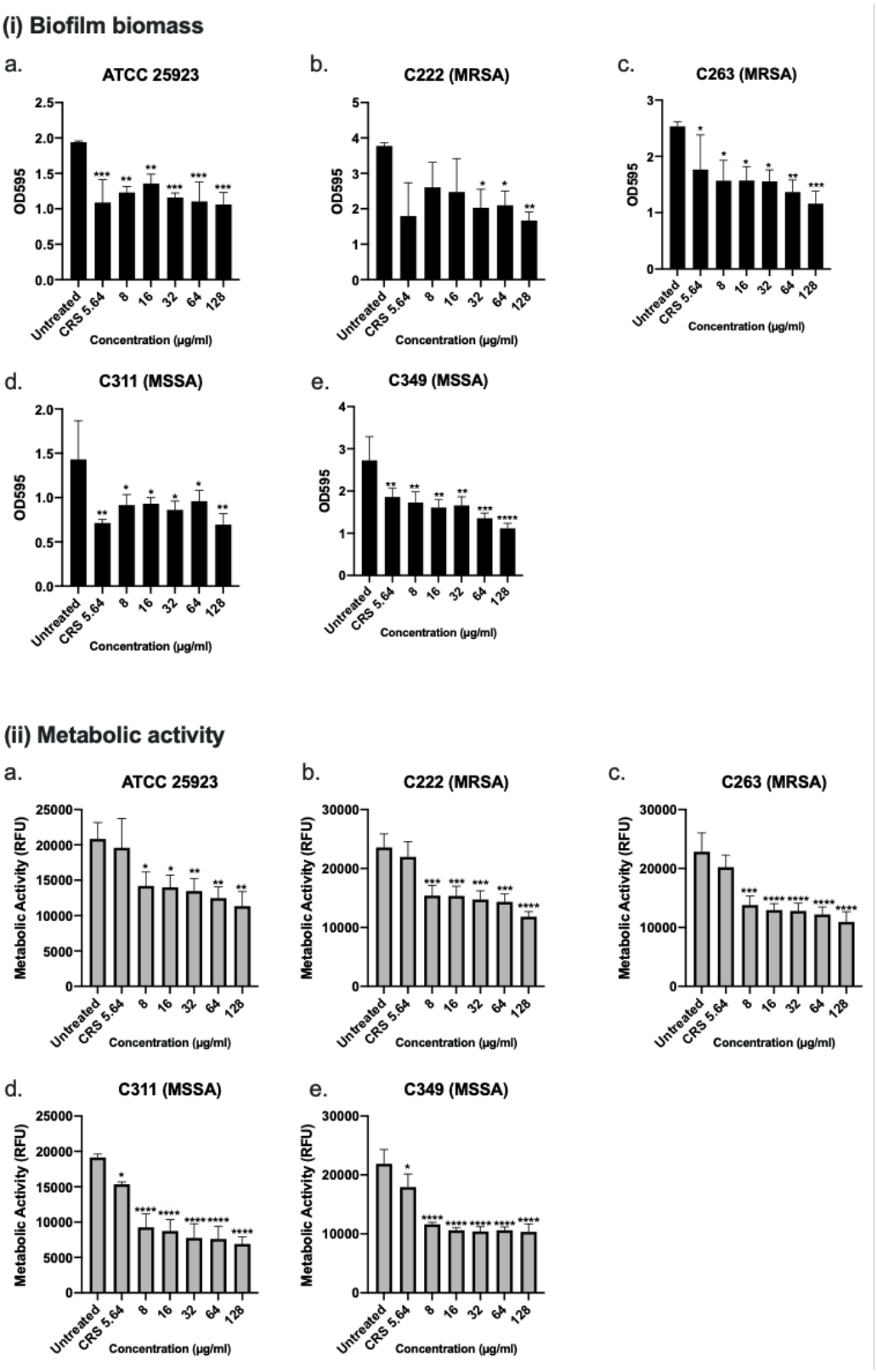

Figure 4. Assessment of (i) biofilm biomass and (ii) metabolic activity for FloCRS diluent at pH 5.64 with and without mupirocin. Biofilm biomass analysis through crystal violet staining (OD 595nm, black bars) and metabolic activity (RFU, grey bars) of five S. aureus bacterial strains (a) ATCC 25923, (b) C222 (MRSA), (c) C263 (MRSA), (d) C311 (MSSA) and (e) C349 (MSSA) after 24 hours treatment with FloCRS (pH 5.64) diluent only and FloCRS (pH 5.64) with five different concentrations of mupirocin. Error bars represent standard deviation of triplicate experiments. CRS 5.64= FloCRS at pH 5.64; One-way analysis of variance (ANOVA) with Dunnett's multiple comparisons test with untreated: ****, p-value < 0.0001; ***, p-value < 0.001; **, p-value < 0.01; *, p-value < 0.05.

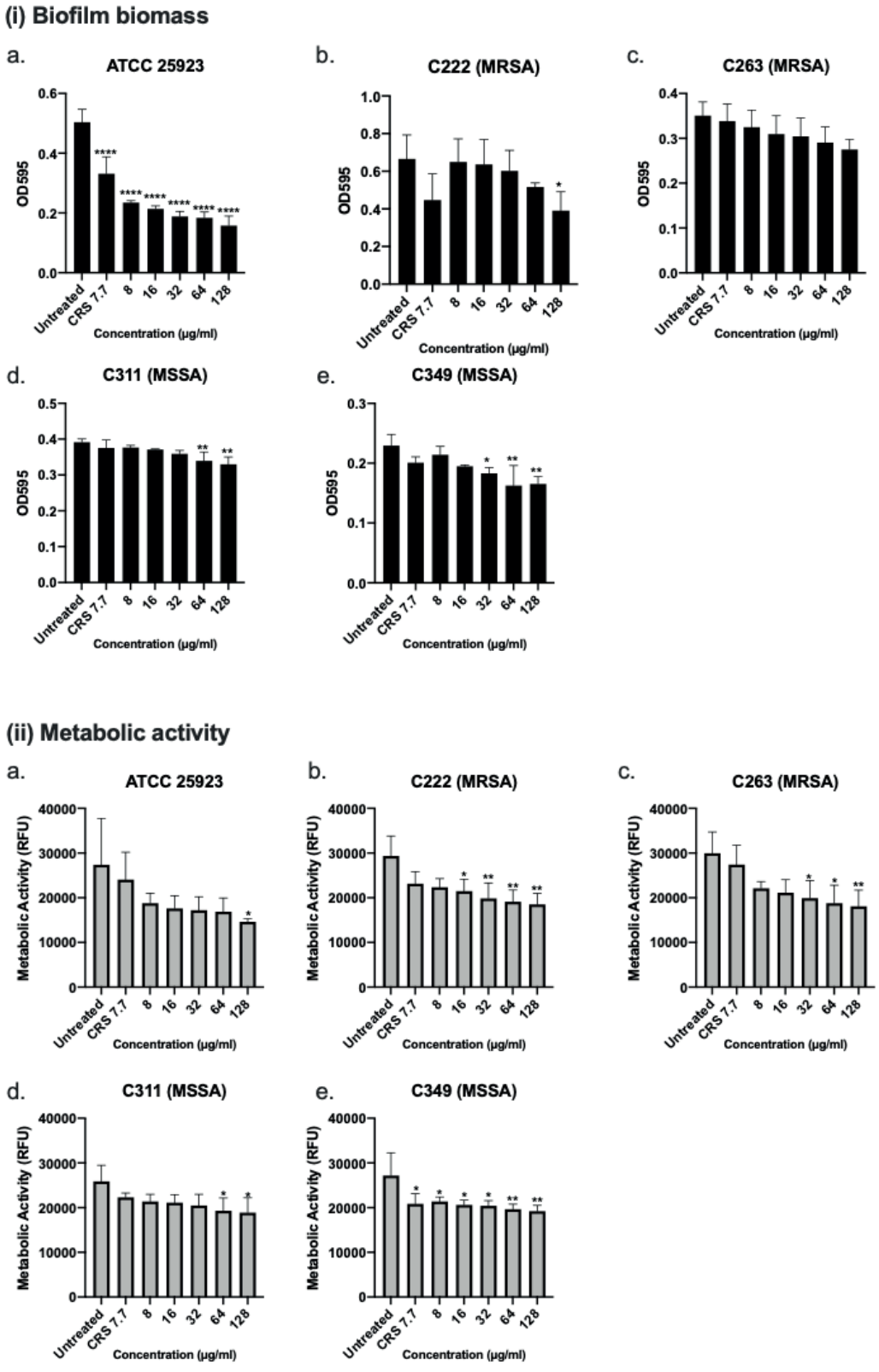

Figure 5. Assessment of (i) biofilm biomass and (ii) metabolic activity for FloCRS diluent at pH 7.7 with and without mupirocin. Biofilm biomass analysis through crystal violet staining (OD 595nm, black bars) and metabolic activity (RFU, grey bars) of five *S. aureus* bacterial strains (a) ATCC 25923, (b) C222 (MRSA), (c) C263 (MRSA), (d) C311 (MSSA) and (e) C349 (MSSA) after 24 hours treatment with FloCRS (pH 7.7) diluent only and FloCRS (pH 7.7) with five different concentrations of mupirocin. Error bars represent standard deviation of triplicate experiments. CRS 7.7 = FloCRS at pH 7.7; One-way analysis of variance (ANOVA) with Dunnett's multiple comparisons test with untreated; **, p-value < 0.01; *, p-value < 0.05.

Table 3. MIC (µg/ml) and MBC (µg/ml) of mupirocin in different diluent carriers on *S. aureus* strains.

	MIC (µg/ml)				MBC (µg/ml)			
	NeilMed	Flo SC	FloCRS (5.64)	FloCRS (7.7)	NeilMed	Flo SC	FloCRS (5.64)	Flo CRS (7.7)
ATCC 25923	0.5	0.5	0.06	0.25	8	8	4	64
C222 (MRSA)	0.5	0.5	0.03	0.5	4	4	4	64
C263 (MRSA)	0.5	0.5	0.03	0.5	4	4	4	1
C311 (MSSA)	0.5	0.5	0.03	0.25	4	4	4	64
C349 (MSSA)	1.0	0.5	0.03	0.25	4	4	4	2

Flo SC = Flo Sinus Care; CRS (5.64) = FloCRS at pH 5.64; CRS (7.7) = FloCRS at pH 7.7.

Table 4. Average value (in bold) for metabolic activity and biomass reduction of biofilm using alamarBlue assay and crystal violet respectively at the concentration of 128 µg/ml of mupirocin in each diluent for five of the *S. aureus* bacterial strains.

Diluent Carrier	Bacterial Strains	Reduction in Biomass				Reduction in Metabolic Activity			
		Rep 1	Rep 2	Rep 3	Average	Rep 1	Rep 2	Rep 3	Average
NeilMed	ATCC 25923	52.4%	51.5%	47.3%	50.4%	50.4%	34.6%	32.1%	39%
	C222 (MRSA)	56.7%	65.8%	44.2%	55.6%	44.9%	45.3%	52.6%	47.6%
	C263 (MRSA)	45.8%	48.5%	40.3%	44.9%	44.6%	33.8%	47.2%	41.9%
	C311 (MSSA)	39%	27.3%	23.4%	29.9%	41.7%	50%	40.9%	44.2%
	C349 (MSSA)	38.8%	48.4%	44%	43.7%	40.1%	38.5%	365	38.2%
Flo Sinus Care	ATCC 25923	13.2%	1.9%	-17.6%	-0.83%	34.1%	23.4%	28.3%	28.6%
	C222 (MRSA)	7.9%	-20%	9.5%	-2.6%	29.8%	33.9%	31.7%	31.8%
	C263 (MRSA)	35.9%	44.4%	50.5%	43.6%	45.1%	53.4%	53.2%	50.6%
	C311 (MSSA)	22.2%	27%	24.6%	24.6%	42.4%	46.6%	50.6%	46.5%
	C349 (MSSA)	47.4%	45.5%	56.4%	49.8%	32.2%	32.1%	28.3%	30.2%
FloCRS5.64	ATCC 25923	43.5%	55.1%	37.1%	45.3%	48.6%	56.1%	29.8%	44.9%
	C222 (MRSA)	50.5%	55.8%	61.3%	55.8%	49.4%	53.2%	46.6%	49.7%
	C263 (MRSA)	53%	56.7%	52.4%	54.1%	59.4%	56.1%	38.6%	51.4%
	C311 (MSSA)	46.8%	41.1%	45.5%	47.7%	70.3%	63.2%	58%	63.8%
	C349 (MSSA)	67.1%	52.7%	54.4%	58.1%	63.4%	48.6%	43.5%	52%
FloCRSpH 7.7	ATCC 25923	60.3%	77.7%	66.7%	68.2%	38.6%	41%	44.2%	41.3%
	C222 (MRSA)	37.9%	45.5%	42.2%	41.8%	48%	25.5%	34.8%	36%
	C263 (MRSA)	25.2%	21.2%	17.8%	21.4%	43.3%	30.2%	44%	39.2%
	C311 (MSSA)	13.2%	15%	18.9%	15.7%	28.3%	24.6%	25%	26%
	C349 (MSSA)	27.1%	28.1%	27.2%	27.5%	28.4%	22.6%	34.2%	27.8%

*All experiments were performed as three independent replicate

fluorescens [24] and is commonly used as a topical agent to treat localized skin and soft tissue infections by acting as a protein inhibitor which binds to the bacterial isoleucyl-tRNA synthetase enzyme which is encoded by the *ile*S gene. It has been demonstrated to be the most successful topical antibiotic for the clearance of both MRSA and MSSA in the nasal vestibule and anterior nasal cavity [25]. Several recent studies have also demonstrated that when delivered in a diluted form in sinus irrigations it appears to also be a clinically effective treatment for *S. aureus* infection in patients with refractory CRS [18, 19]. In this study, we compared the in vitro antibacterial and antibiofilm activity of Mupirocin diluted in three commercially available sinus rinses (FloCRS, Flo Sinus Care, and NeilMed) with different pH.

The results of this study demonstrated that mupirocin diluted in FloCRS at pH 5.64 was effective in reducing the growth of *S. aureus* in vitro and displayed the highest anti-biofilm activity of all three solutions. A lower concentration of mupirocin in FLO

CRS at pH 5.64 was required to inhibit and eradicate *S. aureus* in planktonic growth when compared to the other two solutions tested. We also observed that when diluted in FloCRS (pH 5.64), mupirocin significantly reduced the metabolic activity and biomass of *S. aureus* biofilms compared to FLO CRS at a higher pH (pH 7.7), Flo Sinus Care and NeilMed. Thus, the addition of mupirocin in FloCRS (pH 5.64) appeared to have a higher effect on biofilm eradication.

The improved activity of mupirocin in a low pH, is documented in a review of this antibiotic by Conly et al. [26] and supported by another independent study also demonstrating that mupirocin in normal saline, at a pH of 5.5, significantly reduced the bacterial load of *S. aureus* found in the maxillary sinus mucosa [27]. Interestingly however, studies of the effect of pH on other anti-staphylococcal antibiotics such as gentamicin and oxacillin, show a reduction in their activity at lower pHs [28]. Given that infected sinus cavities typically have reduced pH, this has significant clinical implications in the choice of antibiotic to treat *S. aureus* infections in these regions.

Interestingly, the reduction in biomass was also observed when *S. aureus* strains were treated with FloCRS at both pH (5.64 and 7.7) diluent alone. This could indicate that some of the excipients present in FloCRS, might have an antibacterial or antibiofilm effect. A candidate for this is the osmolyte solute xylitol. Zabner et al. and Weissman et al. indeed demonstrated that xylitol exhibits antimicrobial properties by showing efficacy against chronic bacterial infection [29, 30]. This is also supported by earlier reports which have shown xylitol is commonly used in chewing gum, lozenges or syrup to reduce the risk of caries and prevent acute otitis media and it has been suggested that xylitol could potentially reduce biofilm biomass or inhibit biofilm formation [31]. Xylitol is indeed classified as a non-ionic surfactant and it is well known that such products have antibiofilm properties and can prevent bacterial and biofilm attachment to surfaces [32, 33].

There are some limitations of this study. Firstly, the in vitro and tightly controlled conditions of this study may not be truly representative of what is occurring in CRS patients and further in vivo studies would be required to validate our findings. Secondly, in this study, we did observe some variability of the effect of the low pH on the various isolates tested, with the ATCC strain behaving differently. This could be because the ATCC lab strain is more likely to have a stable adapted phenotype and genotype over time whilst clinical isolates generally alter their genotype and phenotype to accommodate different conditions/stressors of the host. This may be more representative of what is occurring in patients with CRS. A further limitation of this study is that although Neil Med irrigations are available worldwide, Flo Sinus Care and FloCRS are only available in Australasia, thereby limiting the clinical utility of this study to other international centers. We do hope however that the significant finding of this study does prompt similar research to be done in other countries on their commonly available irrigation solutions.

Conclusion

In conclusion, we have used an in-vitro treatment on *S. aureus* clinical isolates to investigate the effect of mupirocin dissolved in three commercially available sinus rinses (FloCRS, Flo Sinus Care, and NeilMed) with different pH. Our results suggest that the addition of mupirocin to a low pH sinus rinse has the highest efficacy in reducing the growth of *S. aureus* planktonic cells and biofilms. This could be useful in eliminating *S. aureus* biofilms present on the sinus mucosa of patients with CRS.

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None.

Authorship contribution

KH: Study design, Experiments, Data Collection, Manuscript preparation; SL: Study Design, Data review, Manuscript review; CC: Study Design, Data Review, Lab Supervision, Manuscript review; SV: Study Design, Data Review, Manusript review; AP: Study Concept, Study Design, Data Review, Manuscript preparation, Review and Correction.

Conflict of interest

Alkis Psaltis - relevant - is a consultant for ENT technologies who provided research grant funding for this project. Non relevant - consultant for Tissium, Medtronic, Fusetec, speakers bureau for Sequiris and Shareholder for chitogel.

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