

The in vitro effect of xylitol on chronic rhinosinusitis biofilms*

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Abstract

Introduction: Biofilms have been implicated in chronic rhinosinusitis (CRS) and may explain the limited efficacy of antibiotics. There is a need to find more effective, non-antibiotic based therapies for CRS. This study examines the effects of xylitol on CRS biofilms and planktonic bacteria.

Methods: Crystal violet assay and spectrophotometry were used to quantify the effects of xylitol (5% and 10% solutions) against *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The disruption of established biofilms, inhibition of biofilm formation and effects on planktonic bacteria growth were investigated and compared to saline and no treatment.

Results: Xylitol 5% and 10% significantly reduced biofilm biomass (*S. epidermidis*), inhibited biofilm formation (*S. aureus* and *P. aeruginosa*) and reduced growth of planktonic bacteria (*S. epidermidis*, *S. aureus*, and *P. aeruginosa*). Xylitol 5% inhibited formation of *S. epidermidis* biofilms more effectively than xylitol 10%. Xylitol 10% reduced *S. epidermidis* planktonic bacteria more effectively than xylitol 5%. Saline, xylitol 5% and 10% disrupted established biofilms of *S. aureus* when compared with no treatment. No solution was effective against established *P. aeruginosa* biofilm.

Conclusions: Xylitol has variable activity against biofilms and planktonic bacteria in vitro and may have therapeutic efficacy in the management of CRS.

Key words: chronic rhinosinusitis, xylitol, biofilms, bacteria, therapeutic irrigation

Introduction

Bacterial biofilms have been implicated in chronic rhinosinusitis (CRS) pathogenesis and disease recalcitrance following endoscopic sinus surgery (ESS) ⁽¹⁻³⁾. Biofilm positive patients have been shown to be more likely to need surgical intervention, worse postoperative symptoms, persistent inflammation and recurrent infections ⁽⁴⁻⁹⁾.

Targeted treatment of sinonasal biofilms with long-term efficacy has not yet been described. Systemic antibiotics such as vancomycin, ciprofloxacin, piperacillin and ceftazidime have been shown to be ineffective at standard concentrations ^(10,11). Topical use of mupirocin has been shown to reduce biofilm mass but is associated with high microbiological failure rate in patients with

surgically recalcitrant CRS ⁽¹²⁾. As rhinosinusitis now accounts for more than 11% of all antibiotic prescriptions in the United States, a non-antibiotic anti-biofilm agent that is inexpensive, effective and safe is required ^(1,13).

Xylitol is a five-carbon sugar alcohol, which occurs naturally in many fruits and vegetables, and is used widely in the food industry as a sweetener. Recently, it has gained more popularity as an anti-biofilm agent, particularly in the field of periodontics ⁽¹⁴⁾. It has been shown to improve CRS symptoms when administered as a sinus lavage solution ⁽¹⁵⁾.

The aim of this study was to assess the effect of xylitol in vitro on established biofilms, biofilm formation and planktonic bacteria

that were either derived from CRS patients or commercial strains of known sinonasal pathogens. Furthermore, this study aimed to determine xylitol's potential as an adjunct to topical therapy.

Materials and methods

All procedures were undertaken at the School of Biological Sciences and Faculty of Medicine and Health Sciences of The University of Auckland, following approval with national and institutional human research ethics committees.

Bacterial isolates

Two sterile rayon-tipped swabs (Copan, Murrieta, CA, USA, #170KS01) were taken from the right and left middle meatuses of two patients with CRS undergoing endoscopic sinus surgery. Swabs were taken following general anesthesia and prior to administration of intravenous antibiotics. These were placed in sterile Eppendorf tubes and transported on ice for immediate culturing on nutrient and Columbia horse blood agar (Fort Richard Laboratories Ltd, New Zealand) for 48 hours at 37°C.

Fourteen bacterial strains were isolated and found amenable to culture in tryptic soy broth (TSB) (Difco Laboratories, Sparks, MD, USA). These strains were investigated further for their biofilm forming capacity. Broth from each isolate (200 µL) was pipetted in triplicate into wells of a flat-bottomed 96-well microtiter plate (Thermo Fisher Scientific Inc, New Zealand), which had been treated for tissue culture to optimise bacterial attachment to well surfaces⁽¹⁶⁾. Negative control wells were instilled with broth media only. Plates were incubated in static conditions for 24 h at 37°C to allow biofilm formation and attachment to well surfaces. After incubation the wells were aspirated and washed with phosphate buffered saline to remove planktonic and non-adherent bacteria⁽¹⁶⁾.

A crystal violet assay with optical density measured at 570 nm (Perkin Elmer Enspire Multimode Plate Reader, Waltham, MA, USA) was used to quantify biofilm biomass as previously described⁽¹⁶⁻¹⁸⁾. Biofilm formation from broth and subsequent optical density measurement was repeated four times. The most reliable and greatest biomass forming isolates were identified by matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry (Bruker UltrafleXtreme, Billerica, MA, USA) and were all found to be *S. epidermidis*, of which the best biofilm-forming isolate was chosen for use in this study.

In addition, commercial biofilm-forming isolates of *S. aureus* New-man and *P. aeruginosa* PAO1 (Institute of Infection, Immunity and Inflammation, University of Nottingham, UK) were also obtained for this study. Biofilm growth was optimised for *S. aureus* and *P. aeruginosa* by addition of 1% (w/v) glucose to overnight broth cultures diluted 1:100 in fresh TSB (15 mL).

Treatment groups

Xylitol (Langdon ingredients, Melbourne, Australia) was obtained in powdered form and diluted with TSB to obtain 5% (w/v) and 10% (w/v) solutions. These were compared with a 0.9% (w/v) solution of NaCl and a TSB only group, which served as a negative control.

Disruption of established biofilms

Biofilms of *S. epidermidis*, *S. aureus*, and *P. aeruginosa* were grown on 96-well plates as described. Each bacterial strain was grown in replicates of six for each treatment group. Following incubation and washing, 200 µL of each treatment solution was added to the six wells corresponding to each group. Plates were covered with a lid and treated for one hour at room temperature in static conditions. After the treatment period, each well was washed three times with 200 µL of phosphate buffered saline (PBS) to remove planktonic and non-adhering bacteria. Biofilms were stained and measured using crystal violet assay. This experiment was repeated four times for each bacterial strain.

Inhibition of biofilm formation

In this study, biofilms were grown on plates in the presence of each of the treatment solutions. In each well, high concentration treatment solutions (50 µL) were added to overnight broth culture for each isolate (200 µL) to make up the working treatment solutions. Each treatment consisted of six replicates. Plates were incubated for 24 h at 37°C to allow biofilms to form. Wells were washed with PBS and underwent crystal violet assay. This experiment was repeated four times for each bacterial strain.

Effects on planktonic bacteria

To assess the effects of treatment on planktonic bacteria, TSB with treatment solutions (100 µL) were added to bacteria grown to mid-exponential phase in TSB (100 µL). Each treatment consisted of six replicates. Plates were incubated at 37°C for four hours. Spectrophotometry was used to measure absorbance at 600 nm and compared to similar treatment TSB solutions without bacterial culture. This experiment was repeated three times for each bacterial strain.

Statistical analysis

Statistical analyses were performed using SPSS (Version 22; IBM, New York, NY, USA). All results were described using mean difference (Δ) \pm standard error. Groups were compared using a one-way analysis of variance (ANOVA) with post-hoc Tukey's HSD to adjust for multiple comparisons. Significance was accepted when P was less than 0.05.

Results

Disruption of established biofilms (Figure 1).

Staphylococcus epidermidis

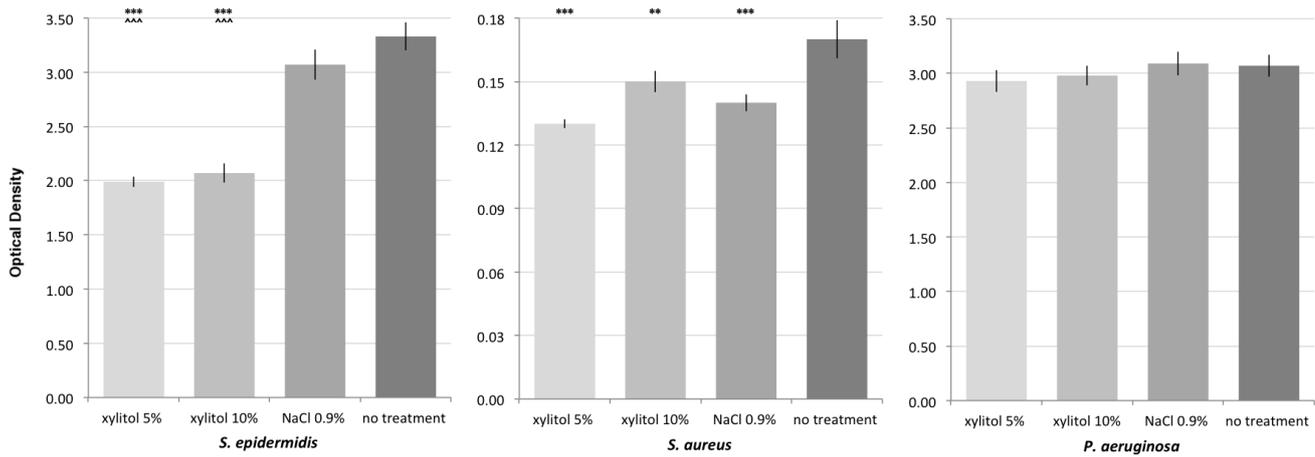


Figure 1. Disruption study: effects of solutions on the optical density of established biofilms after 1 hour of treatment.

n = 24 for each bacteria and treatment combination. Error bars represent standard error of the mean. Significance: *P<0.05, **P<0.01 and ***P<0.001 compared to no treatment, ^P<0.05, ^^P<0.01 and ^^P<0.001 compared to saline.

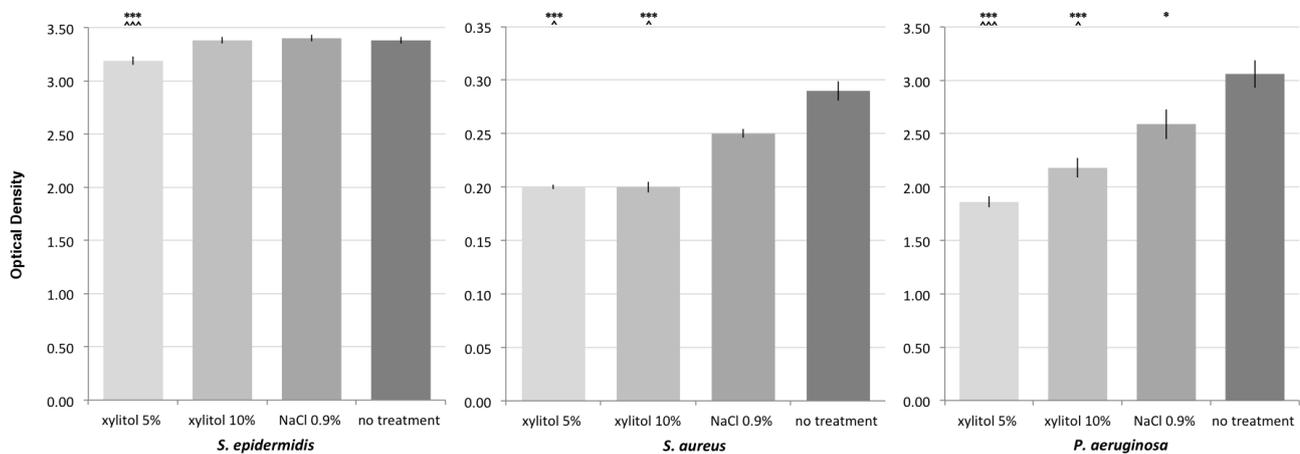


Figure 2. Inhibition study: optical density of bacterial biofilms formed in the presence of solutions after 24 hours.

n = 24 for each bacteria and treatment combination. Error bars represent standard error of the mean. Significance: *P<0.05, **P<0.01 and ***P<0.001 compared to no treatment, ^P<0.05, ^^P<0.01 and ^^P<0.001 compared to saline.

Significant reductions in biofilm biomass were observed with xylitol 5% ($\Delta 1.3 \pm 0.1$, P<0.001) and xylitol 10% ($\Delta 1.3 \pm 0.1$, P<0.001) when compared with no treatment. Both the 5% and 10% solutions were also significantly more effective than saline ($\Delta 1.1 \pm 0.1$, P<0.001 and $\Delta 1.0 \pm 0.1$, P<0.001). There was no difference between xylitol 5% and 10%.

Staphylococcus aureus

Reductions of biomass were seen with xylitol 5% ($\Delta 0.44 \pm 0.01$, P<0.001), xylitol 10% ($\Delta 0.29 \pm 0.01$, P<0.004) and saline ($\Delta 0.31 \pm 0.01$, P<0.001) when compared with no treatment. No differences were seen between the xylitol solutions and saline.

Pseudomonas aeruginosa

No significant differences were seen between the experimental groups.

Inhibition of biofilm formation (Figure 2).

Staphylococcus epidermidis

Only xylitol 5% was shown to significantly inhibit *S. epidermidis* biofilm formation when compared to no treatment ($\Delta 0.19 \pm 0.05$, P<0.001), saline ($\Delta 0.21 \pm 0.05$, P<0.001) and xylitol 10% ($\Delta 0.18 \pm 0.05$, P<0.001). There was no difference between saline and no treatment groups.

Staphylococcus aureus

Inhibition of biofilm formation was seen with both xylitol 5% and xylitol 10% when compared with no treatment ($\Delta 0.09 \pm 0.02$, P<0.001 and $\Delta 0.09 \pm 0.02$, P<0.001) and saline ($\Delta 0.05 \pm 0.02$, P=0.03 and $\Delta 0.06 \pm 0.02$, P<0.03). There was no difference between preparations of xylitol or between saline and no treatment groups.

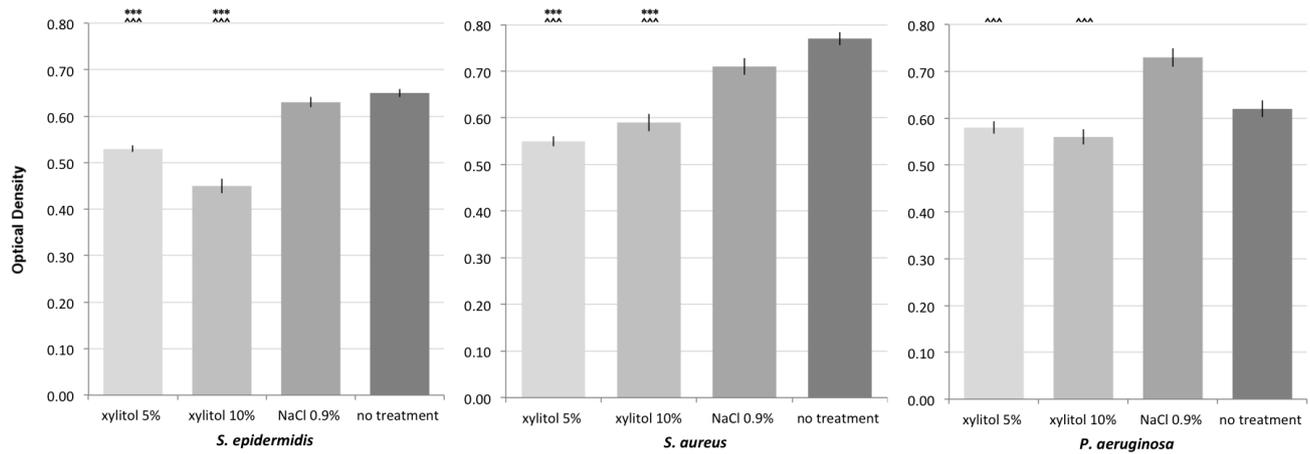


Figure 3. Planktonic study: optical density of planktonic bacteria in the presence of solutions after 4 hours.

n = 18 for each bacteria and treatment combination. Error bars represent standard error of the mean. Significance: *P<0.05, **P<0.01 and ***P<0.001 compared to no treatment, ^P<0.05, ^^P<0.01 and ^^P<0.001 compared to saline.

Pseudomonas aeruginosa

Xylitol 5%, xylitol 10% and saline were able to inhibit *P. aeruginosa* biofilm formation when compared to no treatment ($\Delta 1.2 \pm 0.15$, P<0.001, $\Delta 0.88 \pm 0.15$, P<0.001 and $\Delta 0.47 \pm 0.15$, P<0.02). Xylitol 5% and 10% were also both more efficacious than saline ($\Delta 0.73 \pm 0.15$, P<0.001 and $\Delta 0.41 \pm 0.15$, P=0.04), however there was no significant difference between the two preparations.

Effects on planktonic bacteria (Figure 3).

Staphylococcus epidermidis

Solutions of both xylitol 5% and 10% significantly inhibited planktonic *S. epidermidis* when compared with no treatment ($\Delta 0.12 \pm 0.02$, P<0.001 and $\Delta 0.20 \pm 0.02$, P<0.001) and saline 0.9% ($\Delta 0.1 \pm 0.02$, P<0.001 and $\Delta 0.18 \pm 0.02$, P<0.001). When compared, xylitol 10% was more effective than xylitol 5% ($\Delta 0.08 \pm 0.02$, P<0.001).

Staphylococcus aureus

Solutions of both xylitol 5% and 10% again significantly inhibited planktonic *S. aureus* when compared with no treatment ($\Delta 0.22 \pm 0.02$, P<0.001 and $\Delta 0.18 \pm 0.02$, P<0.001) and saline 0.9% ($\Delta 0.16 \pm 0.02$, P<0.001 and $\Delta 0.12 \pm 0.02$, P<0.001). When compared, there was no difference between xylitol 5% and 10%.

Pseudomonas aeruginosa

Solutions of both xylitol 5% and 10% significantly inhibited planktonic *P. aeruginosa* when compared with saline 0.9% ($\Delta 0.15 \pm 0.02$, P<0.001 and $\Delta 0.16 \pm 0.02$, P<0.001). There was no difference between xylitol 5% and 10%.

Discussion

This study has examined the effects of xylitol on three sinonasal bacterial species, *S. epidermidis*, *S. aureus*, and *P. aeruginosa*, that

are capable of forming biofilms.

Xylitol is an inexpensive and sweet tasting sugar alcohol (or polyol), which has been widely used in the food industry to replace sucrose. More recently xylitol has gained popularity for its antimicrobial effects^(14,19,20). Xylitol is thought to exert its antibacterial activity by a number of mechanisms including reduction of the salt concentration of airway surface liquid, repressing bacterial metabolism of glucose to lactate, and disruption of the biofilm extracellular matrix⁽²¹⁻²⁵⁾.

In this study, xylitol was variously active against the three examined microorganisms. When compared with saline, both xylitol 5% and 10% were able to significantly reduce biofilm biomass (*S. epidermidis*), inhibit biofilm formation (*S. aureus* and *P. aeruginosa*) and reduce planktonic bacteria (*S. epidermidis*, *S. aureus* and *P. aeruginosa*).

Generally, a higher concentration of xylitol was not more effective than the isosmotic 5% solution. Differences in activity between the concentrations of xylitol were only seen for inhibition of *S. epidermidis* biofilms (in which 5% was significantly more effective) and against *S. epidermidis* planktonic bacteria (in which 10% was significantly more effective).

By its in vitro design, the results of this study are limited and may not directly translate to the in vivo effects of xylitol. For example, no solution in this study was effective against established *P. aeruginosa*. Established biofilms in this study were exposed to treatments for one hour. This may not be enough time for xylitol to completely penetrate bacterial membranes. Previous studies assessing the effect of xylitol on *P. aeruginosa* over a 24 h period have suggested that the addition of a syner-

gist such as lactoferrin increases permeability of bacterial membranes and structural disruption of biofilms^(26,27). In vivo, the ability of xylitol to remain on the apical surfaces of respiratory epithelia and lower the airway surface liquid salt concentration without being metabolised by *P. aeruginosa* might increase the antimicrobial effect^(22,28).

The length of exposure time of biofilms to xylitol in vitro studies may be longer than exposure in the paranasal sinuses following nasal lavage, where it is not known how much xylitol would remain in the cavities, or for what duration. The hydrodynamic nature of nasal lavage may also have an effect on biofilm disruption and was not evaluated in this study. Despite this, a pilot randomised control study using a once daily xylitol 5% rinse observed a significant reduction in SNOT-20 scores when compared to saline⁽¹⁵⁾.

Overall, this study has demonstrated the activity of xylitol against three commonly found sinonasal bacteria in both biofilm and planktonic state, which reaffirms its previously described antimicrobial effects. The therapeutic potential of xylitol lies in its utility as a safe, inexpensive, well tolerated, non-antibiotic topical treatment for CRS⁽¹⁵⁾. As a nasal irrigation it may have a stronger regulatory effect on the sinonasal microbiota than

conventionally used saline and have a particular role in the post surgical sinus cavities where control of biofilms may influence clinical outcomes. However, the effects of xylitol on the microbiota in vivo has not yet been established and the effects could be different in the context of a more complex interaction between multiple bacterial populations and innate immune factors, including the airway surface liquid.

Conclusion

Xylitol has reliable antimicrobial activity against established biofilms, inhibiting biofilm formation and reducing planktonic bacteria in vitro. In this study, xylitol was almost universally more effective than saline. Xylitol is a safe and non-antibiotic potential for topical therapy in CRS and deserves strong consideration for further patient based studies.

Authorship contribution

RD: Principal investigator, study design and manuscript review. RJ: Study design, data collection, analysis and manuscript writing. TL, TH: Data collection, analysis and manuscript review. KB, FR: Collaborating investigators, manuscript review.

Conflict of interest

None.

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