



Is There a Role for the Topical Penicillin Treatment of *Staphylococcus aureus* Keratitis Based on Elevated Corneal Concentrations?

Kowalski RP^{1*}, Romanowski EG¹, Yates KA¹, Romanowski JE¹, Grewal A¹, and Bilonick RA²

¹University of Pittsburgh Medical Center (UPMC), The Charles T. Campbell Ophthalmic Microbiology Laboratory, Department of Ophthalmology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

²Department of Biostatistics, University of Pittsburgh School of Public Health, Pittsburgh, PA, USA

*Corresponding Author: Kowalski RP, MS, M(ASCP), The Eye and Ear Institute Bldg, Ophthalmic Microbiology, Room 642, 203 Lothrop Street, Pittsburgh, PA 15213. Tel: (412) 647-7211, Fax: (412) 647-5331, E-mail:

kowalskirp@upmc.edu

Citation: Kowalski RP, Romanowski EG, Yates KA, Romanowski JE, Grewal A, Bilonick RA., et al. (2018) Is There a Role for the Topical Penicillin Treatment of *Staphylococcus aureus* Keratitis Based on Elevated Corneal Concentrations?. J Clin Ophthalmol optom 2(1): 103

Abstract

Purpose: Elevated concentrations of penicillin into the cornea can increase the potency of penicillin as a topical antibiotic for the treatment of *Staphylococcus aureus* (SA) keratitis. We evaluated topical 6% penicillin to treat SA keratitis using isolates deemed resistant to penicillin.

Methods: Rabbit corneas were infected bilaterally by ocular isolates of SA with varying penicillin minimum inhibitory concentration (MIC) levels (0.12, 0.25, 0.5, 16, 32, and 64 µg/ml). The corneal epithelium was abraded in the left eye to mimic ulceration. After 4 hours, four rabbits for each isolate were sacrificed prior to topical treatment to determine the onset colony counts. Each SA isolate was treated topically with 6% penicillin, 2.5% vancomycin, or saline for 5 hours (N=8/group). One hour afterwards, rabbits were sacrificed, corneas were removed, homogenized, and standard colony counts were performed. Data were analyzed to determine bactericidal effect, penicillin efficacy (linear mixed effects model), and a tentative susceptibility standard.

Results: For intact and abraded corneas, a bactericidal effect was noted for SA with MICs of 0.12, 0.25, and 16 µg/ml. Colony counts of the penicillin group was significantly lower than vancomycin (p=0.014) for the abraded group, but not for the intact group (p=0.17). Colony count differences (p=0.00001) indicated that the corneal epithelium hindered penetration of penicillin.

Discussion: Experimentally, penicillin is a potent antibiotic to topically treat SA keratitis even against SA considered resistant. A tentative SA keratitis susceptibility standard could be 16µg/ml, but more work needs to be supportive. Penicillin may be an alternative SA keratitis therapy, but anaphylaxis will remain an issue.

Keywords: Penicillin; *Staphylococcus Aureus*; Keratitis; Rabbit Keratitis Model

Introduction

Staphylococcus aureus (SA) is a frequent bacterial keratitis pathogen as reported by our laboratory (Figure 1) and by others [1-3]. In an era where few new anti-infectives and antibiotics are being developed, it is important to re-evaluate “older antibacterials” [4]. For example, as in this study, penicillin, a beta-lactam antibiotic, could provide alternative topical therapy for penicillin-susceptible SA (PSSA) keratitis, substituting for cefazolin, vancomycin and fluoroquinolone anti-infectives. Penicillin was first reported to be successful to treat conjunctivitis as early as 1930, but topical penicillin is rarely used currently for SA keratitis [5]. There are no *in vitro* antibiotic standards to guide topical therapy in the treatment of bacterial keratitis for penicillin or any other antibiotic. This limitation prevents the use of topical penicillin by preventing laboratory confirmation of ‘*in vitro*’ to ‘*in vivo*’ success. In the case of penicillin, the serum standard for resistance is very low with a minimum inhibitory concentration (MIC) of 0.12 µg/ml [6]. If a correlation could be made with published rabbit corneal concentrations, penicillin can reach concentrations from 28 to 1080 µg/ml by topical administration, 300 µg/ml by subconjunctival injections, and 4-9 µg/ml after systemic therapy

[7]. In light of these elevated concentrations, it appears that there may be an over reporting of bacterial resistance in corneal infections using the serum standard for penicillin. The attainment of high antibiotic concentrations in the cornea indicates that topical penicillin may be able to reach high therapeutic concentrations to treat bacterial keratitis. Support for different standards for ocular topical therapy versus systemic infections was provided by our previous studies [8,9]. We demonstrated that high levofloxacin and gatifloxacin concentrations in the cornea eliminated bacteria that were considered levofloxacin and gatifloxacin resistant based on the serum standards.

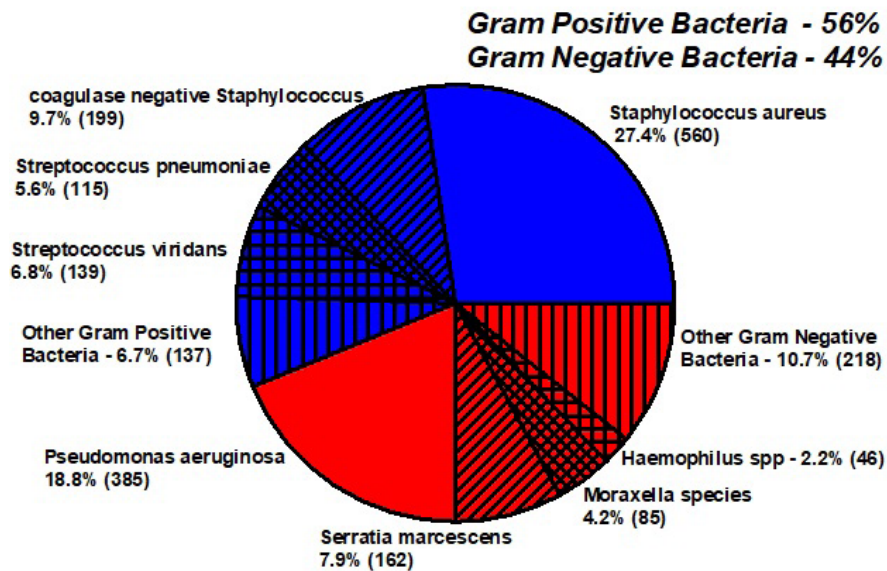


Figure 1: Pictures the distribution of bacteria (1993-2017) (N=2026) from bacterial keratitis. The Charles T. Campbell Ophthalmic Microbiology Laboratory. (<http://eyemicrobiology.upmc.com/AntibioticSusceptibilities/keratitis.htm>) accessed March 13, 2018

Since topical penicillin is rarely (not) used for the treatment of SA ocular infections, the selective pressure for SA to acquire resistance is reduced, and penicillin should be reconsidered as a therapeutic in the treatment of PSSA keratitis. The resurgence of PSSA has been reported from other infection types (i.e. bacteremia) [10-12]. Our laboratory has confirmed the resurgence of PSSA among SA isolated from patients with SA keratitis. (Unpublished) (Kowalski RP (2015) The resurgence of penicillin-susceptible *Staphylococcus aureus* isolated from infectious keratitis. ISOPT, Berlin, Germany, July 11, 2015, and Kowalski RP (2015) Antibiotic resistance and antibiotic alternatives: Looking towards the future. London, England, November 3 -5, 2015). In these reports we demonstrated the resurgence of penicillin susceptibility with 333 SA isolated from the corneas of keratitis patients between 1993 and 2014. The median MIC for '2010-2014' (2 µg/ml) was significantly lower (p=0.005, Mann-Whitney) than '1993-1997' (16 µg/ml) indicating increased penicillin susceptibility.

The aims of this study were

To test the hypothesis that penicillin can be used to treat PSSA keratitis infections. Using our well-established rabbit keratitis models [8,9,13-16]. We tested the efficacy of penicillin against clinical SA with increased resistance to penicillin, based on minimum inhibitory concentrations (MICs). We used established topical dosing with 6% penicillin [17].

1. To create a tentative topical antibiotic susceptibility standard for penicillin to guide the treatment of SA keratitis. We would establish the "Proof of Principle", using a rabbit keratitis model, that a tentative topical antibiotic susceptibility standard for penicillin would be higher than for the approved systemic standard. This would support the assumption, believed by most practicing ophthalmologists, that high concentrations of antibiotics can be attained in the corneal tissue with frequent topical dosing.
2. To introduce a tentative new method for determining susceptibility standards for other topical antibiotics used for the treatment of bacterial keratitis.
3. The expected outcome of this research will provide ophthalmologists another viable therapeutic option for the treatment of a potentially blinding disease, and provide guidance for clinical microbiologists and physicians to determine the susceptibility of SA ocular isolates which in turn can be used in making therapeutic decisions.

Methods

Overview

The purpose of our study was to determine the efficacy of topical penicillin in the treatment of *Staphylococcus aureus* (SA) keratitis in a rabbit model. Rabbit corneas were infected by 8 respective isolates of SA with varying MIC levels. Each SA isolate was treated respectively with topical penicillin, vancomycin, and saline. In each rabbit, the corneal epithelium was abraded in the left eye to mimic corneal ulceration, and the corneal epithelium remained intact in the right eye. This was performed to determine the penetration of antibiotic into the cornea to eliminate SA by comparing the colony counts of the intact and abraded eyes. The corneal infections progressed for 4 hours. A group of rabbits for each isolate was sacrificed prior to topical treatment to determine the colony counts for bactericidal effect. The rabbit eyes were administered topical antibiotic therapy for 5 hours. One hour after topical treatment, the rabbits were sacrificed, the corneas were removed, homogenized, and standard colony counts were performed. The data was statistically analyzed to determine bactericidal effect of topical treatment and the efficacy of penicillin in comparison to vancomycin and saline treatment.

In vivo Animal Testing

Specific pathogen free New Zealand white female rabbits, weighing 1.1 - 1.4 kg, were purchased from Charles River Laboratories' Oakwood Research facility, Oxford, MI. The present study conformed to the ARVO Statement on the Use of Animals in Ophthalmic and Vision Research, and was approved by the University of Pittsburgh Institutional Animal Care and Use Committee (IACUC Protocol #16017384-1 "Development of the First Topical Susceptibility Standard for Penicillin to Predict Successful Treatment of *Staphylococcus aureus* Keratitis").

Experimental Drugs

6% Penicillin (100,000 units/ml)

Penicillin G potassium For Injection (5,000,000 units) (Buffered Pfizerpen®, Pfizer, New York, NY; Lot 33002002) was purchased from the University of Pittsburgh Medical Center (UPMC), Pittsburgh, PA, inpatient pharmacy. The vial was stored at room temperature as directed until reconstituted with 3.2 ml of sterile saline to produce a penicillin G potassium solution of 1,000,000 units per ml. Four ml of the 1,000,000 units per ml penicillin G potassium solution was added to 36 ml of sterile saline to produce 40 ml of 100,000 units per ml penicillin G potassium. The 40 ml was aliquoted into 4 - 10 ml aliquots and stored frozen at -20°C until use within 2 weeks of reconstitution. On the day of the experiment, one of the 10 ml aliquots was thawed and used for dosing. Each topical application was a volume of 37 µl instilled directly onto the cornea using a Rainin electronic pipet set in the multi-dispense mode.

2.5% Vancomycin (25 mg/ml)

Vancomycin Hydrochloride for Injection USP (500 mg) (APP Pharmaceuticals, Fresenius Kabl USA, LLC, Lake Zurich, IL; Lot 2766042) was purchased from the UPMC inpatient pharmacy. Two vials were stored at room temperature as directed until reconstituted with 10 ml of saline to produce a vancomycin solution of 50 mg/ml (5%). The vials were combined. Nineteen mls of the 50 mg/ml vancomycin was added to 19 ml of sterile saline to produce 38 ml of 25 mg/ml (2.5%) vancomycin. The 38 ml of 2.5% vancomycin was transferred into 4 - 9.5 ml aliquots and stored frozen at -20°C until use within 2 weeks of reconstitution. On the day of the experiment, one of the 9.5 ml aliquots was thawed and used for dosing. Topical application was the same as penicillin.

Saline Control

0.9% Sodium Chloride Injection USP (Baxter Healthcare Corp. Deerfield, IL) was used as control drops. Topical application was the same as penicillin.

SA Isolates

Eight patient de-identified SA with varying MICs to penicillin and isolated from the corneas of patients with keratitis were retrieved from a clinical tissue bank which is used for validation of diagnostic testing and antibiotic evaluation. The 8 isolates were designated with penicillin MIC (µg/ml) as follows: I) K62, 0.12, II) K2112, 0.25, III) K1998, 0.5, IV) K950, 16, also methicillin resistant, V) K2561, 32, VI) K2821, 32, also methicillin resistant, VII) K167, 64, and VIII) K176, 64.

The inoculum for injection was created by growing each isolate on trypticase soy agar supplemented with 5% sheep blood (TSA II, Becton, Dickinson, and Company, Sparks, MD) for 24 hours at 37°C. A few colonies were suspended

in tryptic soy broth and the suspension was correlated to a colony count concentration (Colony Forming Units per milliliter) (CFU/ml) that was pre-determined with an optical density value read at 650 nm. This concentration was appropriately diluted in sterile trypticase soy broth to provide the inoculum of approximately 2,000 (2.0×10^3) CFU/eye in 25 μ l. The final concentration was confirmed with another standard colony count.

Experimental Protocol

1) In general, for each SA isolate, 32 rabbits were tested for 4 treatments (N=8) groups:

1. 6% penicillin.
2. 2.5% vancomycin.
3. Saline.
4. Onset.

K2112 and K176 were tested with 16 rabbits for 4 treatments (N=4). The vancomycin and saline groups for K2561 were reduced by 1 (N=7) due to premature rabbit death.

2) All rabbits were systemically anesthetized with intramuscular injections of ketamine (40 mg/kg) and xylazine (4 mg/kg) in the rear flank. Topical anesthesia (0.5% proparacaine) was applied to each cornea prior to proptosing the globe with a dacron-tipped applicator. The corneal epithelium of the left corneas was abraded using an Amoil's epithelial scrubber (Innovative Excimer Solutions, Inc., Ontario, Canada), while the epitheliums in the right eyes remained intact. Abrading the cornea assured de-epithelialization of the cornea. The comparison of the data between the two eyes would determine the ability of antibiotic to penetrate the corneal epithelium into the infected corneal stroma [15,16]. The corneas were intrastromally injected with 2000 CFU of each respective SA isolate. The rabbits were immediately treated with analgesia in the form of intramuscular injections of ketoprofen (1.5 mg/kg). The corneal infections progressed for 4 hours.

3) After 4 hours, the rabbits in the onset group were sacrificed and large 9.5 mm buttons were removed from the corneas. These were placed in 1 ml of phosphate buffered saline (PBS, pH 7.2, Gibco, Grand Island, NY) in Lysing Matrix A 2 ml tubes (MP Biomedicals LLC, Solon, OH). The corneal buttons were homogenized using the MP Fast Prep-24 homogenizer (MP Biomedicals LLC, Solon, OH) for five homogenization cycles (20 seconds) separated by 90 second intervals to keep the samples cool. The speed was set at 4.0 m/s. The homogenizer was kept at 4°C. After homogenization, the homogenates were diluted to 10^{-2} and 10^{-4} , and colony counts were performed on the undiluted sample and the two dilutions per eye using the EddyJet 2 spiral plating system (Neutec Group Inc., Farmingdale, NY) on TSA plates with 5% sheep blood. After 48 hours, the colonies on the plates were counted using the automated Flash and Grow colony counting system (Neutec Group Inc.) and the number of CFU/eye of *Staphylococcus aureus* were determined for each cornea. All colony counts were \log_{10} (CFU+1) transformed for analysis.

4) Also, after 4 hours, topical treatment for the penicillin, vancomycin, and saline groups commenced every 15 minutes for 5 hours (21 total doses).

5) One hour after the final treatment, the treated rabbits (penicillin, vancomycin, and saline) were sacrificed and large 9.5 mm buttons were removed from the corneas. These were placed in 1 ml of PBS in Lysing Matrix A 2 ml tubes. The corneal buttons were homogenized as described previously.

6) After homogenization, colony counts were determined as previously described for the treatment groups and \log_{10} (CFU+1) transformed for analysis.

Statistical Analysis

All statistical analyzes were performed by a trained statistician (RAB). The kill of SA by topical 6% penicillin, 2.5% vancomycin, and saline was determined for all tested SA isolates by subtracting the mean \log_{10} CFU/ml at the onset from the mean \log_{10} CFU/ml after antibiotic treatment. A decrease of 3 \log_{10} or greater denoted a bactericidal effect (99.9% decrease in viable SA). This was calculated for both intact and abraded corneas. A bactericidal kill to the SA with the largest MIC would indicate a tentative level of an *in vivo* susceptibility standard for the topical treatment of SA with 6% penicillin.

The efficacy of topical 6% penicillin against 8 SA isolates with varying MICs to penicillin was compared to 2.5% vancomycin, saline, and at onset, under conditions where corneal epithelium was abraded and left intact. The R language and environment for statistical computing (Version 3.4.1) was used for computations. (URL: <https://www.R-project.org/>) (R Core Team (2017). R: A language and environmental for statistical computing. R Foundation

for Statistical Computing, Vienna, Austria.). The nlme R package was used for the mixed effects modeling. The study design had two crossed factors: 1) topical treatment group with four levels (control, onset, 6% penicillin, and 2.5% vancomycin), and 2) corneal epithelial condition (abraded versus intact). Corneal epithelium condition was nested within the experimental units. The \log_{10} (CFUs + 1) were modelled as a function of antibiotic treatment and corneal epithelial condition using a linear mixed effects model with a random intercept. ([URL://CRAN.R-project.org/package=nlme](http://CRAN.R-project.org/package=nlme)) (Pinheiro J, Bates D, DebRoy S, Sarkar D, and RCore Team (2017). *Nlme: Linear and Nonlinear Mixed Effects Models*. R package version 3.1-131.

Results

Table 1 summarizes the transformed \log_{10} (CFU + 1) of SA from rabbit corneas treated topically with 6% penicillin, 2.5% vancomycin, and saline. For both intact and abraded corneas, a bactericidal effect was noted for SA with MICs of 0.12, 0.25, and 16 $\mu\text{g/ml}$. The colony count decrease was over 90% for the MIC of 0.5 $\mu\text{g/ml}$. This data suggests that a tentative susceptibility standard for keratitis therapy could be high as 16 $\mu\text{g/ml}$.⁵ No bactericidal effects for topical vancomycin treatment of the intact groups were noted. A bactericidal effect was noted for topical vancomycin treatment of the SA with a MIC of 16 $\mu\text{g/ml}$ and an abraded cornea. As expected, there were no bactericidal effects with topical saline treatment.

ID*	K62	K2112	K1998	K950	K2821	K256	K167	K176
Pen MIC ($\mu\text{g/ml}$)†	0.12	0.25	0.5	(MR) 16	#(MR) 32	32	64	64
Penicillin IE[]	1.63	1.89	2.81	0.71	4.97	6.4	3.84	4.29
Penicillin AE[]	0.0	1.0	2.4	0.59	1.21	1.98	2.25	2.42
Vancomycin IE	2.95	2.36	2.36	2.98	3.32	3.386	2.36	2.6
Vancomycin AE	2.36	3.38	2.66	1.1	3.14	2.42	2.67	2.8
Saline IE	4.59	5.52	5.4	7.03	6.87	7.93	7.78	7.4
Saline AE	1.89	3.59	5.18	3.97	2.44	7.17	6.98	6.68
Onset IE‡	4.81	5.24	5.11	4.7	5.32	5.21	4.76	4.94
Onset AE	4.52	4.79	4.76	4.4	3.98	4.42	4.73	4.63
Penicillin IE Minus Onset IE §	-3.18	-3.35	-2.3	-3.99	-0.35	+1.19	-0.92	-0.65
Penicillin AE minus Onset AE§	-4.52	-3.79	-2.36	-3.81	-2.77	-2.44	-2.48	-2.21
Vancomycin IE minus Onset IE§	-1.86	-2.88	-2.75	-1.72	-2.0	-1.83	-2.4	-2.34
Vancomycin AE minus Onset AE§	-2.16	-1.41	-2.1	-3.3	-0.84	-2.0	-2.06	-1.83
Saline IE minus Onset IE§	-0.22	+0.28	+0.29	+2.33	+1.55	+2.72	+3.02	+2.46
Saline AE Minus Onset AE§	-2.63	-1.2	+0.42	-0.43	-1.54	+2.75	+2.25	+2.05

Table 1: Mean Log₁₀ Transformed [Colony Counts + 1] of Staphylococcus aureus from Corneas Treated Topically with % Penicillin, 2.5% Vancomycin and Saline

* - ID is the identification of Staphylococcus aureus from an anonymous clinical tissue bank

†- Pen MIC ($\mu\text{g/ml}$) is the minimum inhibitory concentration of penicillin to each Staphylococcus aureus isolate

‡- Onset is the time that topical therapy commenced

§- (Penicillin or Vancomycin) minus Onset is the determination of the bactericidal action (-3.0 or larger) of topical treatment

[]- IE indicates that the corneal epithelium was not removed (Intact) prior to topical therapy

[]- AE indicates that the corneal epithelium was removed (Abraded) prior to topical therapy

#- MR indicates isolates were methicillin resistant

Figure 2 depicts the interaction plot for the two crossed factors for the treatment groups. For the topical treatment by penicillin of the abraded epithelium group, the colony counts of the penicillin group was significantly lower than vancomycin ($p=0.014$, estimate residual error = -0.88 , 95% CI $[-1.58, -0.18]$), saline ($p=0.005$, estimate residual error = 1.27 , 95% CI $[0.39, 2.15]$) and the onset ($P=0.001$, estimate residual error = 1.13 , 95% CI $[0.46, 1.81]$). For the topical treatment by penicillin of the intact group, the colony counts of the penicillin group was not significantly lower than vancomycin ($p=0.17$, estimate residual error = -0.53 , 95% CI $[-1.28, 0.22]$), but significantly lower than saline ($p=0.00001$, estimate residual error = 3.21 , 95% CI $[2.44, 3.99]$) and the onset ($P=0.00001$, estimate residual error = 1.63 , 95% CI $[0.99, 2.27]$). Figure 3 depicts the interaction plot for the two crossed factors for the corneal epithelium groups. The difference in colony counts between the intact and abraded epithelium groups after topical penicillin was statistically significant ($p=0.00001$, estimate residual error = 0.60 , 95% CI $[0.46, 0.78]$) indicating that the corneal epithelium may hinder the penetration of penicillin. The difference in colony counts between the intact and abraded epithelium groups after topical vancomycin was not statistically significant ($p=0.43$, 2 sample t test, Minitab, State College, PA). This indicated that the corneal epithelium was not a hindrance to vancomycin penetration.

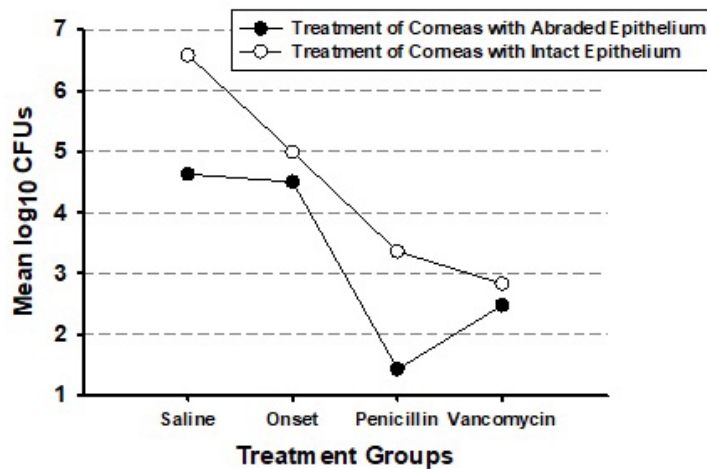


Figure 2: Depicts the interaction plot for the two crossed factors for the treatment groups

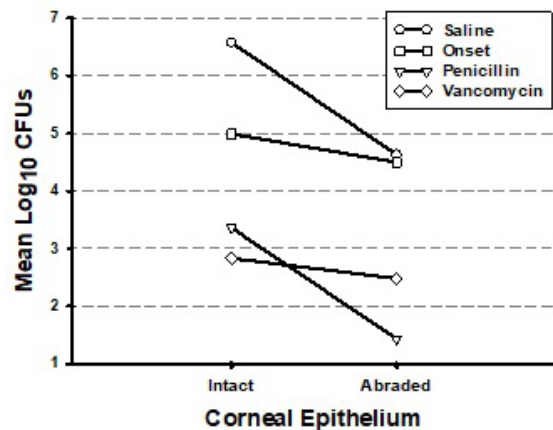


Figure 3: Depicts the interaction plot for the two crossed factors for the corneal epithelium groups

Discussion/Conclusions

Penicillin appears to be a potent antibiotic that could be reconsidered for the topical treatment of SA keratitis. Although the MICs of SA to penicillin will be deemed resistant by the systemic standards, the concentrations reached in corneal tissue will be able to eliminate the growth of SA. Our data indicates that topical treatment with 6% penicillin will reach bactericidal decrease levels, and that the decreases are at least equal to topical treatment with 2.5% vancomycin. It appears that levels of 16 µg/ml may be reached in the corneal tissue which is 133X higher than the systemic susceptibility standard. Our study does not advocate replacing other topical antibiotics with penicillin for treating SA, but it does indicate that penicillin may be considered as an alternative inexpensive antibiotic with a certain key reservation that allergy to penicillin in the form of severe anaphylaxis must be investigated prior to clinical use. Our

data also indicates that the ulceration of the cornea provides elevated penicillin concentrations below the epithelium layer for increased efficacy.

So why is penicillin an interesting antibiotic? Penicillin was an early antibiotic directed to treat predominantly Gram-positive bacteria such as SA. Penicillin and other β -lactam antibiotics act by inhibiting penicillin-binding proteins, which normally catalyze cross-linking of bacterial cell walls which exist in SA. Isolates of SA counteracted the effects of penicillin by producing an enzyme that can resist the effects of penicillin and similar β -lactam antibiotics. This enzyme, β -lactamase, breaks the β -lactam ring of penicillin to disable the penicillin molecule. The serum susceptibility standard indicates that the penicillin concentration in the body tissue by systemic treatment is low and the SA load at the infection site produces high concentrations of penicillinase to catalyze the antibiotic which conveys the resistance.

The concentration of penicillin and other anti-infectives in the cornea tissue have been assumed to be much greater than the concentration that can be delivered systemically, because of frequent topical dosing at the infection site. This is further supported by the fact that bacterial infection causes corneal ulceration and the removal of the corneal epithelium would allow even a higher concentration of penicillin. Our study proposes that the amount of penicillin that gets into the cornea can inundate penicillinase produced by SA allowing excess penicillin to bind to penicillin-binding proteins thus blocking cell wall formation. The near non-existent use of penicillin to treat SA keratitis probably supports the resurgence of lower penicillin MICs among SA over time. It would be reasonable to conclude that penicillinase is not automatically produced by SA in large volumes and the MIC is a function of the amount of penicillinase produced. Less penicillinase thus a lower MIC.

Our data also indicates that methicillin-resistant SA is not automatically resistant to penicillin. Penicillin resistance and methicillin resistance are rendered by two different mechanisms. As penicillin resistance is due to an enzymatic reaction, methicillin resistance is conferred with the acquisition of a nonnative gene that encodes for a penicillin-binding protein (pBp 2') that has a lower affinity for β -lactams. Penicillin would bind to all penicillin-binding proteins as long as copious amounts of β -lactamase is not present. In this study, penicillin was bactericidal to a methicillin-resistant SA that presented with an MIC of 16 $\mu\text{g/ml}$. Based on the bactericidal action produced in our keratitis model, it was reasonably concluded that a tentative susceptibility standard could be 16 $\mu\text{g/ml}$. This concentration would have covered 44% of the methicillin-resistant SA and 64% of the methicillin-susceptible SA from our *in vitro* studies (unpublished) demonstrating increased penicillin susceptibility among SA keratitis isolates.

Our rabbit keratitis model only measured the penetration and efficacy for 5 hours of topical treatment. The rabbit cornea is approximately 350 μm thick in comparison to the human cornea 540 μm , but we assume that frequent dosing would result in final comparable penicillin concentrations [18]. We may have observed a higher bactericidal effect for the SA with higher MICs with longer dosing over 5 hours. This would have resulted in a higher tentative susceptibility standard above 16 $\mu\text{g/ml}$.

The present study supports the "Proof of Principle" that topical penicillin can reach a high concentration into the cornea and overcome resistance that is reported by clinical laboratories using the standards that guide systemic treatment of bacterial infections. There will never be an approved topical standard for treating bacterial keratitis, and a tentative guide will at least provide support of clinical treatment success. Penicillin has lost favor as a topical antibiotic to treat SA keratitis because susceptibility is based on serum concentrations and not corneal concentrations, and the side-effect of severe allergic anaphylaxis. With sensible and careful use, penicillin could find a niche as a potent treatment of SA keratitis based on laboratory support and patient medical history.

Acknowledgements

This study was supported by the PA Lions Sight Conservation & Eye Research Foundation. The Charles T. Campbell Ophthalmic Microbiology Laboratory, Pittsburgh, PA, has received indirect support from: NIH grants P30-EY08098, the Eye and Ear Foundation of Pittsburgh, Pittsburgh, PA; and unrestricted funds from Research to Prevent Blindness Inc.

References

1. F Schaefer, O Bruttin, L Zografos, and Y Guex-Crosier (2001) Bacterial keratitis: a prospective clinical and microbiological study. *Br J Ophthalmol.* 85(7): 842-7.
2. Otri AM, Fares U, Al-Aqaba MA, Miri A, Faraj LA, et al. (2013) Profile of sight-threatening infectious keratitis: a prospective study. *Acta Ophthalmol* 91:643-51.
3. Alexandrakis G, Alfonso EC, Miller D (2000) Shifting trends in bacterial keratitis in south Florida and emerging resistance to fluoroquinolones. *Ophthalmology.* 107(8): 1497-502.

4. Theuretzbacher U, Van Bambeke F, Cantón R, Giske CG, Mouton JW., et al. (2015) Reviving old antibiotics. *J Antimicrob Chemother.* 70(8): 2177-81.
5. M Wainwright and H T Swan (1986) C.G. Paine and the earliest surviving clinical records of penicillin therapy. *Med Hist.* 30(1): 42-56.
6. Clinical and Laboratory Standards Institute (2013). Performance standards for antimicrobial susceptibility testing; Twenty-third informational supplement. Approved standard. Wayne, Pennsylvania. 2013, document M100-S23, 1 (33) 1.
7. Baum J, Barza M. (2000) The evolution of antibiotic therapy for bacterial conjunctivitis and keratitis: 1970-2000. *Cornea* 19: 659-72.
8. Kowalski RP, Romanowski EG, Mah FS, Shanks RMQ, Gordon YJ., et al. (2010) Topical Levofloxacin 1.5% (IQUIX) overcomes in vitro resistance in rabbit keratitis models. *Acta Ophthalmologica.* 88: e120-5.
9. Romanowski EG, Mah FS, Yates KA, Kowalski RP, Gordon YJ., et al (2005) The successful treatment of gatifloxacin-resistant *Staphylococcus aureus* keratitis with Zymar (gatifloxacin 0.3%) in a NZW rabbit model. *Am J Ophthalmol.* 139(5): 867-77.
10. Nissen JL, Skov R, Knudsen JD, et al. (2013) Effectiveness of penicillin, dicloxacillin and cefuroxime for penicillin-susceptible *Staphylococcus aureus* bacteraemia: a retrospective, propensity-score-adjusted case-control and cohort analysis. *J Antimicrob Chemother* 68:1894-900.
11. Crane JK. (2014) Resurgence of penicillin-susceptible *Staphylococcus aureus* at a hospital in New York State, USA. *J Antimicrob Chemother* 69(1):280-1.
12. Chabot M, Larioza J, Friderici J, Stefan M, Schimmel J., (2014) Resurgence of penicillin-susceptible *Staphylococcus aureus* in a tertiary care hospital. *ECCMID Barcelona, Spain.*
13. Kowalski RP, Romanowski EG, Yates KA, Gordon YJ. (2001) Lomefloxacin is an effective treatment for experimental bacterial keratitis. *Cornea* 20: 306-8.
14. Romanowski EG, Mah FS, Kowalski RP, Yates KA, Gordon YJ., (2008) Benzalkonium chloride enhances the antibacterial efficacy of gatifloxacin in an experimental rabbit keratitis model. *J Ocul Pharmacol Ther* 24: 380-4.
15. Romanowski EG, Kowalski TA, O'Connor KE, Yates KA, Mah FS, et al. (2016) The in vitro and in vivo evaluation of tigecycline as an ocular antibiotic. *J Ocul Pharmacol Ther* 32(2): 119-26.
16. Kowalski RP, Romanowski EG, Romanowski, Yates KA, Mah FS. (2016) An Independent Evaluation of a Novel Peptide Mimetic, Brilacidin (PMX30063), for Ocular Anti-Infective. *J Ocul Pharmacol Ther* 32(1): 23-7.
17. Physicians' Desk Reference for Ophthalmics Medicines. Edition 31, 2003. Table 6: Concentrations and Dosage of Principal Antibiotic Agents, page 6, published by Thomson Medical Economics, Montvale, NJ, USA.
18. Li HF, Petroll WM, Moller-Pedersen T, Maurer JK, Cavanagh HD, et al. (1997) Epithelial and corneal thickness measurements by in vivo confocal microscopy through focusing (CMTF). *Curr Eye Res* 16(3): 214-21.