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Report

Targeted Killing of Ocular *Streptococcus pneumoniae* by the Phage Endolysin MSlys

This study describes the translational potential of the pneumococcal endolysin MSlys as a novel approach to uniquely target and kill *Streptococcus pneumoniae* causing ocular infections.

S. pneumoniae is a common cause of ocular infections, including those that present as serious and sight-threatening conditions.[1] Empirical use of topical broad-spectrum antibiotics is the mainstream approach to treat and prevent these infections, a practice that is associated with disruption of the beneficial ocular microbiome and selection of antimicrobial resistances.[2] Because ocular bacteria are becoming increasingly resistant to antibiotics,[3] the efficacy of these approaches is gradually being compromised. Therefore, novel nonantibiotic alternative therapies that are less prone to select for resistance and do not disturb the healthy ocular microbiome are urgently needed. Phage endolysins are peptidoglycan hydrolases encoded by bacteriophages with rapid and specific narrow-spectrum antibacterial activity and low chances of resistance development, which can be used to precisely target the causative agent of an infection while preserving the surrounding microbial ecology. We explore the use of a pneumococcal phage endolysin named "MSlys"[4] to specifically target and kill *S. pneumoniae* lineages that are involved in ocular infections such as conjunctivitis, keratitis, endophthalmitis, dacryocystitis, and periocular cellulitis. The C-terminus of MSlys contains a choline-binding domain that uniquely recognizes and binds to choline residues present in the pneumococcal cell wall, whereas the catalytic domain (N-acetylmuramyol-L-alanine amidase) responsible for bacterial lysis is located in the N-terminus.[4] The amidase catalytic domain cleaves the amide bond between the muramic acid and the L-alanine in the peptidoglycan, leading to cell lysis and death.[4]

The antibacterial activity of MSlys was tested against ocular *S. pneumoniae* isolates (n = 31) molecularly characterized in our previous studies.[5,6] Protocols for obtaining discarded isolates were approved by the Mass General Brigham Institutional Review Board, and the study was conducted in accordance with the Declaration of Helsinki. MSlys was expressed and purified as previously described.[4] Reference strains *S. pneumoniae* R6 (sequence type [ST] 128, nontypeable), *S. pneumoniae* D39 (ST128, serotype 2), and *Staphylococcus aureus* American Type Culture Collection 29213 were used as controls. Frozen isolates were cultured on Trypticase Soy Agar with 5% sheep blood plates (BD Biosciences) and incubated at 37oC with 5% CO_2. Cells were grown overnight in 5 ml of Todd Hewitt Broth with 2% yeast extract, pelleted (5000*g*, 5 minutes, room temperature), and resuspended in phosphate-buffered saline (PBS). In a 96-well plate, MSlys (20 μl, final concentration of 2 μm ≈ 70 μg/ml, which was previously shown to significantly reduce the number of *S. pneumoniae* cells after 30 to 120 minutes)[4] or PBS (20 μl, negative control) was added to 180 μl of the bacterial suspensions and incubated at 37oC with 5% CO_2. After 30 minutes, the optical density at 620 nm (OD_{620}) was measured. Results were expressed as percentage reduction in OD_{620} in comparison with PBS control (Fig 1A).

MSlys was able to reduce the bacterial burden from 21% to 81% after only 30 minutes of incubation for a diverse collection of isolates (Fig 1A). Ocular isolates tested included several strains from the Epidemic Conjunctivitis Cluster, particularly ST448, which is known to cause the majority of conjunctivitis cases in the United States, and several other encapsulated and nonencapsulated strains isolated from various ocular infections. As expected, MSlys did not display any activity against *S. aureus* American Type Culture Collection 29213 used as a negative control.

To further confirm that MSlys is able to rapidly kill pneumococcal cells regardless of the presence of a polysaccharide capsule, a time-kill assay was performed against the nonencapsulated conjunctivitis strain 28/51 (ST448) and the encapsulated keratitis strain 81/79 (ST199, serotype 15B). Overnight grown cells were diluted 1:100 in fresh Todd Hewitt Broth with 2% yeast extract and allowed to grow until exponential phase. Cultures were 100-fold diluted in PBS and incubated at 37oC with 5% CO_2 for 30 minutes, 1 hour, or 2 hours with MSlys (final concentrations of 2 or 4 μm) or PBS (negative control).[4] Colony-forming units were quantified using the track dilution method.

MSlys killing activity was similar against both strains, happened as fast as 30 minutes after contact, and remained similar after further incubation for up to 2 hours (Fig 1B). After 2 hours, an average log reduction of 2.66 (99.78%) and 2.98 (99.90%) colony-forming units/ml was seen for the nonencapsulated strain 28/51 using 2 and 4 μm of MSlys, respectively. For the encapsulated strain 81/79, the logarithmic average reduction in the number of cells was 2.73 (99.81%) and 3.20 (99.94%) after 2 hours with 2 and 4 μm of MSlys, respectively. In a previous study, MSlys at 4 μm was shown to reduce the levels of the unencapsulated *S. pneumoniae* R6st strain by 3.5 log (colony-forming units/ml) or 99.97%.[4] Therefore, the presence of capsule does not appear to impact the lytic activity of the MSlys endolysin, which at a concentration of 4 μm resulted in a 2.9 or > 3 log reduction against both encapsulated and nonencapsulated strains after short exposures (up to 2 hours).

Although not assessed in this study, previous reports have shown that MSlys endolysin has strong activity against not only planktonic *S. pneumoniae* cells but also their biofilms,[4,7] a mode of growth commonly involved in the pathogenesis of ocular infections.[8] Furthermore, the absence of cytotoxicity of the endolysin against fibroblasts and keratinocytes was already demonstrated,[7] showing that MSlys is potentially safe for application in the eye.

With this short report, we aimed to demonstrate that the MSlys endolysin displays rapid killing activity against ocular *S. pneumoniae* strains regardless of the isolation source, genotypes, and encapsulation status, with great potential to translate

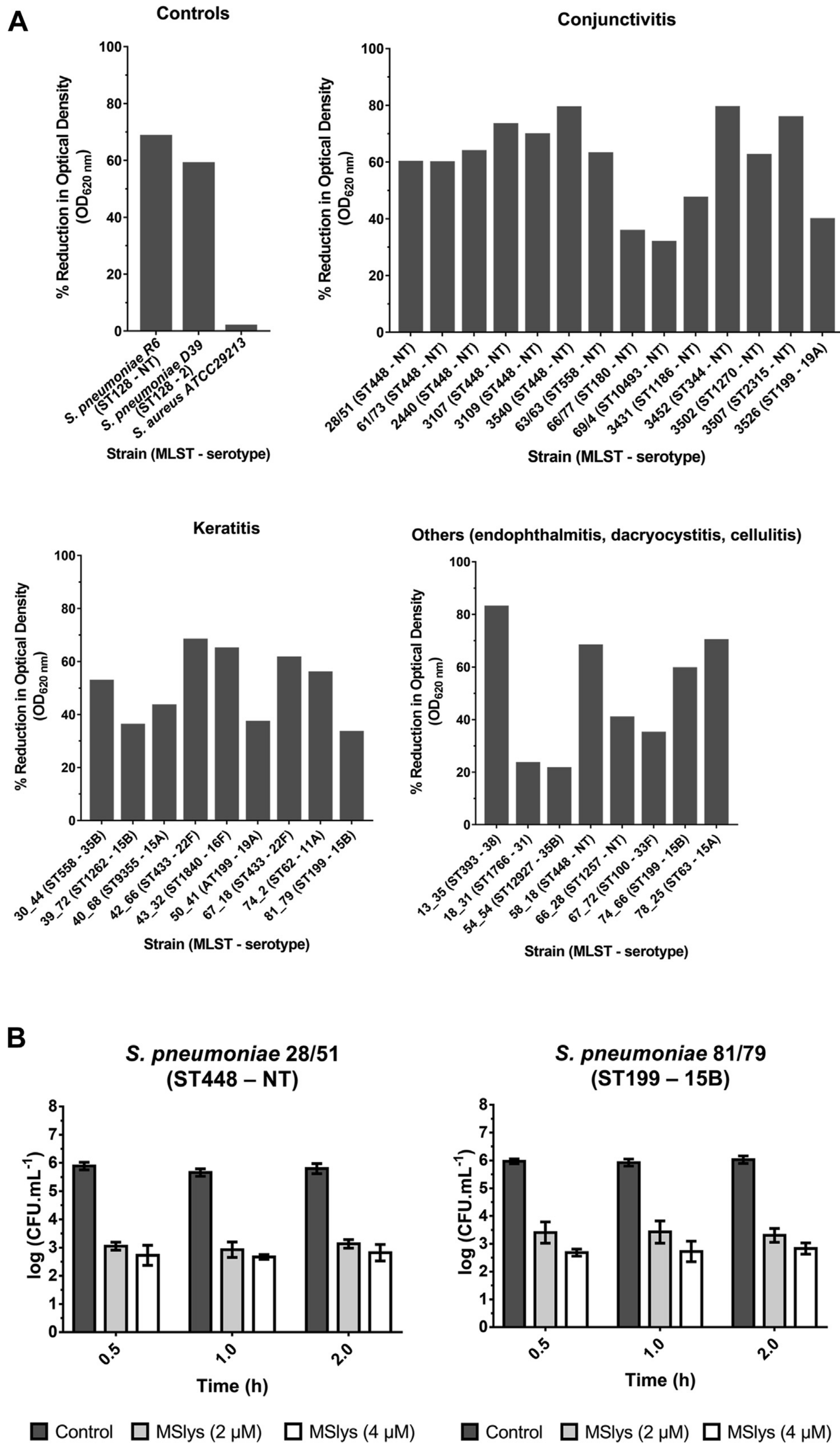

Figure 1. A, Percentage reduction in the optical density at 620 nm (OD_{620}) of bacterial suspensions after treatment for 30 minutes at 37°C with the MSlys endolysin (final concentration of 2 μm) in comparison with phosphate-buffered saline (PBS). **B,** Killing activity of MSlys against nonencapsulated *Streptococcus pneumoniae* strain 28/51 (sequence type [ST] 448) or encapsulated strain 81/79 (ST199, serotype 15B) after 0.5, 1, or 2 hours of treatment (2 or 4 μm) in comparison with control (PBS). MLST = multilocus sequence typing; NT = nontypable.

into improved precision treatments for ocular pneumococcal infections. The development of novel therapies based on narrow-spectrum phage lysins would support the transition from the current one-size-fits-all therapeutic approaches, which are not tailored to an individual's needs and do not work for everyone, to more precise and efficient treatments. These highly targeted therapies also have the added benefits of protecting the beneficial ocular surface microbiome and preventing the selection of resistances across many different commensal species that often occur after the use of broad-spectrum antibiotics. Further in vivo studies are necessary to evaluate the safety and efficacy of the MSlys endolysin as a potential novel topical agent to treat ocular pneumococcal infections.

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HUMAN SUBJECTS: No human subjects were used in the study. Protocols for obtaining discarded isolates were approved by the Mass General Brigham Institutional Review Board, and the study was conducted in accordance with the Declaration of Helsinki. The requirement for informed consent was waived because of the retrospective nature of the study.

No animal subjects were used in the study.

Author Contributions:
Conception and design: Silva, Bispo
Data collection: Silva, André
Analysis and interpretation: Silva, André, Bispo
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Abbreviations and Acronyms:
PBS = phosphate-buffered saline; **ST** = sequence type.

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