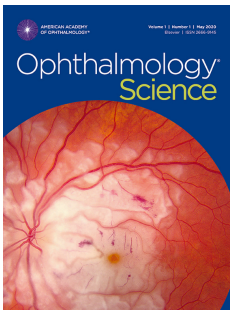


Targeted killing of ocular *Streptococcus pneumoniae* by the phage endolysin MSlys

Maria Daniela Silva, Camille André, Paulo J.M. Bispo

PII: S2666-9145(22)00082-3

DOI: https://doi.org/10.1016/j.xops.2022.100193

Reference: XOPS 100193

To appear in: *Ophthalmology Science*

Received Date: 11 April 2022

Revised Date: 9 June 2022

Accepted Date: 17 June 2022

Please cite this article as: Silva M.D., André C. & Bispo P.J.M., Targeted killing of ocular *Streptococcus pneumoniae* by the phage endolysin MSlys, *Ophthalmology Science* (2022), doi: https://doi.org/10.1016/j.xops.2022.100193.

Targeted killing of ocular *Streptococcus pneumoniae* by the phage endolysin MSlys

Maria Daniela Silva[1,2], Camille André[3], Paulo J. M. Bispo[3*]

[1]CEB−Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal;

[2]Department of Otolaryngology, Massachusetts Eye and Ear, Boston, Massachusetts, 02114

[3]Department of Ophthalmology, Massachusetts Eye and Ear, and Infectious Disease Institute, Harvard Medical School, Boston, Massachusetts, 02114

*corresponding author: paulo_bispo@meei.harvard.edu

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.

Streptococcus pneumoniae is a common cause of ocular infections including those that present as serious and sigh-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, the development of novel non-antibiotic alternative therapies that are less prone to select for resistance and does 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. Here, 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 bind to choline residues present in the pneumococcal cell wall, while 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, non-typeable (NT)), *S. pneumoniae* D39 (ST128, serotype 2) and *S. aureus* ATCC 29213 were used as controls. Frozen isolates were cultured on Trypticase Soy Agar with 5% sheep blood plates (BD Biosciences) and incubated at 37 ºC with 5% CO_2. Cells were grown overnight in 5 mL of Todd Hewitt Broth with 2% yeast extract (THB_{ye}), pelleted (5000 xg, 5 min, room temperature) and resuspended in 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 min) [4] or PBS (20 µL, negative control) were added to 180 µL of the bacterial suspensions and incubated at 37 ºC 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 (Figure 1A).

MSlys was able to reduce the bacterial burden from 21% to 81% following only 30 minutes of incubation for a diverse collection of isolates (Figure 1A). Ocular isolates tested included several strains from the Epidemic Conjunctivitis Cluster (ECC), particularly ST448 that is known to cause the majority of conjunctivitis cases in US, and several other encapsulated and non-encapsulated strains isolated from various ocular infections. As expected, MSlys did not display any activity against *Staphylococcus aureus* ATCC 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 non-encapsulated conjunctivitis strain 28/51 (ST448) and the encapsulated keratitis strain 81/79 (ST199, serotype 15B). Overnight grown cells were diluted 1:100 in fresh THBye

and allowed to grow until exponential phase. Cultures were 100-fold diluted in PBS and incubated at 37 ºC 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 (CFUs) were quantified using the track dilution method.

MSlys killing activity was similar against both strains, happened as fast as 30 minutes following contact and remained similar after further incubation for up to 2 hours (Figure 1B). After 2 hours, an average log reduction of 2.66 (99.78%) and 2.98 (99.90%) CFU/mL was seen for the non-encapsulated 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 of 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(CFU/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 non-encapsulated strains after short exposures (up to 2 h).

Although not assessed in this study, previous reports have shown that MSlys endolysin has strong activity not only against planktonic *S. pneumoniae* cells but also against 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 also already demonstrated[7], showing that MSlys is potentially safe for application in the eye.

In conclusion, with this short report we aimed to demonstrate that the MSlys endolysin display rapid killing activity against ocular *S. pneumoniae* strains regardless of the isolation source, genotypes, and encapsulation status, with great potential to translate 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 that are not tailored to an individual's needs and do not work for everyone, to more precise and efficient treatments. These highly targeted therapies have also the added benefits of protecting the beneficial ocular surface microbiome and preventing the selection of resistances across many different commensal species that often occur following 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.

Funding

This study was partially supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469/2020 unit. MDS was supported from a FCT doctoral fellowship, reference SFRH/BD/128825/2017. CA was supported by a scholarship from Fondation pour la Recherche Médicale (FDM202006011203). This work was also supported in part by the New England Corneal Transplant Research Fund (PJMB). Funding agencies had no role in study design, data analysis, decision to publish or preparation of the manuscript.

References

1. Teweldemedhin M, Gebreyesus H, Atsbaha AH, Asgedom SW, Saravanan M. Bacterial profile of ocular infections: A systematic review. *BMC Ophthalmol*. 2017;17(1):1-9. doi:10.1186/s12886-017-0612-2
2. Ung L, Chodosh J. Foundational concepts in the biology of bacterial keratitis. *Exp Eye Res*. 2021;209:108647. doi:10.1016/j.exer.2021.108647

3. Bispo PJM, Sahm DF, Asbell PA. A Systematic Review of Multi-decade Antibiotic Resistance Data for Ocular Bacterial Pathogens in the United States. *Ophthalmol Ther*. Published online 2022. doi:10.1007/s40123-021-00449-9

4. Silva MD, Oliveira H, Faustino A, Sillankorva S. Characterization of MSlys, the endolysin of Streptococcus pneumoniae phage MS1. *Biotechnol Reports*. 2020;28:e00547. doi:10.1016/j.btre.2020.e00547

5. Andre C, Rouhana J, Scarpa de Mello S, et al. Population structure of ocular Streptococcus pneumoniae is highly diverse and formed by lineages that escape current vaccines. *Microb Genomics*. 2022;8(3). doi:10.1099/mgen.0.000763

6. Valentino MD, McGuire AM, Rosch JW, et al. Unencapsulated Streptococcus pneumoniae from conjunctivitis encode variant traits and belong to a distinct phylogenetic cluster. *Nat Commun*. 2014;5(1):5411. doi:10.1038/ncomms6411

7. Silva MD, Paris JL, Gama FM, Silva BFB, Sillankorva S. Sustained Release of a Streptococcus pneumoniae Endolysin from Liposomes for Potential Otitis Media Treatment. *ACS Infect Dis*. 2021;7(8):2127-2137. doi:10.1021/acsinfecdis.1c00108

8. Bispo P, Haas W, Gilmore M. Biofilms in Infections of the Eye. *Pathogens*. 2015;4(1):111-136. doi:10.3390/pathogens4010111

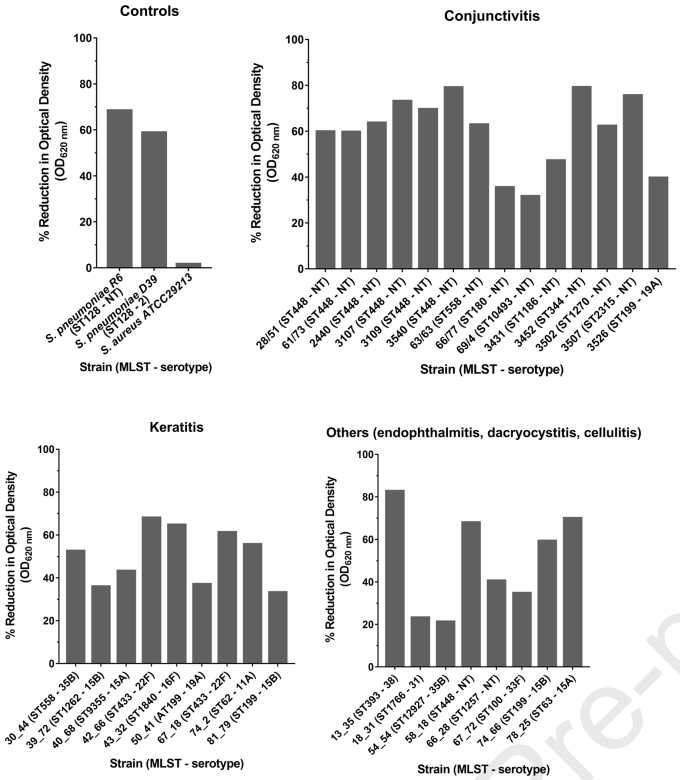
Figure Legend

Figure 1. A) Percentage (%) reduction in the optical density at 620 nm (OD_{620}) of bacterial suspensions after treatment for 30 min at 37 °C with the MSlys endolysin (final concentration of 2 μM) in comparison with PBS. **B)** Killing activity of MSlys against non-encapsulated *S. pneumoniae* strain 28/51 (ST448) or encapsulated strain 81/79

136 (ST199, serotype 15B) after 0.5, 1 or 2 hours of treatment (2 or 4 μM) in comparison with

137 control (PBS). NT, non-typable.

A)



B)