1   **Predatory Bacteria can Reduce *Pseudomonas aeruginosa* Induced Corneal**

2   **Perforation and Proliferation in a Rabbit Keratitis Model**

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7   Short title. Predatory bacteria versus *P. aeruginosa* in the cornea

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21   keratitis, predatory bacteria, *Pseudomonas aeruginosa*

22

Abstract

Purpose: *Pseudomonas aeruginosa* keratitis is a severe ocular infection that can lead to perforation of the cornea. In this study we evaluated the role of bacterial quorum sensing in generating corneal perforation and bacterial proliferation and tested whether co-injection of the predatory bacteria *Bdellovibrio bacteriovorus* could alter the clinical outcome. *P. aeruginosa* with *lasR* mutations were observed among keratitis isolates from a study collecting samples from India, so an isogenic *lasR* mutant strain of *P. aeruginosa* was included.

Methods: Rabbit corneas were intracorneally infected with *P. aeruginosa* strain PA14 or an isogenic ΔlasR mutant and co-injected with PBS or *B. bacteriovorus*. After 24 h, eyes were evaluated for clinical signs of infection. Samples were analyzed by scanning electron microscopy, optical coherence tomography, sectioned for histology, and corneas were homogenized for CFU enumeration and for inflammatory cytokines.

Results: We observed that 54% of corneas infected by wild-type PA14 presented with a corneal perforation (n=24), whereas only 4% of PA14 infected corneas that were co-infected with *B. bacteriovorus* perforate (n=25). Wild-type *P. aeruginosa* proliferation was reduced 7-fold in the predatory bacteria treated eyes. The ΔlasR mutant was less able to proliferate compared to the wild-type, but was largely unaffected by *B. bacteriovorus*.

Conclusion: These studies indicate a role for bacterial quorum sensing in the ability of *P. aeruginosa* to proliferate and cause perforation of the rabbit cornea. Additionally, this study suggests that predatory bacteria can reduce the virulence of *P. aeruginosa* in an ocular prophylaxis model.

1. Introduction

Predatory bacteria, such as *Bdellovibrio bacteriovorus*, have been suggested as an alternative approach for treatment antibiotic resistant bacterial infections in general [1-6]. *B. bacteriovorus* is a Gram-negative bacterium that is an obligate predator of other Gram-negative bacteria. Given the preponderance of Gram-negative bacteria in causing contact-lens related keratitis, predatory bacteria have been hypothesized as treatments for eye infections [7-9], and are largely non-toxic and non-inflammatory when applied in great numbers to a corneal epithelial cell line and intact and wounded ocular surfaces [7, 10]. These have been tested to prevent bacterial proliferation on the ocular surface with some success and to prevent the development of keratitis in a mouse model using *Escherichia coli* as a pathogen [11, 12].

Unlike *E. coli*, *Pseudomonas aeruginosa* is the leading cause of contact lens associated keratitis, a blinding infection [13-15]. Antibiotic resistant *P. aeruginosa* keratitis isolates are associated with worse clinical outcomes [16-19]. Previous work showed that *B. bacteriovorus* predation is not influenced by antibiotic resistance status of its prey [3, 5, 7] and is even effective at clearing antibiotic tolerant biofilms [20, 21]. A recent study showed that around 20% of *P. aeruginosa* keratitis isolates isolated in India from 2006-2010 had mutations in the *lasR* gene and that these strains were correlated with worse visual outcomes in patients [22]. The LasR protein is a major regulator of quorum sensing and important in regulation of numerous virulence-associated genes [23].

In this study we evaluated whether *B. bacteriovorus* strain HD100 could act as a "living antibiotic" in a rabbit keratitis infection model using a clade 2 (cytotoxic/ExoU⁺) and highly virulent *P. aeruginosa* strain. This was a proof-of-concept prevention model rather than a treatment model. The study found that the predatory bacteria caused mild-

71 moderate inflammation, but were able to reduce *P. aeruginosa* proliferation and most

72 importantly significantly reduced the frequency of corneal perforation events caused by

73 *P. aeruginosa*. We also observed that an isogenic *P. aeruginosa* Δ*lasR* mutant was

74 unable to replicate in the rabbit cornea and caused significantly fewer corneal

75 perforations, but was not significantly reduced by *B. bacteriovorus* in the cornea.

76

77 **2. Methods**

78 **2.1. Bacterial strains and culture**

79 *B. bacteriovorus* HD100 (ATCC 15356) [24] was used for this study. Preparation of *B.*

80 *bacteriovorus* followed prior reports [10, 12] where *B. bacteriovorus* was incubated with

81 ~10^9 CFU/ml *E. coli* diaminopimelic acid auxotroph strain WM3064 for 24 h at 30°C. The

82 resulting mixture was passed multiple times through a 0.45-μm Millex®-HV pore-size

83 filter (Millipore, Billerica, MA, USA) to remove remaining prey. Predators were washed

84 with phosphate buffered saline (PBS) and concentrated centrifugation. *B. bacteriovorus*

85 was suspended in PBS to 1.5×10^{10} PFU/ml *B. bacteriovorus*.

86   For a pathogen, the cytotoxic (ExoU+) / clade II strain UCBPP-PA14 (PA14) [25-

87 28] and an isogenic Δ*lasR* mutant were used [29]. These were inoculated from single

88 colonies into LB medium and grown with aeration at 30˚C or 37˚C. For inoculation, *P.*

89 *aeruginosa* were diluted in phosphate buffered saline (PBS) to achieve the inoculum

90 (~2-4x10^3 CFU in 10 μl).

91

92 **2.2. Bacterial keratitis studies**

93 The experiments in this study conformed to the ARVO Statement on the Use of Animals

94 in Ophthalmic and Vision Research and were approved by the University of Pittsburgh's

95 Institutional Animal Care and Use Committee (IACUC Protocols 15025331 and

96 18022194).

97   Female New Zealand White rabbits (1.1-1.4 kg) were obtained from the

98   Oakwood Research Facility through Charles River Laboratories. The rabbits were

99   anesthetized with 40 mg/kg of ketamine and 4 mg/kg of xylazine administered

100   intramuscularly. The corneas of the right eyes only were anesthetized with topical 0.5%

101   proparacaine and injected intrastromally with the 10 µl of *P. aeruginosa* (~2000 CFU) in

102   PBS or PBS alone as a control. Actual inocula for each trial was determined using the

103   EddyJet 2 spiral plating system (Neutec Group Inc., Farmingdale, NY) on 5% trypticase

104   soy agar with 5% sheep's blood plates. Plates were incubated for ~18-20 h at 37°C and

105   the colonies were enumerated (Flash and Grow colony counting system, Neutec Group,

106   Inc). This was followed by a 25 µl injection of *B. bacteriovorus* in PBS (~4×10^{8}) or PBS.

107   At 24 h post-injection, the eyes were evaluated for ocular signs of inflammation

108   using a slit-lamp according to a modified McDonald-Shadduck grading system [30].

109   Rabbits were systemically anesthetized with ketamine and xylazine as described above

110   and euthanized with Euthasol solution following the 2020 AVMA Euthanasia Guidelines.

111   Corneal buttons were harvested using a 10 mm trephine and placed into Lysing

112   Matrix A tubes (MP Biomedicals) containing 1 ml of PBS. The corneas were then

113   homogenized with an MP Fast Prep-24 homogenizer (MP Biomedicals), and the

114   numbers of corneal bacteria were enumerated as described above. The centrifuged and

115   filtered homogenate (Millipore 0.22µm PVDF filter) was frozen for ELISA analysis (IL-1β

116   kit (Sigma-Aldrich RAB1108). MMP9 ELISA) following the manufacturer's guidelines.

117   Additional corneas were fixed with paraformaldehyde (4%) in PBS, embedded in

118   paraffin, and 5 µm sections were stained with hematoxylin and eosin. Sections were

119   viewed using an Olympus Provis AX-70 microscope and images captured with

120   MagnaFire 2.1 software.

121   In another set of animals, the whole eyes were removed following euthanasia

122   and stored individually in 25 mm of PBS and kept on ice. Within one hour, their corneas

5

123   were scanned by optical coherence tomography (OCT) using a Bioptigen Envisu R2210

124   system with InVivoVue software (Leica Microsystems) following the same general

125   approach described elsewhere [31]. Briefly, the system was modified with a broadband

126   superluminescent diode (Superlum, Dublin, Ireland λ = 870 nm, $\Delta\lambda$ = 200 nm, 20,000 A-

127   scans/second). OCT volume scans of the central cornea were acquired using a 10mm

128   telecentric lens for anterior segment imaging and a scan pattern: 6mm x 6mm x 2mm

129   (1000 x 100 x 1024 pixel sampling), with 1 Frame per B-scans, i.e. no repetitions. At

130   least three scans were obtained of each sample and the best quality scan chosen for

131   analysis. The most common issue was a high signal at the cornea apex that reduced

132   visibility directly underneath. Scans of a given eye were completed in less than six

133   minutes. The B-scans were loaded into Fiji for visualization as image volumes and to

134   export stills and movies [32].

135

136   **2.3. Scanning Electron Microscopy**

137   Electron microscopy was performed as previously described [12]. Rabbit corneal buttons

138   were fixed with glutaraldehyde (3%) at room temperature for 24h and washed with PBS.

139   Samples were pinned to prevent curling and post-fixed using osmium tetroxide (1%),

140   dehydrated with ethanol (30–100%), immersed in hexamethyldisilazane and air-dried.

141   Samples were mounted to aluminum stubs and sputter coated with gold/palladium (6

142   nm). Scanning electron microscopy was performed using a JEOL JSM-6335F scanning

143   electron microscope (3 kV).

144

145   **2.4. In vitro predation assay**

146   *P. aeruginosa* were grown at 37˚C in LB broth on a tissue culture rotor, centrifuged and

147   suspended in HEPES buffer (25 mM HEPES with 3 mM $MgCl_2$ and 2 mM $CaCl_2$).

148   Aliquots of *P. aeruginosa* (0.5 ml, ~1×10^9 CFU) and *B. bacteriovorus* (0.5 ml, ~2×10^8

149 PFU) and 4 ml of HEPES buffer were added to 15 ml conical polypropylene tubes and

150 incubated at 30°C. At 24 h bacteria were serial diluted and plated on LB agar plates to

151 determine surviving *P. aeruginosa*. Log reductions were calculated as compared to a

152 predator free control survival at the same time point.

153

154 **2.5. Statistical analysis**

155 Kruskal-Wallis with Dunn's post-test was used for non-parametric analysis and ANOVA

156 with Tukey's post-test or Student's T-tests for parametric analysis. Contingency analysis

157 was performed with Chi-Square test followed by Fisher's Exact test for pair-wise

158 comparisons. All analyses were done with GraphPad Prism software.

159

160 **3. Results**

161 **3.1. Evaluation of the antimicrobial efficacy of *B. bacteriovorus* in a *P. aeruginosa***

162 **keratitis model.**

163 The ability of the predatory bacteria *B. bacteriovorus* strain HD100 to prevent *P.*

164 *aeruginosa* proliferation in a bacterial keratitis model was evaluated. *P. aeruginosa* was

165 used because it is a major cause of corneal infection. In this study either 10 µl of *P.*

166 *aeruginosa* (~2000 CFU) or the PBS vehicle was injected into the corneal stroma (Figure

167 1A), followed by a second injection of 25 µl of either *B. bacteriovorus* strain HD100

168 ($4x10^8$ PFU) or the PBS vehicle into the same needle track.

169 The virulent *P. aeruginosa* strain UCBPP-PA14 (PA14) [25-28] was injected into

170 the cornea at an average of 1,777±353 CFU/cornea. This strain is a clade 2 strain, also

171 known as a cytotoxic strain, that is ExoU positive and ExoS negative [28]. This strain has

172 not been previously reported in a rabbit keratitis model to our knowledge. PA14 was able

173 to replicate 40-fold to $7.25x10^4$ CFU/cornea by 24 hours (Figure 1B). The eyes were

174 evaluated with a slit lamp and fluorescein staining using a MacDonald-Shadduck-based

175 scoring system [30] and there was a clear increase in corneal inflammation score by 24

176 h (Figure 1C-D, 2). This led to corneal perforation in just over half of the infected corneas

177 (54%) (Figure 1E, 2).

178 By contrast, *B. bacteriovorus* caused intermediate inflammation compared to

179 PBS and PA14 groups (Figure 1C-E, 2). Moreover, *B. bacteriovorus* injection did not

180 cause corneal perforations (Figure 1D, 2).

181 *B. bacteriovorus* injected following *P. aeruginosa* correlated with a significant

182 reduction in *P. aeruginosa* proliferation (1.03×10^4 CFU at 24h, p<0.01) but was still

183 higher than the inoculum. This reduction did not significantly influence the overall corneal

184 score, but was associated with a remarkable reduction in the frequency of corneal

185 perforations (4%, p<0.001) (Figure 1D).

186 Fluorescein staining and slit lamp examination 24 h post-inoculation revealed

187 minor pathology of the corneas infected with *B. bacteriovorus* that consisted of redness

188 and a small infiltrate that did not cause a fluorescein stained ulcer (Fig 2). By contrast,

189 the *P. aeruginosa* injected eyes showed major purulent discharge from the large central

190 corneal ulcer and anterior chamber effects (Figure 2). Eyes injected with both had an

191 intermediate phenotype similar to those injected with both the Δ*lasR P. aeruginosa* and

192 predatory bacteria. The eyes injected with just the Δ*lasR* mutant bacteria had minimal

193 inflammation, though had a corneal ulcer of moderate size (Figure 2).

194 Optical coherence tomography (OCT) was done on a subset of eyes following

195 sacrifice of the rabbits at 24h post-inject (Figure 3). This reveals the shape of the cornea

196 and unlike the PBS only injected eye, the *B. bacteriovorus* (HD100) injected eyes had

197 clear inflammation including an infiltrate and overall edema. The loss of the corneal

198 integrity was demonstrated in the *P. aeruginosa* PA14 wild-type injected eyes where the

199 massive infiltrate prevented unobstructed corneal imaging and resulted in shadows. By

200 contrast, the *P. aeruginosa* wild-type and HD100 coinjected eyes had clear damage,

8

201 ulceration, and infiltrate, but maintained overall shape. The ΔlasR mutant with or without

202 HD100 had intermediate phenotypes. Movies depicting OCT volumes of corneal layers

203 are provided in the supplemental information and clearly demonstrate the perforation of

204 the *P. aeruginosa* strain PA14 infected eye (Supplemental movies S1-3).

205   To evaluate whether infiltrates were made by neutrophils, H&E staining of

206 corneas was performed. The histology provided consistent results with the fluorescein

207 staining with respect to maintenance or loss of corneal epithelium (Figure 4). Neutrophils

208 and severe damage of the anterior stroma were observed in corneas infected by PA14

209 and to a lesser extent by *B. bacteriovorus* (Figure 4 and Figure S1).

210   Low magnification scanning electron microscopy showed the perforation in the

211 PA14 infected cornea that was absent in the other corneas (Figure 5). Similar to

212 fluorescein results (Figure 2), a clear ulcer was evident in corneas infected with PA14

213 (PA14 + PBS) and *B. bacteriovorus* (PBS + HD100) (Figure 5). At higher power, red and

214 white blood cells were observed on the ocular surface of most eyes, especially those

215 infected with PA14 (Figure 5).

216   Consistent with the corneal inflammation MMP9 (92-kDa neutrophil gelatinase or

217 gelatinase B), an enzyme that digests gelatin and collagen types IV and V [33], and the

218 pro-inflammatory cytokine interleukin-1β (IL-1β) were highly induced in corneas infected

219 with PA14, and to a lesser extend by *B. bacteriovorus* (Figure 6). The co-infection of

220 PA14 and *B. bacteriovorus* did not significantly alter these pro-inflammatory markers.

221 However, *B. bacteriovorus* by itself was less inflammatory than PA14 for both MMP9 and

222 IL-1β ($p < 0.05$).

223

224 **3.2. LasR promotes corneal pathogenesis by a cytotoxic *P. aeruginosa* strain**.

225 A recent study showed natural *lasR* mutants at high frequency among bacterial keratitis

226 isolates [22]. Therefore, the above experiments were also carried out with an isogenic

9

227   variant of PA14 with a deletion of the *lasR* quorum sensing regulator. This strain was

228   previously validated by restoring the wild-type allele and is defective in expression of a

229   number of potential virulence associated phenotypes such as protease production [22].

230   Though slightly more Δ*lasR* mutant bacteria were inoculated compared to the WT

231   (3,805 ± 1703 CFU/cornea, p=0.03), the CFU per cornea at 24 h was significantly lower

232   at 5.9×10^3 CFU/cornea which is 12-fold lower than wild-type PA14, p<0.01 (Figure 1B).

233   Corneal inflammation scores were also lower, but did not reach significance (p>0.05),

234   (Figure 1C). Nevertheless, the Δ*lasR* mutant was defective in causing corneal

235   perforation compared to the WT (Figure 1D, p=0.002). Histology and SEM analysis

236   demonstrate reduced corneal damage (Figure 2-4), yet the MMP9 and IL-1β levels were

237   similar to the WT (Figure 6). Both the wild-type PA14 and Δ*lasR* mutant was susceptible

238   to *B. bacteriovorus in vitro*. The mean Log_{10} reduction *in vitro* was 4.9±0.05 CFU/ml for

239   PA14 and 4.7±0.15 CFU/ml for Δ*lasR*, p>0.05, n=3. However, the predatory bacteria did

240   not significantly lower the corneal burden. While corneal scores were high, the frequency

241   of perforation was low (0%). However, MMP9 and IL-1β were levels in Δ*lasR* and *B.*

242   *bacteriovorus* inoculate corneas was indistinguishable from PA14 wild-type infected

243   eyes.

244

245   **4. Discussion**

246   This study demonstrated the severe pathogenesis caused by strain PA14 in a rabbit

247   corneal infection model. 54% of corneas perforated by 24 h limiting the length of the

248   study for ethical reasons. While *B. bacteriovorus* on their own caused some

249   inflammation, they prevented *P. aeruginosa* proliferation and corneal perforation.

250   In this study, MMP9 was strongly induced by *P. aeruginosa*. MMP9 is a

251   metalloproteinase secreted by neutrophils, macrophages, and corneal epithelial cells

252   [33]. Transcription of the MMP9 gene was strongly induced when corneal cells are

253   exposed to bacteria [34, 35]. MMP9 is involved in remodeling of the corneal basement

254   membrane and regulates corneal healing, but has also been linked with corneal

255   perforation [36, 37]. Tetracyclines inhibit MMP9 activity, and that is one reason that

256   tetracyclines have been given systemically to patients with corneal ulcers or perforations

257   [38]. In animal models tetracyclines promoted wound healing and prevented corneal

258   perforation [39-41]. Our results suggest that MMP9 may contribute to, but is not fully

259   responsible for corneal perforation caused by *P. aeruginosa* as MMP9 levels remained

260   in infected corneas that did not perforate. Other factors such as neutrophil elastase,

261   MMP8, or bacterial proteases such as elastase B, which is positively regulated by LasR

262   may be key contributors to corneal perforation in our model [42, 43].

263   IL-1 is a major proinflammatory cytokine during bacterial keratitis [44] and

264   promotes neutrophil mediated corneal damage in mouse models of *P. aeruginosa*

265   keratitis [45, 46]. Although a role for IL-1 in preventing corneal perforation in young

266   Swiss mice has also been described [45]. An *in vitro* study showed that keratocytes

267   produced collagenolytic compounds in an IL-1 dependent manner and that collagen

268   degradation could be prevented with an IL-1 inhibitor [47]. IL-1 release from damaged

269   corneal epithelial cells acts as an alarmone and induces increased expression of IL-6

270   and -8 and reduced barrier function in corneal epithelial cells [48]. Similarly, anti-IL-1β

271   antibody pretreatment reduced MMP9 induction in a mouse (C57Black/6) *P. aeruginosa*

272   keratitis model leading to the overall model that *P. aeruginosa* induced IL-1 induces the

273   expression of MMPs resulting in the destruction of corneal tissue [44].

274   In our study, predatory bacteria failed to induce IL-1β production, while this is an

275   unusual for a Gram-negative bacteria in the corneal stroma, *B. bacteriovorus* have

276   unusual physiology that may reduce its ability to activate the immune system. Namely, it

277   has a modified lipopolysaccharide structure that does not activate TLR4 and a

278   membrane sheathed flagellum, so is unlikely to activate TLR5-mediated inflammation

11

279   [49, 50]. By contrast, all other infection groups strongly induced IL-1β that correlated with

280   high levels of MMP9. The intermediate levels of MMP9 induced by *B. bacteriovorus*

281   suggest that there are IL-1-independent mechanisms to induce MMP9. However, only

282   one time point was evaluated, so IL-1 may have been induced at early time point by *B.*

283   *bacteriovorus*. That said, IL-1 induction by bacterial keratitis was reported to be

284   maintained over the course of several days in a mouse model weakening the likelihood

285   that IL-1 levels were higher at earlier time points [45]. Although the levels of MMP9

286   measured in the predator group was significantly lower than that measured in the *P.*

287   *aeruginosa* infected arm*,* one should consider that the concentration of the predator

288   injected was 5 logs higher (~$4x10^8$ vs $2x10^3$ respectively). The low inflammatory

289   stimulating effect of *B. bacteriovorus* seen in this study is in agreement with both *in vitro*

290   [7, 51, 52] and *in vivo* studies that show that pro inflammatory cytokines, including IL-1,

291   are not significantly elevated after exposure to the predator [10, 53-56].

292   The role of the LasR quorum sensing marker in *P. aeruginosa* keratitis has

293   previously been characterized in mouse models using a clade I/invasive/ExoS+ strain

294   PA01 and similar, but not isogenic *lasR* mutant [57]. Prior work has evaluated the

295   importance of LasR in *P. aeruginosa* keratitis. These studies both used PAO1, an ExoS+

296   / invasive / clade I strain and a randomly mutated variant of PAO1 that was selected for

297   streptomycin resistance, PAO1s with the *lasR* gene replaced by a tetracycline resistance

298   cassette. The first study demonstrated that in a murine scratch model, 33-fewer *lasR*

299   mutant bacteria were necessary to establish infections in C3H/HeN mice [58]. The

300   second study used the same bacterial strains with a murine scratch keratitis model using

301   BALB/c mice and did not find a difference in the ability of the two bacteria to cause

302   keratitis, however the *lasR* mutant was not as successful in proliferation with ~10-fold

303   lower CFU/cornea being measured [59]. Our study, by contrast, used a ExoU+ /

304   cytotoxic / clade II strain in a rabbit intrastromal injection model. While both the scratch

12

305   and intrastromal injection models have important limitations, the intrastromal injection

306   model ensures highly similar inoculation numbers, which are important for a treatment

307   study, especially when the *lasR* mutant and wild-type express adhesins at different

308   levels [22]. The results of this study suggest that once past the epithelial barrier the *lasR*

309   mutant is less able to proliferate and this may account for the highly reduced frequency

310   of perforations, additionally, the lower expression of collagen digesting proteases such

311   as elastase B by the *lasR* mutant may also contribute to the highly reduced frequency of

312   corneal perforations [57, 59]. More work is needed to understand the complex role of

313   LasR regulated quorum sensing in *P. aeruginosa* keratitis, nevertheless, this study

314   supports the model that in at least one cytotoxic strain, LasR is required for full levels of

315   pathogenesis.

316

317   This proof-of-concept study supports that predatory bacteria can prevent bacterial

318   proliferation and prevent corneal perforation in a keratitis model, however, further study

319   to test efficacy against established infections by multiple types of *P. aeruginosa* clinical

320   isolates are necessary to move the concept of using predatory bacteria as a therapeutic

321   forward.

322

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338   **6. Disclosure/Conflict of Interest Statement**

339   The authors have no commercial interests regarding this study.

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341   **7. References**

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507

508 **8. Figure Legends**

509 **Figure 1. Predatory bacteria and *lasR* mutation reduce *P. aeruginosa* pathogenesis in
510 a rabbit keratitis model. A.** Diagram of model in which the rabbit corneal stroma (arrow) is
511 injected with *P. aeruginosa* strain PA14, isogenic Δ*lasR* mutant, or PBS followed by PBS or
512 *B. bacteriovorus* strain HD100. **B-D**. Each point represents a rabbit. **a** indicates significant
513 difference from control group, p<0.05. n.s. indicates not significantly different than the control
514 group. **, p<0.01; ***, p<0.001.**B**. CFU from corneas at 24 h post-injection. Means and SDs

17

515   are shown. **C.** Corneal inflammation score based on MacDonnald-Shadduck scoring

516   system. Medians and interquartile ranges are shown and Kruskal-Wallis analysis with

517   Dunn's post-test was performed. **D.** Corneal perforation frequency. Indicated groups were

518   compared by Fisher's Exact test.

519

520   **Figure 2. Bright-field microscopy and fluorescein staining of infected eyes.**

521   Representative images are shown. Fluorescein staining indicates corneal ulceration and

522   loss of corneal epithelium.

523

524   **Figure 3. Optical coherence tomography of infected eyes.** Representative images are

525   shown. Shadows are due to infiltrates that prevent imaging.

526

527   **Figure 4. Histological analysis demonstrates neutrophil infiltrates.** Representative

528   images are shown. Magnification was 10X. Polymorphonuclear neutrophils are evident from

529   inside the stroma and in at the anterior chamber interface.

530

531   **Figure 5. Scanning micrographs of rabbit corneal surfaces.** Representative images are

532   shown. Magnification was 25x (top) and ε500x (bottom). The corneal perforation is clearly

533   shown in the PA14 + PBS injected cornea, and a corneal ulcer was evident in the PA14 with

534   HD100 injected cornea.

535

536   **Figure 6. *P. aeruginosa* strain PA14 stimulation of gelatinase B (MMP9) and pro-**

537   **inflammatory cytokine IL-1® from rabbit corneas.** Medians and interquartile ranges of

538   pro-inflammatory cytokines are shown. Each point represents one rabbit. Kruskal-Wallis with

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539   Dunn's post-test was performed, **a** indicates significant difference from control group,

540      $p < 0.05$. n.s. indicates not significantly different than the control group. *, $p < 0.05$; **, $p < 0.01$.
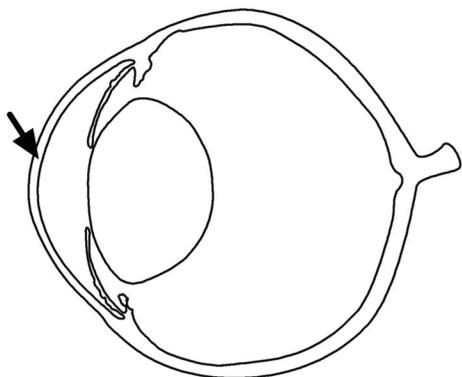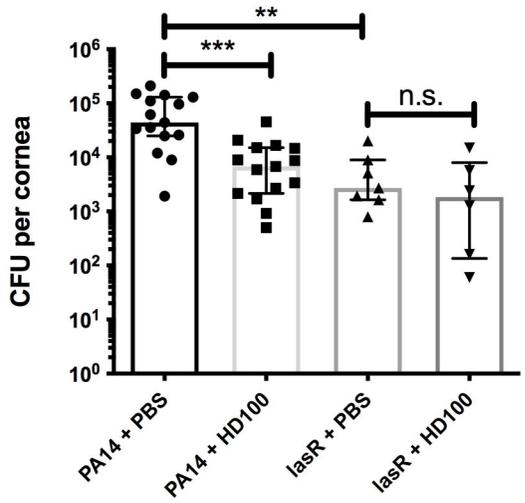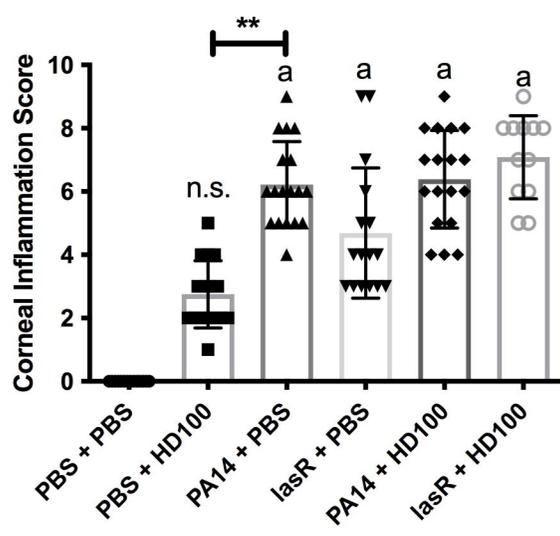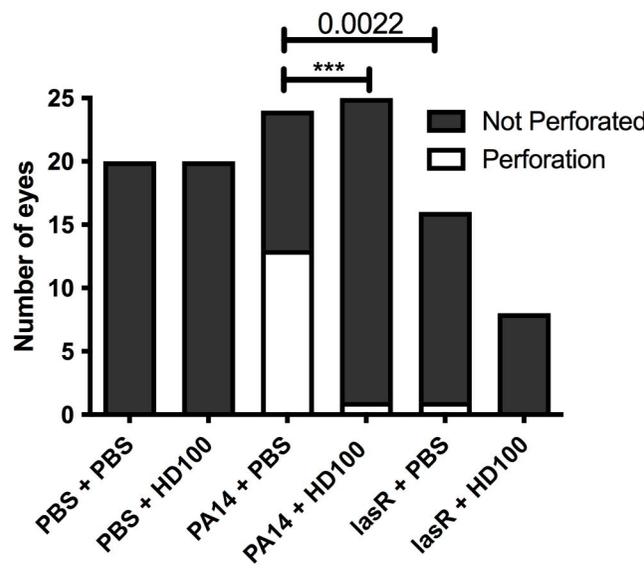
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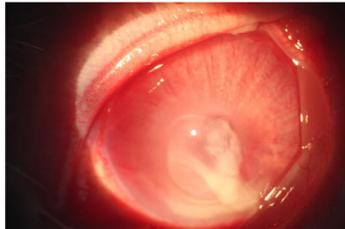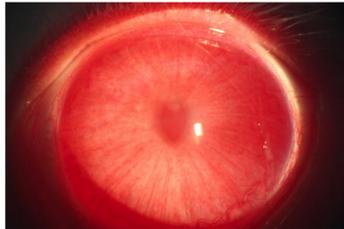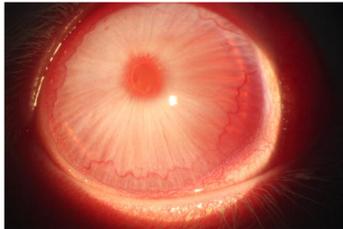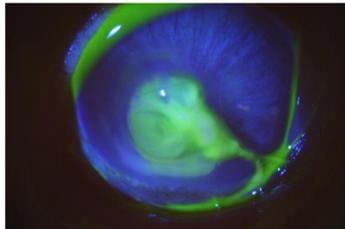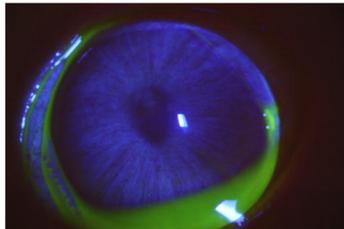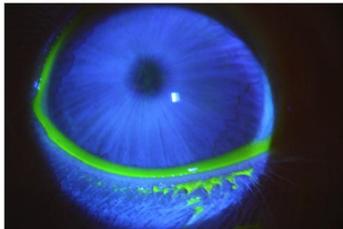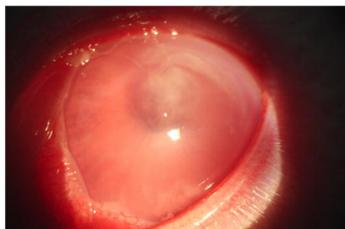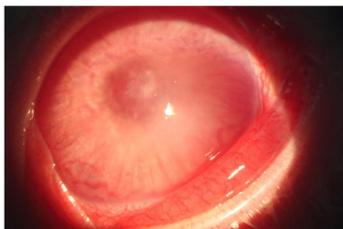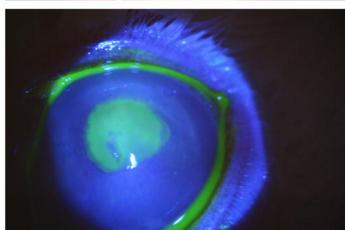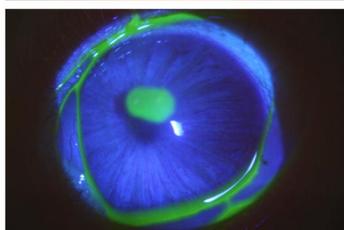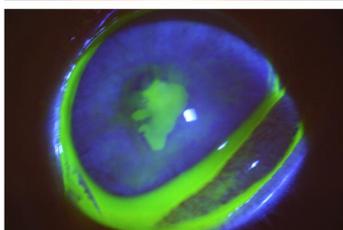
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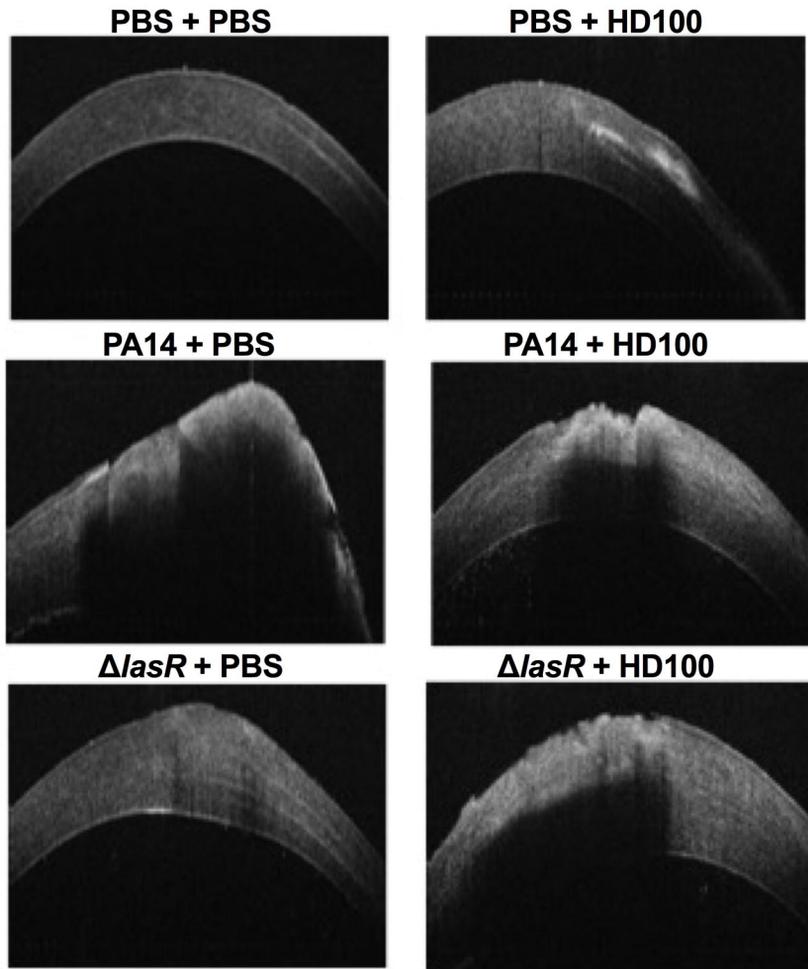
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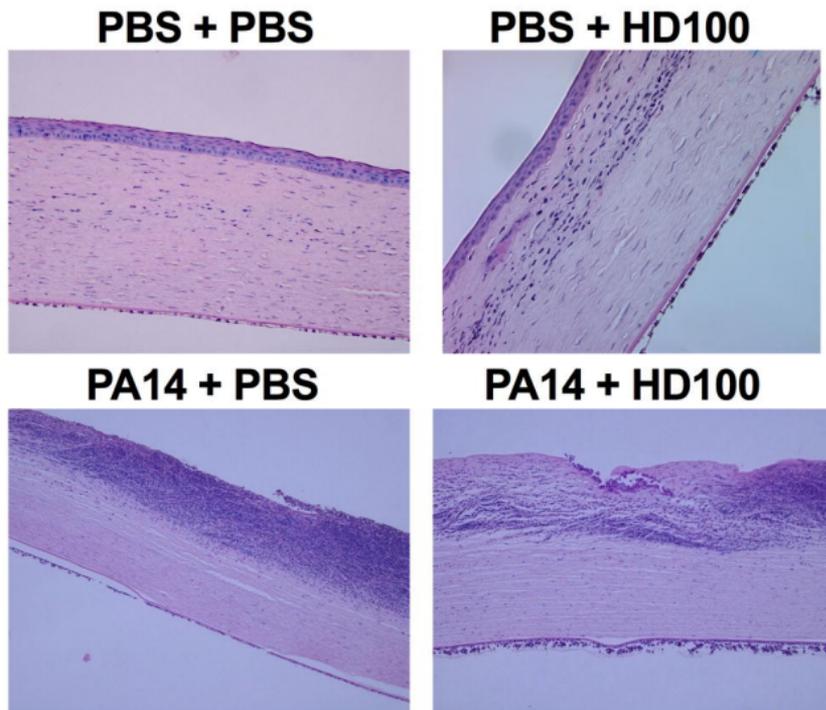
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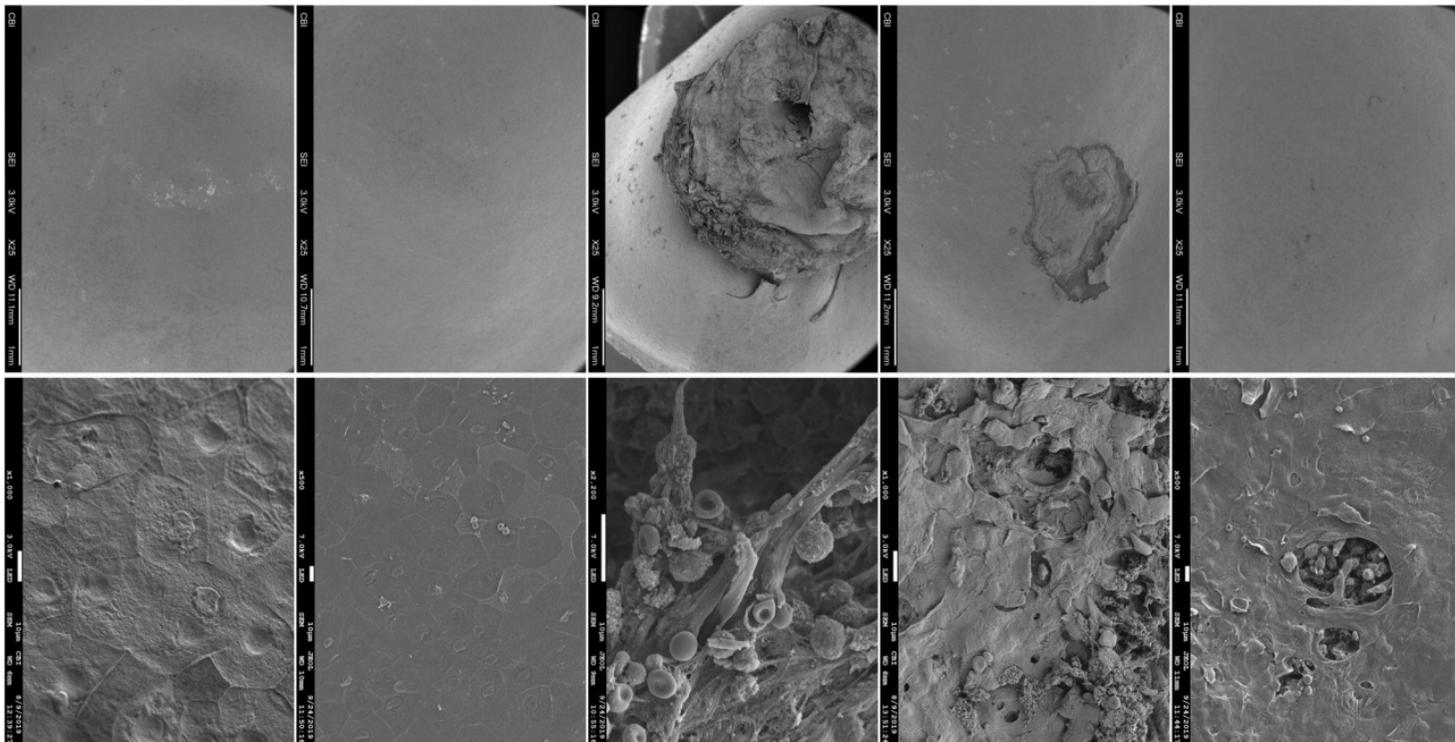
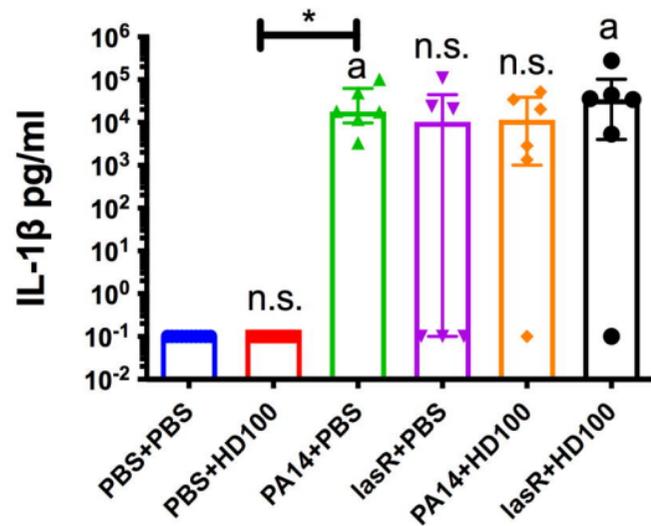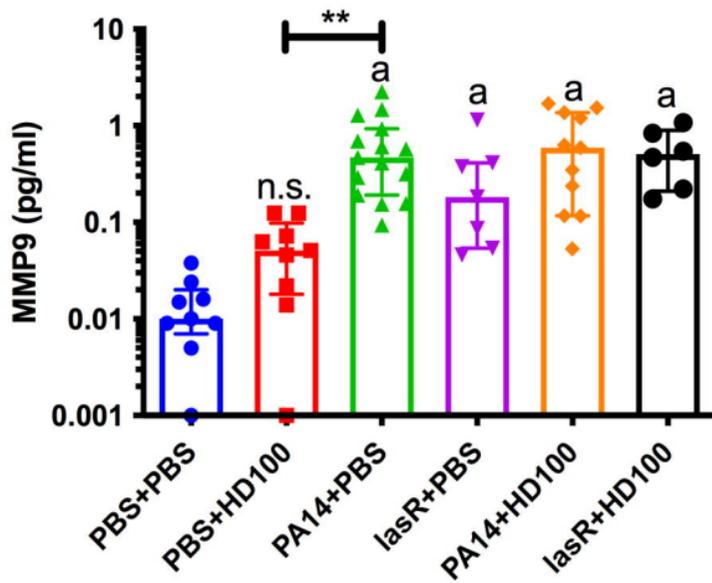**Figure 5. Scanning micrographs of rabbit corneal surfaces.** Representative images are shown. Magnification was 25x (top) and ≥500x (bottom). The corneal perforation is clearly shown in the PA14 + PBS injected cornea, and a corneal ulcer was evident in the PA14 with HD100 injected cornea.

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