



The Effect of Inorganic Ions on *Pseudomonas aeruginosa* Resistance to Disinfection



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Purpose

- During contact lens (CL) wear, bacteria can gain access to the eye via contamination of lenses, cases and lens care solutions.^{1,2} A high incidence of microbial contamination is observed in CL cases and lens care solutions,^{3,5} even in the presence of good compliance with lens care.^{6,7}
- One of the most common pathogens in corneal infection of hydrogel lens wearers is *P. aeruginosa*. Two different genotypes of *P. aeruginosa* have been identified from corneal infections; acutely cytotoxic strains can kill epithelial cells within 3 h of exposure, whilst invasive strains enter epithelial cells and lack acute cytotoxic activity.⁸
- We have shown that *P. aeruginosa* isolates vary considerably in their susceptibility to chemical contact lens disinfectants when basic inorganic ions are made available to the bacteria during testing.^{9,10} Resistance appears to be linked to acute cytotoxicity.
- Our testing methodology included the addition of inorganic ions to the disinfectants under study, as nutrients are plentiful in lens cases during normal use. We have reported that the addition of magnesium ions has a significant effect on bacterial survival.¹⁰
- The aim of this study was to further explore the effect of magnesium and calcium ion availability on *P. aeruginosa* resistance to disinfection:
 - Comparison of cytotoxic and invasive strains
 - Investigation of the role of *exsA*, a gene involved in regulating cytotoxic activity

Table 1: *P. aeruginosa* strains used in this study.

Strain	Isolation source	Isolation date	Phenotype
ATCC 9027	Outer ear infection	1943	Invasive
6294	Cornea – keratitis	Pre-1988	Invasive
CL79	CL case (AOSept)	1994	Invasive
6073	Cornea – keratitis	Pre-1988	Cytotoxic
6206	Cornea – keratitis	Pre-1988	Cytotoxic
PA103	Human sputum	1966	Cytotoxic

Methods

- Susceptibility of 3 cytotoxic & 3 invasive *P. aeruginosa* isolates (laboratory, CL & ocular isolates) assessed (Table 1).
- Comparison of PA103 (functional *exsA* allele; cytotoxic) and PA103 *exsA::Ω* (*exsA*- mutant; invasive)
- Disinfectant under study preserved with: disodium edetate 0.05%, polyquaternium-1 0.001% & myristamidopropyl dimethylamine 0.0005%
- Susceptibility to disinfection tested in the presence or absence of inorganic ions:
 - Disinfectant only
 - Disinfectant + modified Mian's media (7.5 mM NaH₂PO₄, 16.8 mM K₂HPO₄, 10 mM MgSO₄, 0.2% NaNO₃, 10 mM CH₃COONa)¹¹
 - Disinfectant + 10 mM MgSO₄
 - Disinfectant + 10 mM MgCl₂
 - Disinfectant + 10 mM CaCl₂
- CL cases inoculated with 10⁸ CFU/ml & stored at 22°C. Viable counts performed at 6 h post-inoculation.
- Statistical analysis: a log transformation was applied to produce data appropriate for parametric analysis. Comparisons were made using repeated measures ANOVA with appropriate post-hoc tests. *P*<0.05 was considered to be statistically significant.

Results

- Bacterial survival was significantly enhanced when inorganic ions were added to the disinfectant, but varied between strains (*P*<0.05; Figure 1). All bacteria were completely susceptible to the disinfectant alone.
- The greatest effect on survival was observed with the addition of Mian's media, followed by Mg²⁺ & then Ca²⁺ (Figure 2).
 - no significant difference between MgSO₄ & MgCl₂ (*P*>0.05)
 - resistance greater with MgSO₄ & MgCl₂ compared to CaCl₂ (*P*<0.05)
 - cytotoxic strains showed greater resistance with Mg²⁺ than invasive strains (*P*<0.05)
- The *exsA*- mutant was more susceptible to disinfection than the parent strain PA103 (*P*<0.05, Figure 3), again with Mg²⁺ ions playing a greater role than Ca²⁺ ions.

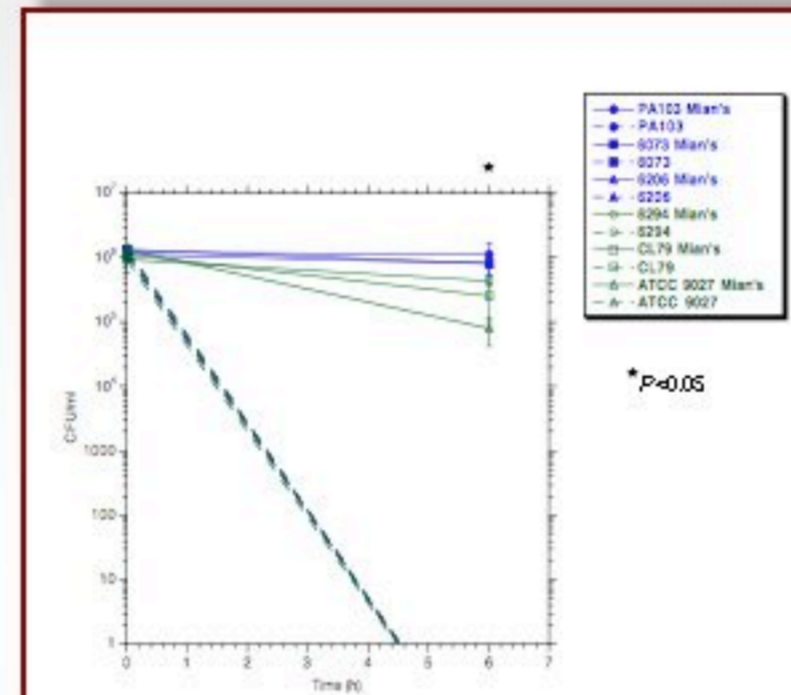


Figure 1: Mean CFU/ml ± SD of *P. aeruginosa* isolates recovered from disinfectant alone (dashed lines) and disinfectant + Mian's media (solid lines), at 6 h post-inoculation. Cytotoxic strains are represented in blue & invasive strains in green. Bacterial survival was significantly enhanced with the addition of inorganic ions (Mian's media), with cytotoxic strains showing greater resistance than invasive strains (*P*<0.05).

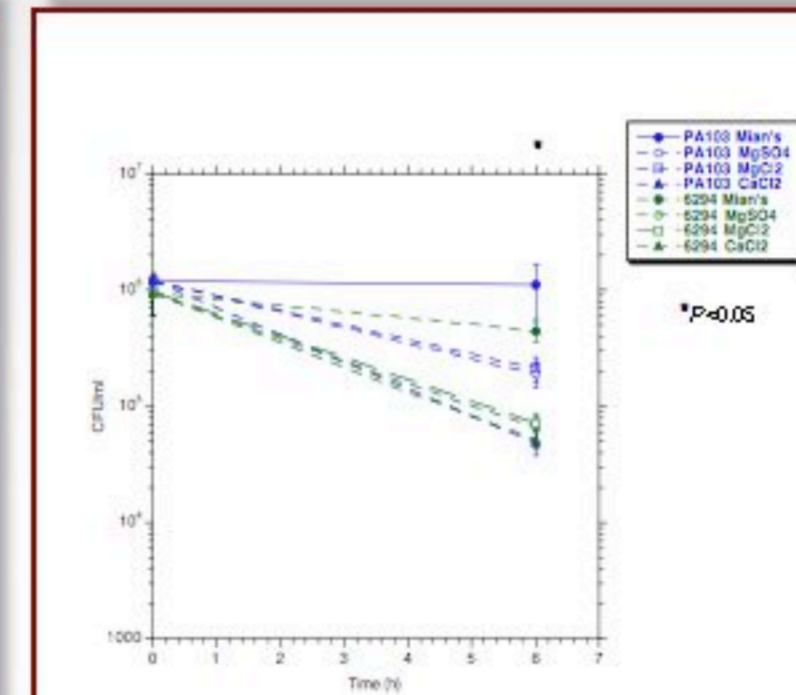


Figure 2: Mean CFU/ml ± SD of *P. aeruginosa* isolates recovered under the difference test conditions at 6 h post-inoculation. There was no significant difference in resistance when MgSO₄ was compared to MgCl₂. Resistance was greater with the addition of Mg²⁺ ions compared to Ca²⁺ ions (*P*<0.05), with cytotoxic strains (shown in blue) showing greater resistance with Mg²⁺ ions than invasive strains (shown in green) (*P*<0.05).

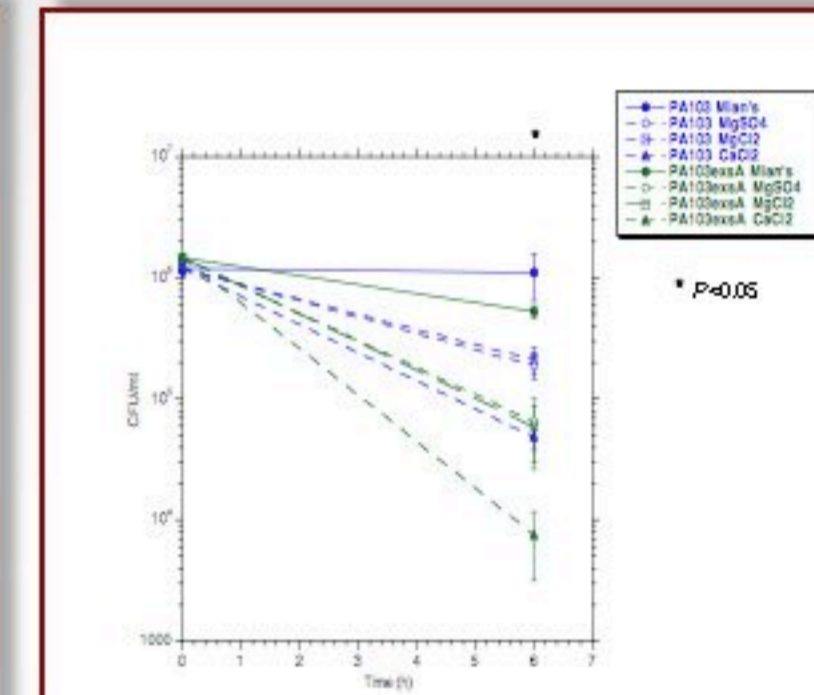


Figure 3: Mean CFU/ml ± SD of parent strain PA103 (cytotoxic, shown in blue) and *exsA*- mutant (invasive, shown in green) recovered from the disinfectants under the different test conditions. The *exsA*- mutant was more susceptible to disinfection than the parent strain (*P*<0.05), with greater resistance observed with the addition of Mg²⁺ ions (no significant difference between MgSO₄ & MgCl₂) compared to Ca²⁺ ions (*P*<0.05).

Conclusions

- The addition of inorganic ions enhanced the ability of *P. aeruginosa* strains to survive disinfectant exposure. This may be the result of an effect on the bacteria, the disinfection solution or a combination of both factors.
- ExsA* was involved in resistance to disinfection, with cytotoxic strains showing greater resistance than invasive strains
 - Mechanisms of *exsA*-regulated resistance require further investigation
 - Potential to modify disinfectants to influence toxin expression and secretion
- Magnesium appeared to play a greater role in resistance to disinfection than calcium. Further exploration of magnesium levels on CLs and in CL cases is warranted.
- Variability between strains should be considered during manufacture and testing of chemical contact lens disinfectants to ensure disinfection efficacy and reduce the risk of CL-related adverse responses

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