*Original Article*

# Susceptibility of various corneal fungal isolates and *Pseudomonas aeruginosa* to contact lens disinfecting solutions

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## Abstract

Introduction: We aimed to investigate the susceptibility of a combined inoculum of *Pseudomonas aeruginosa* and different fungal strains to 6 soft contact lens disinfectants.

Methodology: One corneal isolate of *P. aeruginosa* and 13 corneal fungal isolates (9 *Aspergillus* spp, 3 *Fusarium* spp, 1 *Curvularia* sp.) were used. The following solutions were tested: Arion Cronos, Complete RevitaLens, Dua Elite, Opti-Free Express, Regard, Oxysept Comfort, and Oxysept Comfort without catalase. The effect of the solutions was assessed on a combined inoculum of *P. aeruginosa* plus 1 fungal strain. Suspensions of *P. aeruginosa* and fungi were made in the solutions ($1x10^6$ colony-forming units/mL). After 1 hour (Arion Cronos only), 6, 8, and 24 hours, aliquots of suspension were removed and seeded on Luria-Bertani and Sabouraud agar plates.

Results: After 6 hours' exposure, all the solutions but Dua Elite and Oxysept Comfort eradicated *P. aeruginosa*. Conversely, apart from 3% hydrogen peroxide-based Oxysept Comfort without catalase, which eradicated all the fungi tested after 6 hours, all the other solutions were partly ineffective at killing some of the fungal isolates, even after 24 hours' exposure.

Conclusions: Most contact lens disinfectants may be ineffective if contact lens care systems become co-contaminated with *P. aeruginosa* and fungi. In our experiment, only exposure to 3% hydrogen peroxide without neutralizer for at least 6 hours was always able to kill a combined inoculum of *P. aeruginosa* and different fungal strains.

**Key words:** contact lens-related keratitis; contact lens disinfectants; filamentous fungi; *Pseudomonas aeruginosa*; co-culture; susceptibility

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## Introduction

Microbial keratitis is a potentially blinding condition that represents the most severe complication related to contact lens wear. The vast majority of contact lens-related infections are caused by bacteria, especially *Pseudomonas aeruginosa,* which accounts for up to two thirds of cases [1,2].

Filamentous fungal infections of the cornea are characteristically vision-threatening, can be very difficult to treat, and, in general, carry a poorer prognosis than most other microbial causes of corneal infection. *Fusarium* species represent the most common pathogen for fungal keratitis. Species of *Fusarium* are ubiquitous hyaline filamentous fungi, widely distributed in soil, and commonly associated with plant roots [3]. Outbreaks of fungal eye infections are uncommon; previous cases have been linked to specific circumstances, such as hospital construction [4], contaminated intraocular lens solutions [5], and an environmental reservoir [6]. Fungal keratitis from contact lens wear is rare, constituting less than 5% of microbial keratitis cases in patients who wear contact lenses for refractive errors [7-11]. Recent reports of disproportionate outbreaks of microbial keratitis caused by *F. solani* in Singapore and the United States have led to the removal of the Bausch & Lomb multipurpose lens care solution ReNu with MoistureLoc from the worldwide market in 2006 [12,13].

In a 2007 review of fungal keratitis, Tuli *et al*. [14] suggested that the incidence of fungal keratitis, in particular fungal contact lens-associated keratitis, was rising, and that the rise had begun well before the now well-known ReNu with MoistureLoc *Fusarium*

epidemic. There are several possible reasons for increasing rates of fungal keratitis in contact lens users, including a shift away from thermal disinfection and hydrogen peroxide-based solutions toward multipurpose solutions for storage and cleaning, a trend toward no-rub solutions, and perhaps, worsening lens care by contact lens wearers; however, no evidence has been provided to support the latter idea [14].

Contact lens-related corneal infection has been associated with microbial contamination of the contact lens, contact lens solution, or contact lens storage case [15]. Overall, contact lens disinfectants are more effective against bacteria than fungal species or *Acanthamoeba* [16]. There is no doubt that antimicrobial performance of contact lens disinfection systems is an important factor in reducing contamination.

Little is known about the efficacy of contact lens disinfecting solutions against polymicrobial lens case contamination. The present study was designed to investigate the susceptibility of a combined inoculum of *P. aeruginosa* and different fungal strains (*Aspergillus*, *Fusarium*, and *Curvularia*) to six disinfecting solutions for soft contact lenses.

## Methodology

Thirteen fungal isolates (six *Aspergillus flavus*, three *Aspergillus fumigatus*, three *Fusarium* spp, and one *Curvularia* sp) were used in the present study. All the fungal strains were isolated from corneal specimens at the Institute of Ophthalmology, Joseph Eye Hospital, Tiruchirapalli, India. Along with the fungal strains, we chose to test a corneal isolate of *P. aeruginosa*, one of the most common contaminants of contact lens care systems [17,18]. The soft contact lens disinfectants analyzed in the study are listed in Table 1. Disinfecting solutions available worldwide, containing commonly used active ingredients (*e.g.*, hydrogen peroxide, polyquaternium, polyhexanide), were selected. The one-step hydrogen peroxide system Oxysept was evaluated both with and without the neutralizer. Susceptibility testing experiments were performed at the Department of Biomedical Sciences, Section of Clinical and Experimental Microbiology, University of Sassari, Sassari, Italy.

The susceptibility test was performed as described previously [19]. All the fungal isolates were sub-cultured onto Sabouraud dextrose agar slants and incubated at room temperature for three days, following which conidia were harvested from the cultures using sterile distilled water containing Tween 80 (Sigma Aldrich SRL, Milan, Italy). The conidial suspensions were prepared to an optical density equal to 0.5 McFarland standard (approximately $10^8$ colony-forming units [CFU]/mL). Similarly, 0.5 McFarland standard suspensions of *P. aeruginosa* from overnight cultures of the organism in Luria-Bertani broth were prepared. Then 10 µL of overnight culture of *P. aeruginosa* and 10 µL of each conidial suspension were inoculated into tubes containing 980 µL of each multi-purpose solution (Complete RevitaLens, Dua Elite, Opti-Free Express, Regard), so that each organism had a final concentration of 1 x $10^6$ CFU/mL. Arion Cronos and Oxysept Comfort, two one-step 3% hydrogen peroxide-catalase (0.1 mg/tablet) systems were also tested. *P. aeruginosa* and fungal isolates suspended in hydrogen peroxide (3%) at a concentration of 1 x $10^6$ cfu/mL were placed into the containers provided by the manufacturers, which were filled up to the recommended level. The enzyme catalyst was present during the incubation as instructed by the manufacturers. Arion Cronos and Oxysept Comfort were tested after the completion of the neutralization process (1 hour and 6 hours, respectively). Control tubes received 0.5 mL of *P. aeruginosa* and 0.5 ml of fungi suspended in sterile phosphate buffered saline solution at a concentration of 1 x $10^6$ cfu/mL. Aliquots (5 µL) of suspension were removed for analysis after 1 hour (Arion Cronos only), 6, 8 and 24 hours, seeded on Luria-Bertani agar plates and Sabouraud dextrose agar plates containing antibiotic, and then incubated overnight at 37ºC and for 72 hours at 30°C, respectively. Microbial growth was reported as cfu/mL and the results were finally summarized as "growth positive" or "growth negative". Extreme care was taken to avoid aerial contamination and cross-contamination. The assay was performed in triplicate.

## Results

The ability of the contact lens disinfectants to kill a combined inoculum of fungi and *P. aeruginosa* is summarized in Table 2. Data from one representative assay are shown, as results were consistent on repeated testing. The fungal species recovered from the suspension tubes after incubation were confirmed by growth characteristic to be identical to those used for inoculation.

Only Oxysept Comfort without catalase was able to eradicate *P. aeruginosa* and all the fungi tested after 6 hours' exposure.

Arion Cronos and Oxysept Comfort, containing 3% hydrogen peroxide and catalase, were able to

**Table 1.** Active ingredients and minimum recommended disinfection times of the soft contact lens disinfecting solutions tested

| Contact lens solution | Active ingredient | Minimum recommended disinfection time |
|---|---|---|
| Arion Cronos (Disop, Madrid, Spain) | 3% hydrogen peroxide + microbial catalase (5000 IU/tablet) | 1 hour |
| Complete RevitaLens (AMO, Abbott Park, IL) | polyquaternium-1 0.0003%, alexidine 0.00016% | 6 hours |
| Dua Elite (Disop, Madrid, Spain) | polyhexanide 0.0001% + sodium hyaluronate | 6 hours |
| Opti-Free Express (Alcon Laboratories, Fort Worth, TX) | polyquaternium-1 0.01%, aldox 0.0005% | 6 hours |
| Regard (Advanced Eyecare Research Ltd, High Wycombe, UK) | chlorite/peroxide complex | 6 hours |
| Oxysept Comfort (AMO Ireland, Dublin, Ireland) | 3% hydrogen peroxide + catalase (0.1 mg/tablet) | 6 hours |

eradicate most of the fungal isolates within the minimum recommended disinfection times (1 hour and 6 hours), but failed to kill *P. aeruginosa* in one and two co-cultures, respectively. Conversely, all the other disinfecting solutions were effective against *P. aeruginosa* within the minimum recommended time (6 hours), but were partly ineffective at killing some of the fungal isolates, even after 24 hours' exposure. The worst antifungal performance was given by Regard and Dua Elite, which, after 6 hours' exposure (minimum recommended disinfection time), showed growth of 12 and 10 of the 13 fungal strains tested, respectively.

On the whole, in this study on co-cultures of *P. aeruginosa* and fungi, the susceptibility of the fungal species to the tested disinfection solutions was, in descending order, *Fusarium* spp. > *A. fumigatus* > *A. flavus* > *Curvularia* sp. In our experiment, only Oxysept Comfort was effective against *Curvularia* sp.

Positive controls consistently showed uncontaminated microbial growth at all exposure times.

## Discussion

*P. aeruginosa* is one of the most common contaminants recovered from contact lens cases and one of the most frequent etiological agents of corneal ulcers associated with contact lens wear [1,17,18]. While the pathogenesis of contact lens-related *Pseudomonas* keratitis remains unclear, bacterial contamination of the eye appears to play a major role [2,20]. Although *P. aeruginosa* is the most important cause of contact lens-associated corneal ulcers, other bacteria, fungi, or *Acanthamoeba* may also cause this condition [1,7-16].

Microbial keratitis is an important cause of corneal blindness the world over and is comparatively more prevalent in developing countries. A number of studies from India have reported the epidemiological and microbiological profiles of infectious keratitis [21-25]. Most series on corneal ulcers from tropical countries have highlighted the prevalence of trauma-related fungal keratitis; in addition, 40% of culture-proven corneal ulcers seen in India and other developing countries with similar geographical locations are of fungal etiology [26]. Nevertheless, there is a paucity of data about contact lens-related fungal keratitis from these regions, barring a few anecdotal reports [27-29]. One study from southern India reported infectious keratitis associated with contact lens wear in 35 out of a total of 3,295 patients with infectious keratitis [30].

Fungal keratitis is potentially blinding, but often misdiagnosed among contact lens wearers. In developed countries, the incidence of fungal keratitis is generally low and fungal infection in contact lens wearers is much rarer when compared with bacterial and *Acanthamoeba* keratitis. In July 2005, the Hong Kong Department of Health became aware of an

**Table 2.** Exposure times required by soft contact lens disinfecting solutions to kill a combined inoculum of different fungal isolates and *Pseudomonas aeruginosa*

| | 1 hour | 6 hours | 8 hours | 24 hours |
|---|---|---|---|---|
| **Arion Cronos** | | | | |
| *Aspergillus flavus* | 5/6* | 5/6* | 5/6* | 5/6* |
| *Aspergillus fumigatus* | 2/3 | 3/3 | 3/3 | 3/3 |
| *Fusarium* spp. | 2/3 | 2/3 | 2/3 | 2/3 |
| *Curvularia* sp | 0/1 | 0/1 | 0/1 | 0/1 |
| *Pseudomonas aeruginosa* | growth in 1 coculture with *F. solani* | growth in 1 coculture with *F. solani* | growth in 1 coculture with *F. solani* | no growth |
| **Complete Revitalens** | | | | |
| *Aspergillus flavus* | | 0/6 | 0/6 | 6/6 |
| *Aspergillus fumigatus* | | 3/3 | 3/3 | 3/3 |
| *Fusarium* spp. | | 3/3 | 3/3 | 3/3 |
| *Curvularia* sp | | 0/1 | 0/1 | 0/1 |
| *Pseudomonas aeruginosa* | | no growth | no growth | no growth |
| **Dua Elite** | | | | |
| *Aspergillus flavus* | | 0/6 | 1/6 | 1/6 |
| *Aspergillus fumigatus* | | 1/3 | 1/3 | 1/3 |
| *Fusarium* spp. | | 2/3 | 3/3 | 3/3 |
| *Curvularia* sp | | 0/1 | 0/1 | 0/1 |
| *Pseudomonas aeruginosa* | | no growth | no growth | no growth |
| **Optifree Express** | | | | |
| *Aspergillus flavus* | | 2/6 | 5/6 | 6/6 |
| *Aspergillus fumigatus* | | 2/3 | 3/3 | 3/3 |
| *Fusarium* spp. | | 3/3 | 3/3 | 3/3 |
| *Curvularia* sp | | 0/1 | 0/1 | 0/1 |
| *Pseudomonas aeruginosa* | | no growth | no growth | no growth |
| **Regard** | | | | |
| *Aspergillus flavus* | | 0/6 | 0/6 | 0/6 |
| *Aspergillus fumigatus* | | 0/3 | 0/3 | 0/3 |
| *Fusarium* spp. | | 1/3 | 2/3 | 2/3 |
| *Curvularia* sp | | 0/1 | 0/1 | 0/1 |
| *Pseudomonas aeruginosa* | | no growth | no growth | no growth |
| **Oxysept Comfort** | | | | |
| *Aspergillus flavus* | | 5/6 | 5/6 | 5/6 |
| *Aspergillus fumigatus* | | 3/3 | 3/3 | 3/3 |
| *Fusarium* spp. | | 2/3 | 2/3 | 2/3 |
| *Curvularia* sp | | 1/1 | 1/1 | 1/1 |
| *Pseudomonas aeruginosa* | | growth in 2 cocultures (with *F. solani* and *A. flavus*) | growth in 2 cocultures (with *F. solani* and *A. flavus*) | growth in 2 cocultures (with *F. solani* and *A. flavus*) |
| **Oxysept Comfort without catalase** | | | | |
| *Aspergillus flavus* | | 6/6 | 6/6 | 6/6 |
| *Aspergillus fumigatus* | | 3/3 | 3/3 | 3/3 |
| *Fusarium* spp. | | 3/3 | 3/3 | 3/3 |
| *Curvularia* sp | | 1/1 | 1/1 | 1/1 |
| *Pseudomonas aeruginosa* | | no growth | no growth | no growth |
| **Phosphate-buffered saline solution** | | | | |
| *Aspergillus flavus* | | 0/6 | 0/6 | 0/6 |
| *Aspergillus fumigatus* | | 0/3 | 0/3 | 0/3 |
| *Fusarium* spp. | | 0/3 | 0/3 | 0/3 |
| *Curvularia* sp | | 0/1 | 0/1 | 0/1 |
| *Pseudomonas aeruginosa* | | growth in all cocultures | growth in all cocultures | growth in all cocultures |

*number of susceptible isolates/total number of isolates tested

increased incidence of *Fusarium* keratitis, which they ascribed to the use of ReNu with MoistureLoc [31]. Likewise, in 2006, Khor *et al*. [13] reported on an outbreak of contact lens-related *Fusarium* keratitis in Singapore. Initially, the epidemic was believed to have followed contamination of the Bausch & Lomb multipurpose solution by a single strain of *Fusarium* during manufacture or storage. However, subsequent molecular studies revealed that the *Fusarium* strains involved in the outbreak were derived from the patients' own environments. Bullock *et al*. [32] investigated the effect of storage temperature on the ability of contact lens solutions to inhibit the growth of *Fusarium* species and reported that ReNu with MoistureLoc, when exposed to prolonged temperature elevation, loses its *in vitro* fungistatic activity. In a former study, Leung and co-workers [33] studied the effect of storage temperature and time on the efficacy of four multipurpose solutions for soft contact lenses, including ReNu MultiPlus. The investigators noted that the antimicrobial activity of ReNu MultiPlus on *P. aeruginosa* dropped below the FDA guideline when stored at 30$^{o}$C for two months. They also noted decreased activity of ReNu MultiPlus and Complete Multi-Purpose (containing polyhexamethylene biguanide 0.0001% [Allergan, Irvine, California]) toward *P. aeruginosa* when the solutions were stored at 4$^{o}$C. They concluded that the stability of multipurpose contact lens solutions may be adversely affected by higher temperatures, lower temperatures, and fluctuating temperatures, as well as by prolonged use of the same bottle and by the presence of air within the bottle.

The multicountry outbreak of *Fusarium* keratitis emphasizes that contact lens wear is a major risk factor for infectious keratitis [23]. It is obvious that the use of contact lenses in developed countries exceeds that in developing countries, such as India. However, even in developing countries, the situation is changing due to awareness about contact lens use, urbanisation, and improved socioeconomic status. If the antimicrobial efficacy of contact lens solutions is vulnerable to temperature changes, the climatic conditions of countries such as India may adversely affect the quality of the solution. Contamination of the contact lens case and solution, lens material, wearing schedule and disinfection techniques are important factors that influence infections related to contact lens use. Non-compliance with contact lens care and poor hygiene may result in their contamination, thus predisposing the eye to infections irrespective of geographical regions.

Contact lens wear is the major risk factor for the development of microbial keratitis [10,20]. During contact lens wear, organisms can gain access to the eye from the environment via contamination of the lens, lens case, and lens care solution. Studies of patients with contact lens-associated corneal ulcers have shown contamination of the care systems [17,18]. Microbial contamination of the care system may represent the source of the infecting organisms in corneal ulcers associated with contact lens wear [15]. Contamination rates range from 20% to 80% in asymptomatic and symptomatic patients, with *P. aeruginosa* emerging as the predominant pathogen in the presence of other bacteria, fungi, and *Acanthamoeba* [1,17,18,20]. The presence of micro-organisms in the contact lens cases does not necessarily always imply the occurrence of keratitis. Various factors could explain the discrepancy between case contamination rates and the occurrence of contact lens-related keratitis. On the one hand, in most cases the host reaction could overcome a microbial infection in its initial phase and prevent the development of true keratitis. On the other hand, some organisms may be poorly adherent to the corneal epithelial cells and, therefore, not capable of colonizing the ocular surface [2].

In general, proper contact lens disinfection is essential in preventing contact lens-associated corneal infection. Nevertheless, despite the apparent adherence to recommended disinfecting regimes, several studies have shown a significant degree of microbial contamination of the contact lens cases [18,20]. Biofilm formation on the internal surfaces of the contact lens case may be responsible for disinfectant failure by providing a continuous seed inoculum [18]. In addition, we have previously shown that the currently available contact lens disinfecting solutions have different antimicrobial activity on different organisms [15,19,34-36]. Overall, solutions are more effective against bacteria than they are against fungal organisms or *Acanthamoeba*.

Relatively little attention has been paid to the efficacy of disinfecting solutions against fungi [19,37-43]. In the U.S., only 1-log reduction of fungal organisms within the recommended disinfection time is required by the FDA to meet the primary Stand Alone Test criteria for contact lens disinfection. However, the recent appearance of reports showing insufficient antifungal activity of multipurpose solutions is of great concern [41,42].

Co-contamination of contact lens care systems with *Acanthamoeba* and bacteria capable of supporting

amoebic growth may be the first step in the pathogenesis of *Acanthamoeba* keratitis by the provision of large inocula of amoebae [44]. Conversely, little is known about the role played by fungal and bacterial co-contamination of contact lens care systems in the pathogenesis of fungal keratitis. The paucity of studies addressing this topic is rather surprising, as co-contamination of contact lens care systems with bacteria and fungi is relatively common [17,18].

In a former study, investigating the susceptibility of *P. aeruginosa* and *F. solani* to four disinfecting solutions, we found that disinfectants may act differently, depending on whether the organisms are tested alone or together [19]. Interestingly, we found that ReNu with MoistureLoc showed reduced fungicidal activity in the presence of both organisms, a result which may contribute to explain the ReNu with MoistureLoc – *Fusarium* epidemic. Indeed, it is possible that co-contamination of the lens case with *P. aeruginosa* and *F. solani* may decrease the antifungal activity of ReNu with MoistureLoc. Numerous researchers have attempted to explain the epidemic; causative factors hypothesized include direct uptake of alexidine by the contact lenses (thereby reducing its antimicrobial efficacy) [44], exposure of ReNu with MoistureLoc to prolonged temperature elevation [32], reduced antimicrobial activity of evaporated ReNu with MoistureLoc [45], enhanced growth of *Fusarium* spp on contact lens and lens case biofilm [46], direct penetration of *Fusarium* spp into soft contact lenses [47], and patient non-compliance with manufacturer's recommendations (*e.g.*. storing the lenses without emptying and replacing the solution in the case every day, just adding extra solution to the case, *etc*.) [14,45].

Current contact lens solutions cause fewer toxic and hypersensitivity reactions and are easier to use than previous products, but they are less effective in killing organisms, especially those producing resistant forms, such as *Bacillus cereus* and *Acanthamoeba* [15,34]. Hydrogen peroxide 3%, one of the oldest disinfecting solutions, has good antimicrobial activity, but it is toxic to human cells as well. Therefore, it is necessary to neutralize fully any hydrogen peroxide adherent to the lens before the lens is reapplied to the eye. This can be done by enzymatic means (catalase) in a one- or two-step process. In one-step systems, such as Arion Cronos and Oxysept Comfort, a catalase tablet is added to the lens case at the beginning of disinfection. This system, generally active against bacteria, proved to be ineffective at killing *B. cereus* and *Acanthamoeba* within the minimum recommended disinfection time [15,34]. Because the enzyme catalyst is present from the very beginning of the disinfection step, the hydrogen peroxide is neutralized long before complete disinfection can occur. Conversely, complete disinfection may be accomplished by using the two-step system, with neutralization occurring after 9 hours' exposure (overnight) to hydrogen peroxide.

In the present study, all the solutions showed good antibacterial activity against *P. aeruginosa*, even though the one-step hydrogen peroxide systems Arion Cronos and Oxysept failed to kill the organism within the minimum recommended time in one and two co-cultures, respectively. These results suggest that co-contamination of the lens case with *P. aeruginosa* and fungi may somewhat decrease the antibacterial activity of disinfecting solutions containing hydrogen peroxide plus catalase. On the other hand, apart from Oxysept Comfort without catalase, which eradicated all the fungi tested after 6 hours, all the other solutions were partly ineffective at killing some of the fungal isolates, even after 24 hours' exposure. The least effective antifungal performance was given by Regard and Dua Elite, which, after 6 hours' exposure, showed growth of 12 and 10 of the 13 fungal strains tested, respectively.

Furthermore, our experiment also showed that different clinical isolates belonging to the same fungal species may show different susceptibilities to the same disinfecting solution. This observation must be taken into consideration while testing the antifungal activity of new disinfecting solutions.

Even though we tested a significant number of clinical fungal isolates, a clear limitation of our study is that we used an *in vitro* model, which may not reflect exactly the real situation in contaminated contact lens cases.

## Conclusion

In conclusion, our findings suggest that most contact lens disinfecting solutions may be ineffective if contact lens care systems become contaminated with *P. aeruginosa* and fungi. Only exposure to 3% hydrogen peroxide without catalase for at least 6 hours was always able to kill a combined inoculum of *P. aeruginosa* and different fungal strains. In the light of these results, the need for a complete re-evaluation of the real antifungal efficacy of currently available contact lens disinfecting solutions must be stressed. This is crucial to reduce the risk of fungal keratitis in contact lens wearers, especially in tropical and sub-tropical regions, where keratomycoses are common.

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