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Status of the effectiveness of contact lens solutions against keratitis-causing pathogens

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ABSTRACT

Purpose: The aim of this study was to assess the antimicrobial effects of marketed contact lens disinfecting solutions.
Methods: Using ISO 14729 Stand-Alone Test for disinfecting solutions, bactericidal, fungicidal and amoebicidal assays of eight different contact lens solutions including: ReNu MultiPlus, DuraPlus, Ultimate Plus, OptiFree Express, Kontex Clean, Kontex Normal, Kontex Multisol extra^{+}, Kontex Soak were performed. The efficacy of contact lens solutions was determined against keratitis-causing microbes, namely: *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, *Fusarium solani* and *Acanthamoeba castellanii*.
Results: The results revealed that ReNu MultiPlus, DuraPlus and OptiFree Express were effective in killing bacterial and fungal pathogens as per manufacturer's minimum recommended disinfection time. Ultimate Plus was effective against *F. solani* and MRSA but ineffective against *P. aeruginosa*, *S. marcescens* and *S. aureus.* Of concern however, is that none of the locally formulated contact lens disinfecting solutions from Pakistan, i.e., Kontex Clean, Kontex Normal, Kontex Multisol extra^{+} and Kontex Soak were effective against any of the keratitis-causing organisms tested. All eight contact lens disinfecting solutions were unable to destroy *Acanthamoeba* cysts.
Conclusions: Because such ineffective contact lens disinfection solutions present a major risk to public health, these findings are of great concern to the health officials and to the manufacturers of the contact lens disinfection solutions and effective solutions are needed, along with emphasis on proper hygiene for contact lens care and special guidelines for developing countries regarding the manufacture and storage of contact lens disinfecting solutions.

1. Introduction

Microbial keratitis is a devastating ocular infection and an important cause of visual impairment/blindness that is frequently associated with contact lens (CL) wear [1–4]. The majority of cases are attributed to *Pseudomonas aeruginosa*, *Serratia*, *Fusarium* spp., and *Acanthamoeba* spp. [1–4]. The recognition and management of microbial keratitis require suspicion, early differential diagnosis and aggressive treatment for successful prognosis otherwise it often has vision-threatening consequences.

With over 120 million people wearing CL for refractive correction and cosmetic purposes throughout the world, the associated risk factors are a cause for concern. Recently the anti-amoebic effects of marketed CL disinfecting solutions were assessed [5]. The efficacies of different CL disinfecting solutions manufactured locally in Pakistan and internationally were evaluated against *Acanthamoeba castellanii* of the T4 genotype. Surprisingly, none of the solutions tested had any potent cysticidal effects [5]. Following this alarming finding, the aim of the present study was to determine the antibacterial and antifungal efficacy of different CL disinfecting solutions against a range of bacterial and fungal pathogens: *P. aeruginosa*, *Serratia marcescens*, *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus* and *Fusarium solani*. Using the ISO 14729 Stand-Alone Test for CL disinfecting solutions, bactericidal and fungicidal effects of eight different CL disinfecting solutions including: ReNu MultiPlus, DuraPlus, Ultimate Plus, OptiFree Express, Kontex Clean, Kontex Normal, Kontex Multisol extra^{+}, Kontex Soak was performed. To allow comparison to our previous data of *Acanthamoeba* cysts, the CL disinfecting solutions were tested against *A. castellanii* of the T4 genotype.

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2. Materials and methods

2.1. Contact lens disinfecting solutions

The CL disinfection solutions used in this study along with their active ingredients and manufacturers' instructions are listed in Table 1. All CL disinfection solutions were tested within their stated expiry date. Eight different CL disinfection solutions were tested: ReNu MultiPlus, DuraPlus, Ultimate Plus, OptiFree Express, Kontex Clean, Kontex Normal, Kontex Multisol extra^{+}, Kontex Soak. These solutions are commonly available in Pakistan and were purchased from local retailers. ReNu MultiPlus and OptiFree Express were imported from the USA, whereas both DuraPlus and Ultimate Plus were manufactured locally, though were from international manufacturers'. Kontex Clean, Kontex Normal, Kontex Multisol extra^{+}, and Kontex Soak were formulated and manufactured in Karachi, Pakistan.

2.2. Test organisms and growth conditions

All chemicals were purchased from Sigma Labs (Poole, Dorset, England), unless otherwise stated. *P. aeruginosa*, *S. marcescens*, *S. aureus*, MRSA and *F. solani* were used in this study. *P. aeruginosa* was isolated from a clinical sample at the Aga Khan University hospital. *S. marcescens* was isolated from the gut of Black Cobra [6]. *S. aureus* was obtained from an environmental sample at the Aga Khan University [7]. MRSA was isolated from the blood sample of a sepsis patient [8]. *F. solani* was purchased from the First Fungal Culture Bank of Pakistan (FCBP0055). All bacterial strains were grown on nutrient agar at 37 °C for 24 h. Cultures were prepared by inoculating each bacterial strain in Luria–Bertani (LB) broth overnight and incubating at 37 °C. Cultures were centrifuged 10,000 × *g* for 10 min and resuspended in phosphate-buffered saline (PBS), and adjusted to optical density of 0.2 spectrophotometrically, which is equivalent to approximately 10^8 colony forming units (CFU)/mL as well as enumerated by plating on nutrient agar plates using serial dilution [9,10]. *F. solani* was grown for 7–10 days on potato dextrose agar at 30 °C [11]. Following incubation, conidia were harvested by scraping the surface of fungal colony with a cell scraper and hyphae were removed via filtration using sterile gauze [9–11]. The conidia were washed with sterile PBS and counted using a haemocytometer. *A. castellanii* belonging to the T4 genotype (ATC 50492) sourced from a keratitis patient was grown in PYG medium [proteose peptone 0.75% (w/v), yeast extract 0.75% (w/v) and glucose 1.5% (w/v)] at 30 °C as previously described [12]. To prepare *A. castellanii* cysts, encystation was induced by inoculating 5×10^6 *A. castellanii* trophozoites onto non-nutrient agar plates and incubating at 30 °C for up to 14 days [13]. Food deprivation resulted in trophozoite transformation into the cyst form. Next, 10 mL of dH_2O was added to each plate. Cysts were then scraped off the agar surface using a cell scraper, enumerated using a haemocytometer and 5×10^4 *A. castellanii* cysts were used.

2.3. Bactericidal activity of contact lens disinfection solutions

To determine the effects of various CL solutions against *P. aeruginosa*, *S. marcescens*, *S. aureus* and MRSA, bactericidal assays were performed. The assay method was based on ISO 14729 Stand-Alone Test criteria for testing CL disinfecting solutions [9,10]. Briefly, 10^6 CFU/mL of test organisms were exposed to 10 mL of different CL disinfection solutions and incubated at room temperature as per manufacturer's minimum recommended disinfection time. Following Kontex products were used: Kontex Clean is used to disinfect; Kontex Normal is used for rinsing; Kontex Multisol extra^{+} is used for extra rinsing; and Kontex Soak is used for soaking/storage. The tested organisms were first suspended in Kontex Clean for 30 s followed by centrifugation at 10,000 × *g* for 5 min. The supernatants were aspirated and organisms rinsed in Kontex Normal and/or Kontex Multisol extra rinsing solutions as recommended by the manufacturer. Next, 10-fold serial dilutions were made using Dey–Engley neutralizing broth after the manufacturer's minimum recommended disinfectant time. The bacterial (CFU) were determined by plating dilutions on nutrient agar plates and incubating at 37 °C for 24 h. All experiments were performed at least 3 times, in duplicate.

2.4. Fungicidal and amoebicidal activity of contact lens disinfection solutions

To study the effects of CL disinfection solutions against *F. solani*, fungicidal assays were performed. The assay method was based on ISO 14729 Stand-Alone Test criteria for testing CL disinfecting solutions [9,10]. Briefly, 10^6 CFU of fungal conidia were exposed to 10 mL of different CL disinfecting solutions and incubated at room temperature as per the manufacturer's minimum recommended disinfection time. In the case of Kontex Multisol extra^{+} and Kontex Soak, conidia were first exposed to Kontex Clean solution for 30 s followed by centrifugation at 4500 × *g* for 5 min. The supernatants were aspirated and conidia rinsed in Kontex Normal and/or Kontex Multisol extra rinsing solutions as recommended by the manufacturer. Next, 10-fold serial dilutions were made using Dey–Engley neutralizing broth and mixtures allowed to stand at room temperature for 10–15 min to neutralize the preservatives. The conidia were enumerated by plating the dilutions on potato dextrose agar plates and incubating at 30 °C for up to 5 days. All experiments were performed at least 3 times, in duplicate. Additionally, the cysticidal effectiveness of these solutions was determined against *A. castellanii*. Briefly, 5×10^4 *A. castellanii* cysts were pelleted by centrifugation at 2500 × *g* for 5 min. The supernatants were aspirated and pellet resuspended in 0.5 mL of each CL disinfection solution listed in Table 1. In the case of Kontex Multisol extra^{+} and Kontex Soak, amoebae were first suspended in Kontex Clean for 30 s followed by centrifugation at 2500 × *g* for 5 min. The supernatants were aspirated and amoebae resuspended in Kontex Normal and/or Kontex Multisol extra^{+} as recommended by the manufacturer. Next, amoebae counts were performed using a haemocytometer after the manufacturer's minimum recommended disinfectant time as well as after 24 h. The viability of trophozoites was determined by inoculating CL disinfection solution-treated amoebae in the growth medium, i.e., PYG and incubating at 30 °C for 72 h [5].

3. Results

3.1. Bactericidal effects of contact lens disinfection solutions tested

The results revealed that ReNu MultiPlus, DuraPlus and OptiFree Express killed *P. aeruginosa*, *S. marcescens*, *S. aureus* and MRSA when tested for the manufacturers' recommended disinfection time. More than 3-log reduction was observed in case of these CL disinfection solutions against the tested bacteria (Fig. 1 and Table 2). On the other hand, Ultimate Plus was effective against MRSA with a 3-log reduction, but not against *P. aeruginosa*, *S. marcescens* and *S. aureus*. When tested against *P. aeruginosa*, *S. marcescens* and *S. aureus*, 2.7-, 2.6- and 1.7-log reductions were observed respectively (Fig. 1 and Table 2), which is considered ineffective according to ISO Stand-Alone Test primary acceptance criteria [9]. Notably, the locally formulated solutions: Kontex-CLS (i.e., Kontex Clean, Kontex Normal, Kontex Multisol extra^{+} and Kontex Soak) did not exhibit any bactericidal activity against any of the bacterial