Sir,

Fungal keratitis is an infection that is difficult both to diagnose and to treat. Candida albicans, other yeasts and the septate moulds, Fusarium spp. and Aspergillus spp., are common causes of this disease. Treatment options are limited and include topical formulations of natamycin (5%), amphotericin B (0.15%) and fluconazole (1%). However, the efficacies of these agents are compromised by poor ocular penetration, poor tolerability and/or poor in-vitro activity.\(^1\) Polyhexamethylene biguanide (PHMB), which is currently used as an environmental biocide and contact lens disinfectant, has been shown to have excellent in-vitro activity against a broad range of fungal pathogens\(^2\) and to be effective and well tolerated at concentrations of 200 mg/L (0.02%) when used as treatment of patients with keratitis caused by Acanthamoeba spp.\(^3\) The present study was undertaken to evaluate the in-vitro activity of PHMB against fungal isolates associated with infective keratitis.

The organisms used in the study included 25 isolates of C. albicans which were isolated from patients at the University of Illinois Hospital, Chicago, IL, USA (n = 10), Columbia Wesley Medical Center, Wichita, KS, USA (n = 10) and St Vincent’s Mercy Medical Center, Toledo, OH, USA (n = 5), a single isolate of Aspergillus niger which was recovered from a patient at the University of Illinois Hospital, and the following four strains which were provided by the American Type Culture Collection, Rockville, MD, USA: C. albicans 24433; Candida parapsilosis 20219; Candida krusei 6258 and Fusarium solani 44366. PHMB 20% was obtained from Zeneca Biocides, Wilmington, DE, USA.

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In-vitro activity of polyhexamethylene biguanide (PHMB) against fungal isolates associated with infective keratitis


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References

MICs for the yeasts were determined by a macrobroth dilution method recommended by the National Committee for Clinical Laboratory Standards (NCCLS), while those of the filamentous fungi were determined by the methods of Espinel-Ingroff et al. The medium used in all cases was RPMI 1640 containing glutamine but without sodium bicarbonate (ICN Biomedicals Inc., Costa Mesa, CA, USA). Two-fold serial dilutions of PHMB were prepared in RPMI 1640, the final concentrations ranging from 0.78 to 200 mg/L (0.00078–0.02%). An inoculum of each yeast strain was prepared from a 24 h culture grown at 35°C on Sabouraud dextrose agar plates (Remel, Lenexa, KS, USA). Colonies were suspended in 0.85% sterile saline and the turbidity of each suspension was adjusted with a spectrophotometer at 530 nm so that it was equivalent to that of a 0.5 McFarland standard. The suspensions were diluted 1:100 and then 1:20 with RPMI 1640, thereby producing inocula of 0.5–2.5×10^6 cfu/L; the size of each inoculum was confirmed by determining viable counts. The F. solani and A. niger isolates were grown at 30°C on potato dextrose agar (PDA) slants (Remel) and suspensions were prepared from mature 5-day-old cultures by covering the slants with 2–3 mL of 0.85% sterile saline and gently probing the colonies with the tip of a pipette. Each suspension was then drawn into a sterile tube and mixed vigorously with a vortex. The heavy particles were allowed to settle for 3–5 min and the upper homogeneous suspension was used as the inoculum. The density of the suspension was read in a spectrophotometer at 530 nm and adjusted to 75% transmittance. The suspensions were further diluted 1:100 with RPMI 1640 to give inocula of 0.5–5.0×10^6 cfu/L, the size of each inoculum was confirmed by determining viable counts. The fungal suspensions were combined with the PHMB dilutions in sterile polystyrene tubes. C. albicans isolates were incubated at 35°C for 48 h, and the F. solani and A. niger isolates at 30°C for 72 h. In accordance with NCCLS guidelines, the MIC was taken as the lowest concentration of each drug that prevented visible growth.

The MIC(50) of PHMB for the yeasts was 1.56 mg/L (0.000156%), with a range of 0.78–1.56 mg/L (0.000078–0.000156%). The MICs for the A. niger and F. solani isolates were 6.1 mg/L (0.00061%) and 2.4 mg/L (0.00024%), respectively.

The data reported here confirm that PHMB is active in vitro against isolates of C. albicans, A. niger and F. solani. These results are in accord with those of Liu et al., who described MICs ranging from 0.32 to 0.71 mg/L for C. albicans, and similar to those of Myers et al. who recently reported MIC_{c50} of 20 mg/L (0.002% and <2 mg/L (<0.0002%) for Aspergillus spp. and Fusarium spp., respectively. Moreover, the concentrations of PHMB shown here to inhibit the growth of all of the isolates tested were markedly lower than that present in the formulation (200 mg/L or 0.02%) used to treat patients with infections caused by Acanthamoeba spp.

In conclusion, we have demonstrated that PHMB is active in vitro against C. albicans, F. solani and A. niger strains, all of which are commonly isolated from patients with fungal keratitis. Further in-vitro and clinical studies are warranted in order to determine its role in the treatment of patients with this disease.

References

Antimicrobial resistance levels of enterobacteria isolated from minced meat


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Sir, Antibiotic resistance among the members of the family Enterobacteriaceae is often high and can cause major clinical problems. In the normal flora they have also been found to harbour resistant strains at high frequencies. Some of this resistance could originate from food. In a study of enterobacteria on vegetables, we found very low