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## Biofilm testing of microbiota: An essential step during corneal scrap examination in Egyptian acanthamoebic keratitis cases

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**Purpose:** To detect co-infections in the culture-proven acanthamoebic keratitis (AK) cases, and to test the capability of biofilm formation in the isolated microbiota. The clinical findings, habit of wearing contact lens and in-vitro antibiotic resistance were analyzed further according to the biofilm formation capability.

**Methods:** After clinical examination, corneal scraps and swabs were taken from 240 clinically suspected AK cases, for Acanthamoeba and microbiological cultures. In cases of keratoplasty, trimmed corneal tissue was collected and sent for histopathological examination. Scanning electron microscopy was done for some samples. Biofilm formation capability was investigated using a tissue culture plate method. Antibiotic resistance pattern was determined using a modified-Kirby-Bauer disc diffusion method.

**Results:** In 102 AK culture proven cases, 11 had no co-infection, 74 had a single co-infection and 17 had double co-infections. Enterobacteriaceae and Aspergillus were the commonest bacterial and fungal isolates, respectively. Regarding the biofilm formation, 64.7% of Enterobacteriaceae, 50% of *Pseudomonas aeruginosa*, 43.75% of *Staph aureus*, 76.92% of *Streptococcus pneumoniae*, 28.57% of *Corynebacterium*, 60% of  $\alpha$ -haemolytic streptococci, 40% of *Acinetobacter*, 100% of *Candida* and 77.8% *Aspergillus* isolates were biofilm producers. Severe manifestations were more frequently reported in cases co-infected with biofilm producers than with non-biofilm producers. Generally, high percentages of the biofilm forming bacterial isolates were sensitive to antibiotics in-vitro.

**Conclusions:** Routine investigations for co-infection and biofilm formation in addition to *Acanthamoeba* culture are strongly recommended in suspected AK cases. Co-infection with biofilm producers may precipitate extrinsic in-vivo drug resistance despite of the in-vitro sensitivity. Designing a biofilm-dissolving topical drug is highly recommended to enhance the response to the standard therapeutic regimen especially in the resistant AK cases.

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