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CASE REPORT

# Adventitious sporulation in *Fusarium* keratitis

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## Introduction

*Fusarium* species may form reproductive structures in tissue, a phenomenon known as `adventitious sporulation' [1]. If *Fusarium*-specific adventitious sporulation (phialides and phialoconidia morphologically suggestive of *Fusarium* species, and irregular hyphae with both 45° and 90º branching) is seen in a tissue lesion, a preliminary diagnosis of *Fusarium* infection can be made [2, 3], and specific therapy can be rapidly initiated while waiting for the culture results.

*Fusarium* species, the principal causes of fungal keratitis in many countries [4], are typically relatively resistant to most antifungal agents. Natamycin is currently the drug of choice to treat fusarial keratitis [5, 6], while topical and oral voriconazole may also be effective [7]. If keratitis due to *Fusarium* species is diagnosed rapidly, specific therapy can be started promptly.

This report describes how the phenomenon of *Fusarium*–specific adventitious sporulation in corneal scrapings allowed a rapid presumptive diagnosis of *Fusarium* keratitis in six patients.

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## Case report

Between July and December 2005, each of 152 patients with suspected microbial keratitis underwent scraping of the base and edges of the ulcerated corneal lesion [4]. The corneal material was stained by lactophenol cotton blue (LPCB wet film) and Gram's stain (dry smear) for direct microscopic examination; fusoid macroconidia with foot cells were specially looked for, since this is an important feature of the genus *Fusarium* [8]. *Fusarium*-specific adventitious sporulation was said to be present in corneal scrapings exhibiting such reproductive structures. Corneal material was also inoculated as `C-streaks' onto sheep blood agar and Sabouraud glucose neopeptone agar and broth; the media were incubated at 30°C and 37°C. Fungi isolated were deemed significant if isolated on multiple media or on one medium with direct microscopic evidence of fungal hyphae. Filamentous fungi were identified according to standard procedures.

During the study period, a diagnosis of mycotic keratitis was established by isolation of fungi alone in culture in 49 patients (32.3 % of 152 patients). *Fusarium* species (20 strains) were isolated from 20 patients [*Fusarium solani* {13}, *Fusarium dimerum* {three}, undefined *Fusarium* species{four}].

*Fusarium-specific* adventitious sporulation (Fig. 1) was detected in the corneal scrapings of six patients. Five of these were males; all six were adults (age range: 30 to 52 years). Three patients had had the symptoms for 3 days or less, while the other three had had symptoms for 6 days or more. The principal risk factor was trauma in four

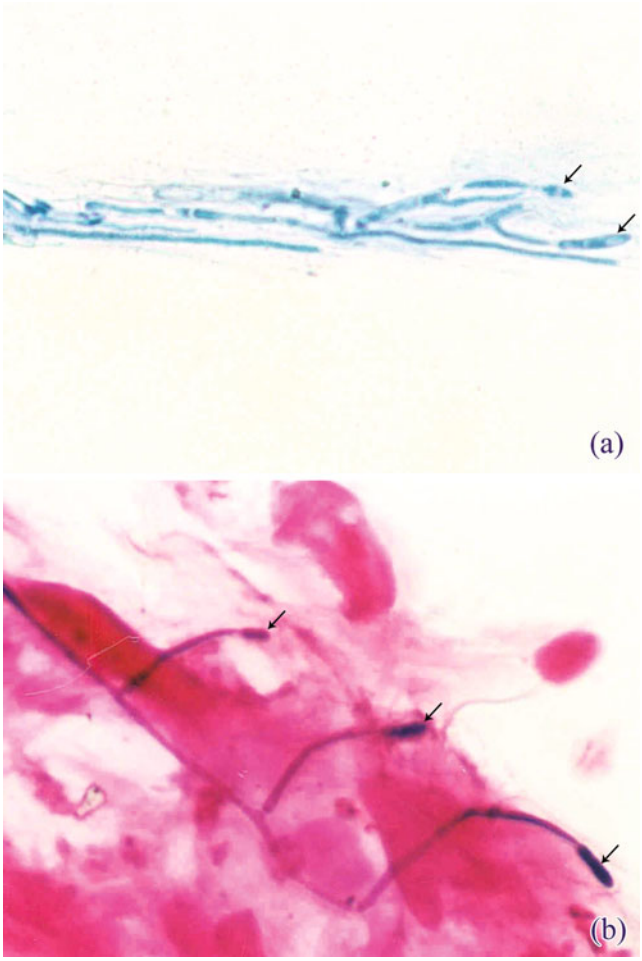


**Fig. 1** **a** Lactophenol cotton blue (LPCB) wet film of corneal scrape material showing *Fusarium*-specific adventitious sporulation (×400); *Fusarium solani* was isolated in culture of the corneal material. **b** Gram-stained smear of corneal scrape material exhibiting *Fusarium*-specific adventitious sporulation (×400); *Fusarium solani* was isolated in culture of the corneal material

patients (due to vegetable material in one, mud or dust in two and an unknown agent in one), while the other two patients did not report any specific risk factor. The right eye was affected in five patients, and the left in one patient. Two ulcers had a smallest diameter of 5 mm, while the smallest diameter of each of the other four ulcers was 3 mm or less. Four patients had good vision (20/60 or better) in the affected eye, while two patients had poor vision (20/200 or worse).

Fungal hyphae were detected by Gram's stain in corneal scrapings of all six patients, and in the LPCB wet films of corneal scrapings of five patients. Since *Fusarium*-specific adventitious sporulation was noted in the corneal scrapings, hourly topical natamycin (5%) was commenced without waiting for the culture results. With this therapy, the corneal ulceration completely healed in three patients, and was healing in one patient when last reviewed. One patient was lost to follow-up before the status could be evaluated, while in one patient, the ulcer was still active when last seen. Interestingly, *Fusarium solani* was isolated from the corneal scrapings of all six patients.

## Discussion

Adventitious sporulation is known to occur in the course of infections due to *Fusarium, Paecilomyces* or *Acremonium* [2]. The exact significance of this phenomenon is yet to be established [9]. However, it can be used for a rapid presumptive diagnosis of certain fungal infections, although definitive identification requires culture. Liu et al. [2] reported adventitious sporulation in histological sections of nine of nine infections caused by *Paecilomyces* species, and in seven of ten caused by *Fusarium* species.

Direct microscopy is an important diagnostic modality in investigating suspected microbial keratitis, since the detection of fungal hyphae or yeast cells in corneal scrapings permits a rapid presumptive diagnosis of fungal keratitis and initiation of antifungal therapy. However, it is difficult to determine which genus of filamentous fungi is involved just by looking at the fungal hyphae in a corneal scraping. This has important implications for treatment. If specific anti-*Fusarium* therapy could be confidently initiated based on the findings in the corneal scraping, it would greatly

**Table 1** Presence of *Fusarium*-specific adventitious sporulation as a diagnostic aid for *Fusarium* infection in patients with keratitis

| *Fusarium*-specific adventitious sporulation in microscopy | Fusarium isolated in culture | Fusarium not isolated in culture | Total |
|---|---|---|---|
| Present | 6 | 0 | 6 |
| Not present | 14 | 132 | 146 |
| | 20 | 132 | 152 |

Sensitivity: 30 pe cent

Specificity: 100 percent

False-positive rate: 0 pe cent

False-negative rate: 9 percent

Agreement of microscopy with culture: 91 percent

benefit the clinician. Hence, we tried to assess the significance of *Fusarium*-specific adventitious sporulation in corneal scrapings as a diagnostic aid for presumed *Fusarium* keratitis.

We observed that in relation to a culture-proven diagnosis of *Fusarium* infection (the 'gold' standard), the occurrence of *Fusarium*-specific adventitious sporulation had a sensitivity of 30 percent, a specificity of 100 percent, a false-positive rate of 0 percent and a false-negative rate of 9 percent (Table 1). The results of direct microscopy (presence of *Fusarium*-specific adventitious sporulation suggesting *Fusarium* keratitis, and absence of such sporulation excluding *Fusarium* keratitis) were in agreement with the results of culture in 138 (91%) of the 152 patients investigated.

Our results suggest that if *Fusarium*-specific adventitious sporulation is observed in corneal scrapings from a patient with keratitis, a relatively reliable diagnosis of presumed *Fusarium* keratitis can be made, and topical eyedrops active against *Fusarium* species (e.g., natamycin, econazole, voriconazole) can be rapidly instituted while awaiting the culture results. The low sensitivity of this technique is currently a concern, but improvement is possible with refinements in the techniques used.

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