Fungal Keratitis Due to *Exophiala Phaeomuriformis*

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American Society of Cataract and Refractive Surgery Annual Meeting Boston 2014

None of the authors have any financial disclosures related to the presentation

Introduction

- Fungal keratitis is a growing cause of microbial keratitis.
- Common causative organisms are filamentous fungi, such as Fusarium and Aspergillus, and yeast, mostly Candida sp.
- Yeast infections occur when host defenses are compromised, such as ocular surface disease, surgery, and chronic topical steroid use.
- Filamentous fungi tend to be associated with ocular trauma or contact lens wear.
- Recent advances in molecular diagnostic studies like DNA sequencing in addition to the standard techniques of microbial staining and culture have led to the recognition of newer and rare fungal species.

- *Exophiala pheomuriformis* is one such rare organism belonging to the dematicious fungal genus.
- Phenotypic and genotypic similarities to *Sarcinomyces pheomuriformis* has resulted in the name of this particular strain.
- Although few other *Exophiala sp, E. dermatidis* and *E.jeanselmei* have been reported to cause corneal infection, fungal keratitis by *E.pheomuriformis* has never been reported.
- Here we report the first case of *Exophialaia phaeomuriformis* fungal keratitis

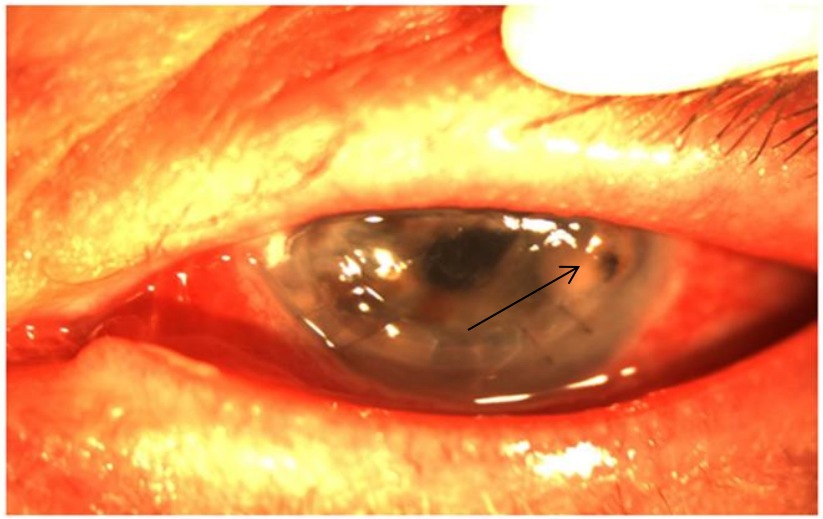
Case Report

84 year-old female

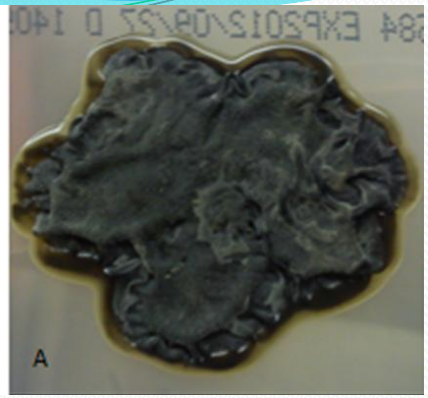
- Presentation: Pain, redness and itching in the left eye (OS) for 2 weeks. Had experienced a dishwasher water spill into her eyes 3 weeks back.
- Past ocular history: Penetrating keratoplasty (PKP) for pseudophakic bullous keratopathy OS 4 years back , using 1% prednisolone acetate once daily since PKP, in the left eye.
- Examination:

  BCVA – 20/30 right eye; Hand motion at 1 foot left eye

Slit lamp examination -
2.0 x 2.0 mm deep black infiltrate at the 4 o'clock position, inside the graft, with dark pigmentation on the superior area of the lesion without overlying epithelial defect.

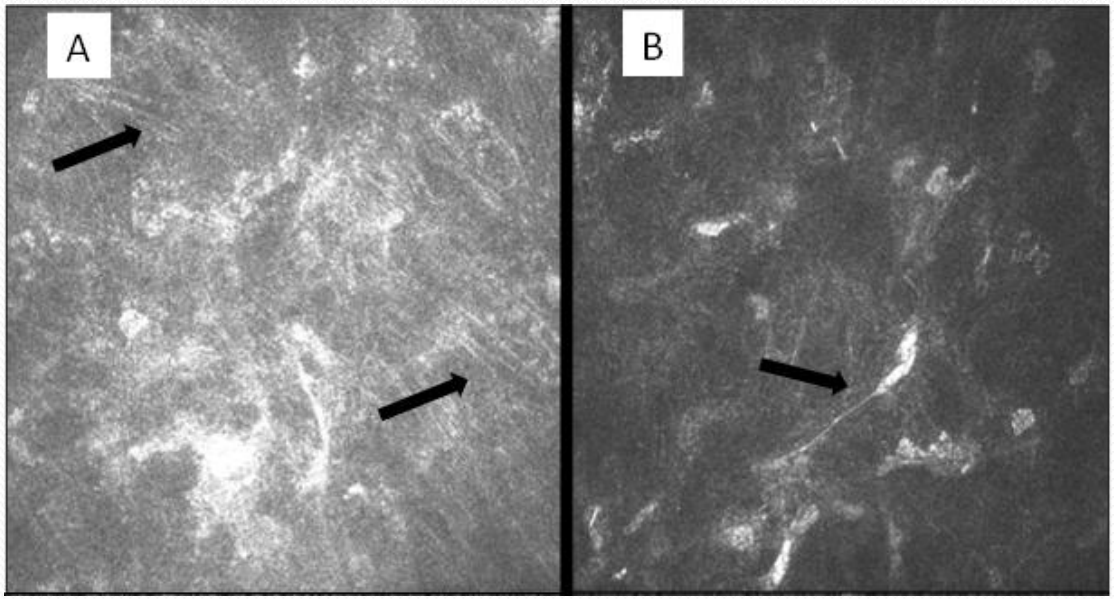


- Microbiological investigations:
  - No organisms on gram or fungal KOH stain.
  - Corneal cultures in Sabouraud dextrose agar (SDA) grew black yeast like colonies.



- Laser in vivo confocal microscopy (IVCM) of the cornea : HRT 3/RCM (Germany)

Thin filamentous structures in the stroma at a depth of 95 - 100µm which were highly suspicious for fungal elements (A, B).



- Treatment:
  - Hourly amphotericin B 0.15% ; voriconazole 1%; fortified antibiotics topically

- Progression:
  - BCVA worsened from HM at 1 foot to HM at 1 inch
  - Slit-lamp - black infiltrates of 2.0 x 2.0 mm at 9 o'clock with a 3.4 x1.2 mm temporal epithelial defect. Central KPs and a hypopyon were noted.

- Therapeutic PKP with intra-cameral injection of ceftazidime (2mg), vancomycin (1mg), and amphotericin B (10mcg) was done. During surgery the infiltrate was seen to extend to the inferior scleral margin.

- Post-surgical therapy: Amphotericin B 0.15% , voriconazole 1% drops every 2 hours, cyclosporine 2% 4x/d and moxifloxacin drops 4x/d,systemic voriconazole (200mg 2x/d).

- Follow-up:
  - Sequential IVCM imaging showed no fungal elements.
  - One year follow up showed a clear graft with no recurrence, BCVA 20/40.
  - The topical and systemic antifungal therapy was tapered over 5 months.
  - Prednisolone drops 1x/d and topical cyclosporine 2%4x/d continued.

Identification of organism:

- Fungus testing laboratory :

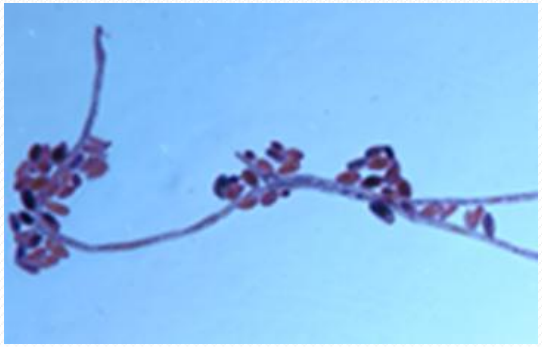
Physiologic features - temperature tolerance , nitrate assimilation

Molecular characterization - sequencing of the ribosomal DNA (rDNA) targeting internal transcribed spacer regions (ITS) and the D1/D2 domains.

Fungus identified as *Exophiala phaeomuriformis*.

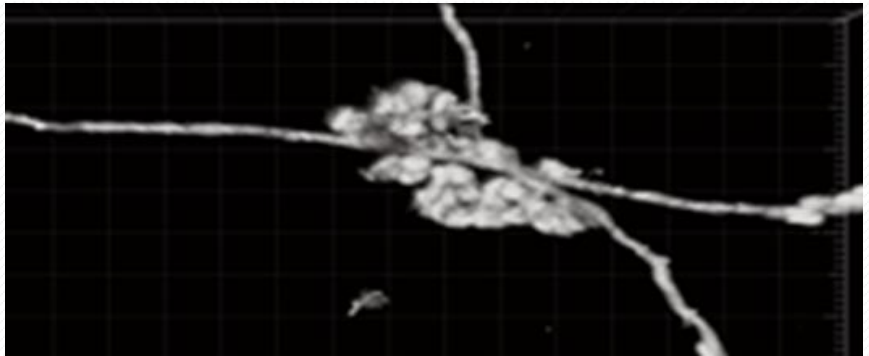
- Microscopic examination of the gram-stained smear:

Dimorphic fungi, septate, branched hyphae,
pigmented muriform cells with conidia. (600X Mag.)



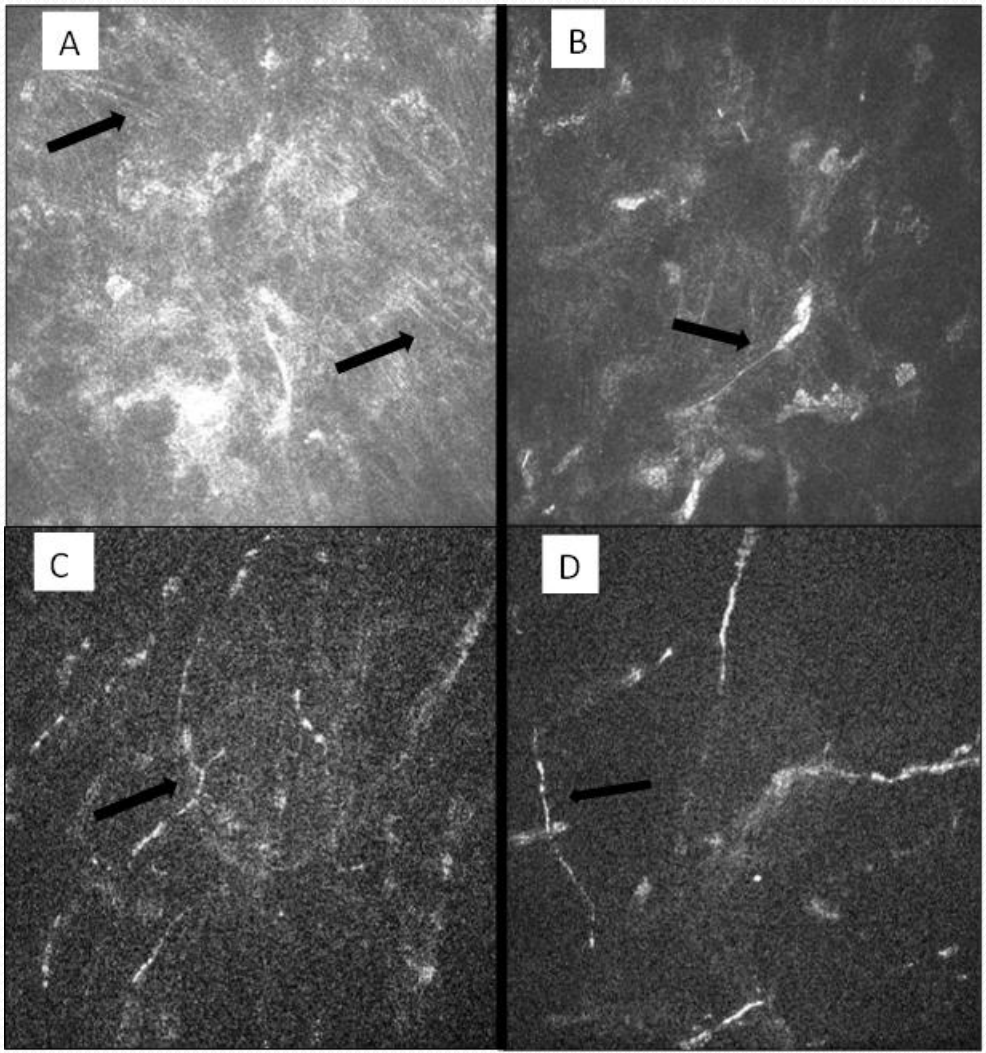
Multi-photon microscopy imaging of sealed gram-stained fungal smear:

Snapshot of 2Photon Microscopy (400X) with optical zoom 200%



- Validation of imaging to aid diagnosis in clinical suspicion:

IVCM done on patient cornea and on the sealed SDA culture plate.



Laser *in vivo* confocal microscopy of the patient's cornea showing thin filamentous structures at a depth of 95-100 µm in the stroma, suspicious of fungal elements. (A,B) and of the sealed Sabouraud dextrose agar culture plate with growth of *Exophiala pheomuriformis* showing filaments similar in thickness to the ones seen in patient's cornea. (C, D)

Discussion

- *Exophiala sps.* are melanized fungi belonging to the order Chaetothyriales , called “black yeasts” due to black yeast-like colonies on culture.
- Species are very similar microscopically, dimorphous having hyphae formed by chains of cells with conidia.
- Species differentiation is facilitated by physiologic features and molecular characterization.
- They are characterized as “microextremophiles” as they have them ability to tolerate extreme temperatures and saline environments*. E.sps. have* been recovered from hot and humid environments, such as saunas and steam baths.
- Household appliances, most commonly dishwashers, have been shown to harbor *E. dermatidis and E. phaeomuriformis* and are thus a potential source of fungal infections.

- Numerous members of the genus *Exophiala* are potential agents of human and animal mycoses.

- *E.sps.* are known to cause deep systemic infections (39.9%), cutaneous infections including skin, mucous membranes, nail, and corneal epithelial lesions (38.3%), superficial infections (0.5%, including hair) and subcutaneous infection (12.0%).

- The most common strains isolated from clinical infections have been *E. dermatitidis* (29.3%) and *E. xenobiotica* (19.7%). *E. phaeomuriformis* has been isolated in only 6.4% cases.

- To date, few cases keratitis due to *E. dermatidis* and E.jeanselmei have been reported.

- Our case, to the best of our knowledge, is the first case of keratitis caused by E. phaeomuriformis.
- Chronic topical steroid use (1% prednisolone 4 times a week) for over 4 years, and chronic dry eye disease were the risk factors in our patient.
- Interestingly, she was exposed to dirty water from dishwater spill about a week before developing the infection, which could be a likely source of exposure to E. phaeomuriformis.
- She was successfully treated with a therapeutic corneal transplant, followed by a 5 month course of antifungal therapy with systemic voriconazole, and has demonstrated no recurrence since one year.

Conclusion

- Imaging modalities like IVCM are being increasing used in infectious keratitis. We demonstrate through this case that IVCM is useful in not supporting diagnosis when there is clinical suspicion of infectious keratitis, but also to monitor treatment and evaluate for persistence or recurrence of infection.

- Diagnosis depends on a high degree of clinical suspicion, particularly when cultures show black yeast.

- Moreover, *in vivo* confocal microscopy and molecular diagnostic techniques are useful to suspect an early diagnosis and to identify the fungal species.

- Prompt and aggressive treatment is essential to prevent devastating ocular consequences and blindness.