Characterization of Ocular Fungal Infections in Egypt

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Ocular fungal infections, or oculomycosis, are being increasingly recognized as an important cause of morbidity and blindness. Keratitis is the most frequent presentation, but the orbit, lids, lacrimal apparatus, conjunctiva, sclera and intraocular structures may also be involved. The true extent of visual impairment is thought to far exceed the recognized prevalence, particularly among agricultural workers in the developing world, where a “silent epidemic” of corneal blindness has been postulated. Mycotic keratitis may account for more than 50% of all cases of culture-proven microbial keratitis, especially in tropical and subtropical areas. An overwhelming number of fungal genera and species have been implicated as causes of ophthalmic mycoses, depending on the geographical location and this number is steadily increasing. A rapid and accurate identification of the fungal species causing an ocular infection will permit the immediate institution of specific antifungal therapy. The aim of the present study was to evaluate the usefulness of polymerase chain reaction (PCR) compared with the conventional mycologic methods in the diagnosis of ocular fungal infections. Fifty subjects, in whom oculomycosis was suspected, and 20 controls were enrolled in the study. Specimens were properly collected and tested for the presence of fungi by microscopy, culture and PCR. Direct microscopy and culture were positive in 32 (64%) and 25 (50%) of the cases, respectively. Among the control group, direct microscopy and culture showed positive results in only 2 (10%) and 4 (20%) subjects, respectively. Species-specific PCR was positive for *C. albicans* and *A. fumigatus* in 6 (12%) and 7 (14%) cases, respectively, whereas it was positive for *A. fumigatus* in only one (5%) of the controls. The nonspecific fluorescent staining techniques and PCR are very promising methods, however, culture continues to provide many advantages.

INTRODUCTION

Ocular fungal infections (oculomycosis) remain a diagnostic and therapeutic challenge to the ophthalmologist. Difficulties are related to establishing a clinical diagnosis, isolating the etiological fungal agent in the laboratory and treating effectively with topical antifungal agents. Mycotic keratitis is undoubtedly the most common form of oculomycosis, whereas mycotic endophthalmitis most often follows trauma or intraocular operations. Mycotic keratitis has a worldwide distribution and its incidence is higher in tropical and subtropical countries; particularly among agricultural workers in rural areas; following traumatic implantation of fungal spores from soil or plant matter into the corneal stroma. More than 105 species of fungi; belonging to 56 genera; have been reported to cause oculomycosis. However, species of *Aspergillus, Candida* and *Fusarium* are the usual isolates from patients with mycotic keratitis. Diagnosis of oculomycosis is often delayed because of the poor availability of infected material and the slow growth of a large number of fungi on routinely used culture media. This has led to the development of culture-independent diagnostic tests such as fluorochromatic stains (e.g., Calcofluor white), immunological detection, identification of distinctive metabolites, nucleic acid probes and polymerase chain reaction (PCR).

The current study aims to (1) use PCR to detect fungal DNA in ocular specimens from patients clinically suspected to have oculomycosis; (2) compare the diagnostic accuracy of PCR analysis and standard microbiological techniques for diagnosing oculomycosis; (3) evaluate the practicality of PCR as a diagnostic method in studies of oculomycosis.

SUBJECTS & METHODS

Subjects:

Seventy subjects attending the outpatient clinic of Kasr El-Aini Teaching Hospitals were selected for this study, during the period from March through October 2005. They included 50 patients (39 cases of suspected keratomycosis and 11 cases of suspected endophthalmitis) as well as 20 healthy controls, belonging to similar socio-economic status, to determine the prevalent fungi. Cases were clinically, provisionally diagnosed, as having ocular mycosis and most of them...
came from agricultural areas with a history of trauma with vegetable or animal matter. Full clinical history was obtained as well as full ophthalmic examination and any previous treatments were reported. Patients already receiving antifungal drugs were excluded from the study. Informed consent was obtained from all subjects participating in this study.

**Specimens:** Three samples were taken from each subject: one sample for direct staining, the 2nd sample for culture and the 3rd sample was collected in sterile phosphate buffered saline (PBS, Dulbecco's GIBCO, BRL, Paisely, Scotland) and preserved at -70°C for PCR. Specimens included conjunctival swabs from cases of conjunctivitis (and also from controls), scrappings from the base, margin and the leading edge of the infiltrate from cases of corneal ulcers (by means of Kimura-like platinum spatula, using slit lamp bimicroscopy) and vitreous aspirate from cases of endophthalmitis (using sterile disposable 27 gauge needle with a tuberculin syringe).

**Methods:**

I. **Identification of fungi by direct smear using Spot Test Calcofluor White Reagent** (Difco Lab., Detroit, Michigan, USA), according to manufacturer's instructions. Briefly, the spot test was done by transferring the specimen on a clean glass slide and gently mixing it with two drops of 10% KOH. An equal volume of Calcofluor white reagent was dispensed and mixed onto the slide then mounted with a cover slip. The specimen was finally examined by a fluorescent microscope (Olympus BX40). Fungal elements display a brilliant apple-green fluorescence.

II. **Culture:** Specimens were immediately inoculated onto Sabouraud's dextrose agar (SDA, Oxoid) and Chromagar Candida (Chromagar Microbiology, France). Plates were incubated aerobically at 37°C. Chromagar Candida plates were incubated for 48 hours whereas SDA plates were incubated for 3 weeks at least. Plates were examined every 48 hours for evidence of growth. Suspected colonies on Chromagar Candida were identified according to manufacturer's instructions.

III. **Staining of the suspected fungal colonies by Lactophenol Cotton Blue** (Becton Dickinson Microbiology Systems, Maryland, USA) and visualization by light microscopy (Olympus BX50).

IV. **Identification of the different yeast colonies by API Candida** (Biomerieux, Lyon, France) using a 3 McFarland turbidity standard yeast suspension. The strips were incubated aerobically at 37°C for 24-48 hours.

V. **Detection of fungal DNA by semi-nested PCR:** A rapid semi-nested PCR approach for the detection of *C. albicans* or *A. fumigatus* was applied for all specimens (from cases and controls). A first round PCR was achieved using two outer primers corresponding to nucleotides 40 to 63 (forward primer, UNI-F) and 637 to 654 (reverse primer, UNI-R), derived from the large subunit ribosomal DNA (rDNA) complex of *Saccharomyces cerevisiae* V3 region. Identification of *C. albicans* or *A. fumigatus* was then achieved by a 2nd amplification reaction using species-specific inner forward primers (Ca-F or Af-F, respectively) in combination with the outer reverse primer (UNI-R). The following selected oligonucleotide primers were synthesized by a commercial vendor (Biosynthesis, Germany): Outer forward primer (UNI-F), 5'- GCA TAT CAA TAA GCG GAG GAA AAG -3'; Outer reverse primer (UNI-R), 5'- GGT CCG TGT TTC AAG ACG -3'; *C. albicans* (inner) forward primer (Ca-F), 5'- TTG GAG CGG CAG CAG GAT AAT GG -3' and *A. fumigatus* (inner) forward primer (Af-F), 5'- GCA TTC GTG CCG AAT TTG GAT CAT GAT AAT GG -3'.

DNA extraction was done with a QIAamp DNA Mini Kit (Qiagen, UK). Outer primers were included in a 50 µl first PCR reaction which contained 3 µl of extracted DNA, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 1.0 mM MgCl2, 20 µM of each dATP, dCTP, dGTP and dTTP, 0.5 unit of Taq DNA polymerase (all Fermentas, Germany) and 2 µM of each primer. The cycling parameters consisted of 30 cycles: 94°C for 30 sec (except for 5 minutes for the first cycle), 66°C for 90 sec and 72°C for 15 sec (except for 5 min for the last cycle; final extension). Species-specific PCR (30 cycles) was performed in exactly the same way as the first round PCR except that UNI-F was replaced with either Ca-F or Af-F primer, in addition to UNI-R primer. Aliquots of 1 µl of the 1st
PCR amplification products were used as targets in the species-specific PCR.

Post-PCR gel electrophoresis was performed on 2% agarose gel (Hispan agar, Spain), containing 0.5 µg/ml ethidium bromide (Amresco, USA) in 1% TBE buffer (GIBCO, BRL, Life Technologies, USA). The amplification products were visualized using Spectroline Bio-Vision UV/White Light Transilluminator and compared to a commercial molecular weight marker (ΦX174 DNA-HaeIII Digest, New England BioLabs), which gives bands at 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72 bp (N.B., 281 and 271 bp bands appear as a single band). The expected PCR product lengths were 156 bp and 192 bp for C. albicans and A. fumigatus, respectively. Negative (sterile distilled water) and positive controls [C. albicans standard strain (ATCC 10232) and A. fumigatus standard strain (ATCC 10827)] were included in each PCR run.

**Statistical Analysis:** Data were analyzed using Statistical Package for Social Sciences (SPSS) software, Version 11. P value < 0.05 was considered significant.

**RESULTS:**

The present study included 50 cases (30 males and 20 females) of clinically suspected oculomycosis [39 cases (78%) of keratoconjunctivitis and 11 cases (22%) of endophthalmitis] as the study group (cases), in addition to 20 healthy individuals (10 males and 10 females) serving as the control group.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Cases (N° = 50)</th>
<th>Controls (N° = 20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
<td>No</td>
</tr>
<tr>
<td>Yeasts</td>
<td>12</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>Hyphae</td>
<td>20</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>32</td>
<td>64</td>
<td>2</td>
</tr>
</tbody>
</table>

![Figure (1): Calcofluor white stained specimens showing:](image)

1-a: Budding oval cells of C. albicans
1-b: Germ tube of C. albicans
1-c: Hyphal elements of septate filamentous fungi
Table (2): Comparison between the isolation rates of different fungal species on Sabouraud's dextrose agar (SDA) and Chromagar Candida among cases and controls

<table>
<thead>
<tr>
<th></th>
<th>SDA</th>
<th>Chromagar Candida</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td></td>
<td>N°</td>
<td>%</td>
</tr>
<tr>
<td>No growth</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Candida Spp.*</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Aspergillus spp.**</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Penicillium</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Alternaria</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Curvularia</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Rhodotorula rubra</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

N.B.: *Isolated candida species included 4 C. albicans, 2 C. krusei, 1 C. parapsilosis and 1 C. tropicalis among cases, whereas only 1 C. parapsilosis was identified among controls (confirmed by API Candida).

** Isolated Aspergillus species included 4 A. fumigatus, 2 A. niger and 2 A. flavus among cases, whereas only 1 A. fumigatus was isolated from controls (confirmed by lactophenol cotton blue stain).

Figure (2): Lactophenol cotton blue stain of:
2-a: A. fumigatus (showing conidiophore having a flask-shaped swollen end called vesicle).
2-b: A. flavus (showing conidiophore arising from a foot cell and terminating in a vesicle, which produces phialides in one or two series and unicellular conidia).
2-c: A. niger (showing black spore heads).
2-d: Penicillium (showing the brush appearance)
2-e: Alternaria (showing multi-celled conidium).
2-f: C. albicans (showing the characteristically asexual budding).
Table (3): Results of culture in relation to the clinical diagnosis of cases

<table>
<thead>
<tr>
<th>Culture Results</th>
<th>Keratoconjunctivitis</th>
<th>Endophthalmitis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>No growth</td>
<td>22</td>
<td>56.4</td>
</tr>
<tr>
<td>Aspergillus species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. fumigatus</td>
<td>6</td>
<td>15.4</td>
</tr>
<tr>
<td>A. niger</td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td>A. flavus</td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td>Candida species</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. albicans</td>
<td>4</td>
<td>10.3</td>
</tr>
<tr>
<td>C. krusei</td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Penicillium</td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td>Alternaria</td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td>Curvularia</td>
<td>2</td>
<td>5.1</td>
</tr>
<tr>
<td>Rhodotorula rubra</td>
<td>1</td>
<td>2.6</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>100</td>
</tr>
</tbody>
</table>

N.B.: - Positive fungal culture was obtained in 17 cases (43.6%) of keratoconjunctivitis and in 8 cases (72.7%) of endophthalmitis.

Comparison between keratoconjunctivitis and endophthalmitis cases, regarding the common predisposing factors, revealed that out of the 39 cases of keratoconjunctivitis, 22 cases (88%) had a definite history of trauma and 3 cases (12%) were post-surgical. However, out of the 11 cases of endophthalmitis, 9 cases were exogenous endophthalmitis [6 cases (54.5%); following cataract operations and 3 cases (27.2%); following trauma] and 2 cases (18.2%) were endogenous endophthalmitis.

Table (4): Comparison between direct smear and culture, regarding detection of mycosis among cases

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N°</td>
<td>%</td>
<td>N°</td>
</tr>
<tr>
<td>Direct Smeara</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>24</td>
<td>48</td>
<td>8</td>
</tr>
<tr>
<td>Negative</td>
<td>1</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>50</td>
<td>25</td>
</tr>
</tbody>
</table>

a Sensitivity = 96% Specificity = 68% Accuracy = 82%
Positive Predictive Value (PPV) = 75% Negative Predictive Value (NPV) = 94.4%

API Candida results revealed 4 isolates of C. albicans, 2 isolates of C. krusei, 1 isolate of C. tropicalis and 1 isolate of C. parapsilosis (a total of 8 Candida isolates) among cases, whereas among controls, API Candida could identify only one Candida species (C. parapsilosis). There was a complete agreement (100%) between the results of Chromagar Candida and API Candida in the identification of the different Candida species.

Among the 50 cases of suspected ocular mycosis, PCR - performed directly on clinical specimens - revealed 6 positive (12%) C. albicans and 7 positive (14%) A. fumigatus. However, among controls, only one positive A. fumigatus (5%) was detected by PCR. No statistically significant difference was found - regarding the detection rates of A. fumigatus by PCR - between cases and controls (P value 0.28). Compared to culture, PCR proved to be 100% sensitive in the detection of C. albicans and A. fumigatus.
Figure (3): Gel electrophoresis showing C. albicans and A. fumigatus - PCR products
Lane 1: positive control for A. fumigatus (ATCC 10827), giving band at 192 bp. Lanes 2 and 3: positive samples for A. fumigatus. Lane 4: PCR molecular weight marker (ΦX174 DNA-HaeIII Digest, New England BioLabs), which gives bands at 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72 bp (281 and 271 bp bands appearing as a single band). Lane 5: positive control for C. albicans (ATCC 10232), giving band at 156 bp. Lanes 6 and 7: positive samples for C. albicans. Lane 8: negative control (H2O).

Table (5): Results of culture and PCR analysis for C. albicans of specimens from patients with presumed oculomycosis

<table>
<thead>
<tr>
<th>Species-Specific PCR for C. albicansa</th>
<th>C. albicans Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>N°</td>
<td>%</td>
</tr>
<tr>
<td>-----</td>
<td>-------</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
</tr>
</tbody>
</table>

a Sensitivity = 100% Specificity = 95.7% Accuracy = 97.9%
Positive Predictive Value (PPV) = 66.7% Negative Predictive Value (NPV) = 100%

Table (6): Results of culture and PCR analysis for A. fumigatus of specimens from patients with presumed oculomycosis

<table>
<thead>
<tr>
<th>Species-Specific PCR for A. fumigatusa</th>
<th>A. fumigatus Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>N°</td>
<td>%</td>
</tr>
<tr>
<td>-----</td>
<td>-------</td>
</tr>
<tr>
<td>Positive</td>
<td>4</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
</tr>
</tbody>
</table>

a Sensitivity = 100% Specificity = 93.5% Accuracy = 96.8%
Positive Predictive Value (PPV) = 57.1% Negative Predictive Value (NPV) = 100%

DISCUSSION
Ocular infections due to opportunistic fungal pathogens are steadily increasing. Fungal keratitis is a major ophthalmologic problem in the tropical regions of the world and it is one of the frequent causes of corneal damage in developing countries.
Preservation of vision requires early diagnosis and early institution of fungal therapy. Non-specific fluorochromatic stains have become popular for the detection of fungi in ocular samples. Calcofluor white appears to be the most widely used of these stains. In the present study, 50 cases of suspected oculomycosis were investigated. Direct microscopic examination with Calcofluor white stain, mounted with 10% KOH revealed fungal elements in 32 cases (64%), whereas positive fungal cultures; on different culture media; were obtained in 25 cases (50%). Direct smear and fungal culture matched (were both positive or negative) in 41 (82%) of the 50 specimens from cases. Similar results were reported by Nag et al. who stated that a wet mount with 10% KOH showed fungal elements in 68% of corneal ulcers, while culture was positive in only 26.4% of the cases. Moreover, other studies reported higher sensitivity of fungal detection using KOH and Calcofluor white stain than culture. Calcofluor white sensitivity of 80–90% in culture-proven mycotic keratitis has been reported. This agrees with our results, where Calcofluor white stain showed a sensitivity of 96% in culture-proven cases of oculomycosis. Calcofluor white fluorescent staining proved to be a simple technique that overcomes the difficulties and time involved in staining.

Even with the advent of many new techniques, culture remains the “gold standard” and the cornerstone of the diagnosis of most ophthalmic mycoses, except for rhinosporidiosis, where direct microscopic examination of samples yields more reliable results. Wherever possible, it is best to use more than one culture medium, and to incubate these at 37°C and at 25-30°C for the optimal recovery of ocular fungi.

Our culture results among cases (showing 50% positivity) are similar to those of El-Sawy et al. and El-Mowafy et al. who detected fungal isolation from cultures in 45% and 47.5% of cases of clinically suspected fungal keratitis, respectively.

In the present study, positive fungal culture was obtained in 17 cases (43.6%) of keratoconjunctivitis. The most prevalent isolates were Aspergillus species then Candida species, followed by Penicillium, Alternaria, Curvularia and lastly Rhodotorula rubra. This agrees with the results of Tanure et al. On the other hand, different fungal species were isolated in 8 cases (72.7%) of endophthalmitis; the most common were Candida species followed by Aspergillus fumigatus and lastly Penicillium.

Internationally, Aspergillus species is the most common isolate (27%-64%) in cases of fungal keratitis worldwide, followed by fusarium (6%-32%) and penicillium (2%-29%). Similar studies also concluded that filamentous fungi are the principal causes of mycotic keratitis in most parts of the world; either Aspergillus spp. or Fusarium spp. were the most common isolates. Dematiaceous fungi, such as Curvularia spp. and Bipolaris spp., are the third most important cause of keratitis in a number of studies. A study conducted in Saudi Arabia, concluded that the higher prevalence of Aspergillus species; among cases of keratitis; could be explained by the fact that its spores could survive the hot dry weather, as is the case in Egypt.

Keratitis; due to yeasts and yeast-like fungi; is most frequently caused by C. albicans. Keratitis due to this organism tends to occur more frequently in areas where traumatic keratitis is uncommon, but where other predisposing factors causing epithelial or stromal ulceration are important, for example, due to previous herpes simplex keratitis, contact lens-induced corneal abrasions or in patients with dry eyes. C. albicans and related fungi have been infrequent isolates in most studies performed in tropical countries, possibly due to the predominance of livelihoods, such as agriculture, which carry a higher risk for the occurrence of trauma-related keratitis caused by filamentous fungi than for keratitis due to C. albicans.

In the present study, of the 39 cases of keratoconjunctivitis, 22 cases (88%) had a definite history of trauma and 3 cases (12%) were post-surgical. Moreover, of the 11 cases of endophthalmitis, 6 cases (54.5%) were clinically manifested following cataract operations, 3 cases (27.2%) followed trauma and 2 cases (18.2%) occurred secondary to hematogenous dissemination in patients having debilitating diseases (endogenous endophthalmitis).

Many studies reported that a definite history of ocular trauma (usually with vegetable matter) was the most common...
predisposing factor for mycotic keratitis, (occurring in 44 to 55% of patients); less frequently reported risk factors include prolonged use of topical corticosteroids or anti-bacterials, systemic diseases such as diabetes mellitus, pre-existing ocular diseases, contact lens wear and previous ocular surgery. On the other hand, mycotic endophthalmitis has been reported to occur after cataract surgery (61%), trauma (28%) or as metastatic endogenous endophthalmitis (11%). The yeast *C. albicans* is the most common cause of endogenous endophthalmitis. Moreover, *Candida* species are particularly likely to cause exogenous endophthalmitis. In this setting, infection may be due to peri-operative contamination of lens prostheses or contamination of fluids used for irrigation of the eye. Infection may be also enhanced by the pre- and post-operative use of topical corticosteroids and anti-bacterial agents. However, *Aspergillus* endophthalmitis is the commonest type of vision-threatening fungal endophthalmitis encountered in India.

Our results revealed that Sabouraud's dextrose agar allowed for the recovery of most fungal pathogens within 2 weeks of incubation. Generally, the duration of incubation - for most yeasts - is one week, although the range of time for recovery of dimorphic fungi may extend to 4-6 weeks. The present study also showed a complete agreement (100%) between the results of Chromagar Candida and API Candida in the identification of the different *Candida* species, however, the yeast colony color development (on Chromagar Candida) or its proper identification (by API Candida) was better reported after 48 hours of incubation, as previously noted. This delay in the identification by cultural and/or biochemical procedures or the limited yield of vitreous cultures (in cases of endophthalmitis) has led to a search for more rapid techniques. The speed and sensitivity of PCR makes it an ideal choice for the basis of a rapid identification system. PCR has been reported to be a more sensitive and rapid diagnostic tool, compared to the conventional mycologic methods in the diagnosis of oculomycosis, where 55.8% of cases of fungal endophthalmitis were positive by conventional methods versus 74.4% by PCR. Similarly, Hidalgo *et al.* and Lohmann *et al.* concluded that PCR had a higher rate of positive fungal identification than by microscopy or diagnostic culture. This is similar to our results.

Using species-specific primers in the present study, PCR could detect 6 positive *C. albicans* (12%) and 7 positive *A. fumigatus* (14%) among cases of suspected oculomycosis; versus only 1 positive *A. fumigatus* (5%) among the control group. No statistically significant difference was found regarding the detection rates of *A. fumigatus* by PCR - between cases and controls (P value 0.28), which might be explained by the small sample size included in the study. However, using culture, 4 *C. albicans* (8%) and 4 *A. fumigatus* (8%) isolates were recovered from cases and only one *A. fumigatus* isolate was recovered from controls. Among the 50 cases in the present study, PCR and fungal culture for *C. albicans* and *A. fumigatus* matched in 48 (96%) and 47 (94%) of the specimens, respectively. In 2 (4%) and 3 (6%) case specimens, PCR detected *C. albicans* and *A. fumigatus*, respectively, where no organism was found in culture. All of these patients appeared clinically to have ocular fungal infections, and fungi were present on Calcofluor white smear for all.

PCR would probably be most valuable in providing a positive result in a shorter period than that required for culture and in identification of a fungal isolate which does not sporulate. Moreover, PCR offers the ability to analyze specimens far from where they are collected. Eventually, PCR might solidly complement the current “gold standard” diagnostic techniques for guiding management or supporting research studies of oculomycosis. However, concern persists regarding the specificity of this technique and the problems that may arise from the production of false-positive results. PCR does not distinguish viable from non-viable organisms; it may therefore be difficult to assess the relevance of a positive PCR result in a healing corneal ulcer, where culture is negative, or in locations such as the conjunctival sac, where fungi may be found as transient commensals. Although PCR is extremely sensitive and specific, it cannot be used to monitor the patient’s response to treatment. Moreover, a few culture media will suffice to detect and grow the common
ocular pathogens, but PCR must be multiplexed for each microorganism that is suspected. The difficulty of DNA extraction (some filamentous fungi have a sturdy cell wall; which is resistant to standard DNA extraction procedures) and the presence of PCR inhibitors in human specimens are some of the difficulties encountered with fungal detection in ocular samples. Finally, PCR can detect only fungi for which the DNA sequence is known and primers are available.

To conclude, the increased awareness of ocular fungal infections, the better recognition of their clinical features and the improved laboratory diagnostic techniques, will all lead to an increase in the frequency of correct diagnosis. A rapid and accurate diagnosis of oculomycosis will improve the chances of a complete recovery, especially in the tropics, where patients may delay presenting to an ophthalmologist. Calcofluor white fluorescent staining proved to be a simple and reliable technique for initiation of antifungal therapy. Culture methods remain important diagnostic tools that add to the proper identification and characterization of oculomycosis, however, new culture media need to be developed. PCR is a potentially valuable technique for diagnosing ocular fungal pathogens, particularly, if panfungal primers are used and optimized. Although PCR is expensive and requires laboratory expertise, yet the loss of an eye, due to delay in laboratory diagnosis, is devastating.

References:
36. Srinivasan M, Gonzales CA, George C, et al. (1997): Epidemiology and
دراسات

القصة: 


الشرح: 


الاستنتاج: 


الاستشارة: 

تفضلون، إذا كنت بحاجة إلى مزيد من المعلومات، فلقد نشرنا هذا البحث في [نسبة] من الحالات. 

ال受访者: 