Utility of Molecular Sequence Analysis of the ITS rRNA Region for Identification of *Fusarium* spp. from Ocular Sources

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** Purpose.** Fungal ocular infections cause significant ocular morbidity, particularly when diagnosis and treatment are delayed. Accurate morphologic identification of *Fusarium* spp. beyond the genus is time-consuming and insensitive. It was the purpose of this study to examine the usefulness of the nuclear ribosomal RNA (rRNA) internal transcribed spacer regions (ITS1 and -2) to detect and differentiate *Fusarium* spp. responsible for ocular infections.

**Methods.** Fifty-eight archived isolates from ocular sources of 52 patients diagnosed with *Fusarium* keratitis at the Bascom Palmer Eye Institute (Miami, FL) from April 2000 to May 2007 were analyzed. The archived samples, which were initially classified according to morphologic characteristics, were analyzed by DNA sequence data generated from the ITS regions of the rRNA genes.

**Results.** Fifteen distinct sequences were identified among the 58 isolates. Sequence analysis identified the isolates as *Fusarium solani* (75%), *F. oxysporum* (16%), *F. incarnatum-equiseti* (5%), *F. dimereum* (2%), and one *Fusarium* sp. (2%) that was not classified within any species complex. Species identification based on sequence data correlated well with the morphologic classification when performed by a mycology reference laboratory, but a higher rate of mismatch was observed based on identification by a nonreference laboratory.

**Conclusions.** Most of the isolates of *Fusarium* ocular infections belong to the *F. solani* or *F. oxysporum* species complexes. Morphologic classification at the species level yielded inconsistent results at a general microbiology laboratory. In contrast, the sequence variation within the ITS region allowed reliable and faster discrimination of the isolates at both the genus and species level. (Invest Ophthal Vis Sci. 2009;50:2230–2236) DOI:10.1167/iovs.08-2757

Fungal corneal ulcers are a prevalent cause of ocular morbidity throughout the world,1 and the incidence has increased significantly over the past three decades,2,3 largely as the result of increases in patient risk factor profiles. The etiology of infectious keratitis is variable and largely dependent on geographic and climatic variables, as well as patient risk factor profiles. Fungal organisms are the etiologic agent in 1.2% to 62% of infectious keratitis case and are significantly more common in tropical and subtropical regions.4–8 *Fusarium* is the most common genus isolated in cases of fungal keratitis in tropical and subtropical regions, where they represent as much as 45% to 73% of fungal keratitis cases.2,4–7,9

Diagnosis of fungal keratitis is challenging due to limitations in culture- and microscopy-based methods of identification. Culture-based methods are labor intensive and time consuming, requiring specially trained mycologists to properly identify isolates at the species level, typically yielding positive results 5 to 7 days after initial presentation. Identification based on histopathologic examination of corneal scrapings allows for a rapid diagnosis of a fungal infection, but has highly variable sensitivity and is less effective at differentiating isolates at the species level.8 This delay in identification of the etiologic agent and initiation of appropriate antimicrobial coverage undoubtedly results in increased ocular morbidity. Molecular diagnostic techniques have been suggested to address these limitations and can significantly improve our diagnostic capabilities as well as patient outcomes in the future.

The increasing reports of *Fusarium* as a human pathogen in ocular infections have generated an interest in finding a more rapid, accurate, and consistent method for diagnosis and classification. Fortunately, numerous molecular techniques, including PCR-based technology and microarray technology have shown promise in offering a solution in both human and animal experimental models.10–20 Recent genotyping studies have shown that DNA sequence-based methods are useful for species identification and subtyping of *Fusarium*.11–17,21–22

Accurate species identification of *Fusarium* spp. is important not only in promoting our understanding of the spectrum of fusaria that are pathogenic to the eye, but in gaining important prognostic and therapeutic information for the patient and clinician in the future.

Many different genes have provided the basis for the development of molecular-based identification techniques, including ribosomal RNA (rRNA), translation elongation factor (EF-1α), the second largest subunit of RNA polymerase II (RPB2), and domains D1/D2 of the nuclear large subunit (LSU).17,21,23,24 Many fungal genotyping studies have relied on the rRNA gene complex for rapid species identification.15,14,23–30 This gene family comprises four ribosomal

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rRNA genes: the 26 to 28S, 18S, 5.8S, and 5S genes, which are arranged as head-to-tail tandem repeats separated by the spacer regions, the internal transcribed spacer region (ITS) and the intergenic spacer region (IGS; Fig. 1). Although rRNA genes are highly conserved, the ITS regions are typically variable and rich in informative sites; hence, their usefulness for phylogenetic classification. Although the ability of the ITS region to differentiate medically important species within *Fusarium* species, complexes have been reported, and no study has been undertaken to fully examine the utility of the allelic variability within this single locus to differentiate *Fusarium* spp. isolates from ocular sources alone. In this study, we employed sequence data analysis of the ITS region as well as traditional morphologic classification to elucidate the phylogenetic diversity of *Fusarium* spp. strains isolated from ocular tissue and contact lens paraphernalia of patients with *Fusarium* keratitis in south Florida.

**METHODS**

**Fungal Isolates**

A total of 58 fungal isolates from ocular sources in 52 patients were selected from the archived samples at the Bascom Palmer Eye Institute’s (University of Miami) ocular microbiology laboratory (BPEI-OML). The isolates were obtained from patients with culture-proven infectious keratitis, which had been examined at BPEI between April 2000 and May 2007. The isolates were obtained from 41 corneas, four aqueous humor samples, one vitreous sample, eight contact lenses, and four contact lens cases (Table 1). The archived isolates were retrieved from their lyophilized state at BPEI-OML and grown in pure culture supplemented with peptone-yeast-glucose (PYG; Remel, Lenexa, KS) media or on Sabouraud dextrose agar. Species identification was provided for 30 isolates at BPEI-OML. In addition, 30 of 58 isolates (due to funding restraints) were sent to the reference fungal laboratory, Texas Fungus Testing Laboratory (TFTL; University of Texas Health and Science Center, San Antonio, TX) for species identification after molecular characterization. The 30 isolates were selected after genotypic characterization to include all the non-*F. solani* isolates as well as 17 randomly selected *F. solani* isolates. Traditional identification of filamentous molds were performed by using a combination of macroscopic (texture, color, growth rate, and media) and microscopic techniques (conidia formation).

**DNA Extraction**

The fungi were grown on Sabouraud dextrose agar plates for 7 days, transferred to 15-mL centrifuge tubes containing 2 mL of PBS solution, and centrifuged at 3000 rpm for 10 minutes. The supernatant was removed, and DNA was isolated by a modified UNSET procedure. Briefly, 1 mL of UNSET lysis buffer (urea 8 M, sodium dodecyl sulfate [SDS] 2%, NaCl 0.15 M, EDTA 0.001 M, Tris [pH 7.5] 0.1 M) was added to the fungal pellets and vortexed for 5 seconds. An equal volume of phenol/chloroform/isooamyl alcohol (25:24:1) was added to the solution, along with 0.3 g of acid-washed glass beads, and the tubes vortexed for 5 minutes. The samples were centrifuged for an additional 5 minutes at 13,000 rpm, and the aqueous phase was transferred to a new 1.5-mL tube (Eppendorf, Westbury, NY). The phenol/chloroform/isooamyl alcohol extraction procedure was repeated until satisfactory removal of the protein interface was achieved. The aqueous phase was precipitated with a 1/10th volume of 3 M sodium acetate and 1 volume of isopropanol addition. The tubes were centrifuged for 10 minutes at 13,200 rpm at 4°C and the supernatant was decanted. The DNA pellets were rinsed with 1 mL of 70% ethanol, and the tubes were centrifuged again for 10 minutes at 13,200 rpm. The supernatant was then decanted and residual ethanol was removed. The remaining ethanol was evaporated by using a vacuum centrifuge at 45°C for 2 minutes. The resultant DNA pellet was resuspended in 30 μL of distilled water and the genomic DNA was stored at −20°C.

The quality and concentration of the genomic DNA was assessed through spectrophotometric determination of the UV absorbance 260/280 nm ratio (Bio Photometer; Eppendorf) and by gel electrophoresis of the sample with a 1-kb molecular weight ladder (Invitrogen, Carlsbad, CA).

**DNA Amplification**

PCR reactions were performed with 200 ng of genomic DNA. The primers F18S (5′-CCGAGGGATCATTACCGAGTT-3′) and F28S (5′-CAGCGGGATTCTACTGAGTC-3′) (Invitrogen) were designed to specifically target *Fusarium* spp. F18S primer is located at the end of the 18S ribosomal DNA at position 520 to 540 of *F. solani* reference strain Fs-27 (GenBank EF432243; http://www.ncbi.nlm.nih.gov/Genbank; provided in the public domain by the National Center for Biotechnology Information, Bethesda, MD), and F28S is located at the beginning of the 28S rDNA at position 41-62 of the sequence EF432243. The described set of primers amplified the internal transcribed spacer region comprising ITS1, ITS2, and 5.8S rRNA. The PCR reaction was performed with a polymerase system kit (Accuprime *Taq* DNA Polymerase System; Invitrogen). The 25-μL PCR mixture contained 1 μL (200 ng) of DNA template, 2.5 μL of buffer II solution (containing the dNTPs and MgCl2), 1 μL of each 10 μM primer (F18S and F28S), 1 μL of *Taq* DNA polymerase (Invitrogen), and 18.5 μL of distilled water. PCR reactions were performed in a thermocycler (iCycler; Bio-Rad, Hercules, CA) and used 1 cycle at 95°C for 3 minutes, followed by 45 cycles with a denaturation step at 95°C for 30 seconds, an annealing step at 55°C for 30 seconds, and an extension step at 68°C for 2 minutes. A negative control was included in all experiments. The detection of amplified products was performed by electrophoresis of an aliquot of 5 μL of each amplion with an agarose gel with ethidium bromide 0.02% in 1× Tris-acetate-EDTA (TAE) buffer. The DNA bands were visualized under UV illumination (Universal Hood II; Bio-Rad). A 1-kb molecular weight ladder (Invitrogen) was included in each run. PCR products were purified with a spin kit (GeneClean; MP Biomedicals, Solon, OH) according to the manufacturer’s instructions.

**DNA Sequencing**

Direct sequencing of PCR products was performed by Genewiz, Inc. (South Plainfield, NJ, with BigDye version 3.1; Applied Biosystems, Inc. [ABI], Foster City, CA). The reactions were run on a DNA analyzer (model 3730; ABI). The PCR amplification primers, F18S and F28S, were used as the sequencing primers, and the PCR products were sequenced on both strands in duplicate or triplicate to assure sequence fidelity.

**Phylogenetic Analysis**

Two hundred sixty-three sequences, which varied in size from 509 to 535 base pairs (bp), were aligned (MegaAlign; DNASTar, WI) and adjusted manually to form the consensus sequences for the 58 isolates. The percent identity of the aligned sequences was also determined with the same program. The phylogenetic trees were computed (MEGA 4.0; Molecular Evolutionary Genetic Analysis software, ver. 4.0; http://www.megasoftware.net) using maximum-parsimony analysis. The sequence of the ITS region from the pathogenic fungus, *Lecanicillium lecanii* was used as the outgroup sample (GenBank accession number, DQ007051). For the purposes of comparison and to have a

![Figure 1](image-url)
more complete representation of the spectrum of ophthalmologically important fusaria, the ITS region from 11 isolates, deposited into GenBank by O’Donnell et al.,21 were included in the data set (USDA identification codes: NRRL 43529, 43536, 43445, 43468, 43489, 43375, 25483, 43656, 13604, 43504, 43726). The neighbor-joining tree used the Kimura two-parameter distance algorithm, produced in MEGA 4. Bootstrap values for the MPT were obtained from a consensus tree based on 100 randomly generated data sets with jumbled sequence addition. All positions containing gaps were eliminated from the dataset.

Table 1. Summary of Fusarium spp. Isolates Examined

<table>
<thead>
<tr>
<th>Patient</th>
<th>Source</th>
<th>Year</th>
<th>Classification</th>
<th>Reference Lab Classification</th>
<th>Genotype</th>
<th>Haplotype</th>
<th>GenBank Accession Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cornea</td>
<td>2005</td>
<td>F. solani</td>
<td>FSSC</td>
<td>FSSC</td>
<td>a</td>
<td>EU71672*</td>
</tr>
<tr>
<td>2</td>
<td>CL case</td>
<td>2006</td>
<td>Fusarium sp.</td>
<td>FSSC</td>
<td>FSSC</td>
<td>a</td>
<td>EU71721</td>
</tr>
<tr>
<td>3</td>
<td>Cornea</td>
<td>2006</td>
<td>F. oxysporum</td>
<td>FSSC</td>
<td>FSSC</td>
<td>b</td>
<td>EU71688*</td>
</tr>
<tr>
<td>4</td>
<td>CL</td>
<td>2003</td>
<td>Fusarium sp.</td>
<td>—</td>
<td>FSSC</td>
<td>a</td>
<td>EU71708</td>
</tr>
<tr>
<td>5</td>
<td>Cornea</td>
<td>2006</td>
<td>Fusarium sp.</td>
<td>FSSC</td>
<td>FSSC</td>
<td>a</td>
<td>EU71704</td>
</tr>
<tr>
<td>6</td>
<td>CL</td>
<td>2006</td>
<td>Fusarium sp.</td>
<td>FSSC</td>
<td>FSSC</td>
<td>b</td>
<td>EU71683</td>
</tr>
<tr>
<td>7</td>
<td>Cornea</td>
<td>2004</td>
<td>Fusarium sp.</td>
<td>—</td>
<td>FSSC</td>
<td>b</td>
<td>EU71701</td>
</tr>
<tr>
<td>8</td>
<td>Cornea</td>
<td>2006</td>
<td>Fusarium sp.</td>
<td>—</td>
<td>FSSC</td>
<td>b</td>
<td>EU71671</td>
</tr>
</tbody>
</table>

CL, contact lens; AC, anterior chamber. The remaining abbreviations are defined in Figure 2.

* Used to build the maximum parsimony tree (Fig. 2).
† Patients with two isolates from the same eye.

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Nucleotide Sequence Accession Numbers

All DNA sequence data reported in this article have been deposited in GenBank under accession numbers: EU721670 to EU721727.

RESULTS

The 58 isolates analyzed in this study are shown in Table 1. Morphologic classification at BPEI-OML identified 28 isolates to the genus level (Fusarium spp.), 19 as F. oxysporum, and 11 as F. solani. Thirty of the isolates were sent to the TFTL for morphologic confirmation. Of these, 17 were classified as belonging to the F. solani species complex (FSSC), 9 to the F. oxysporum species complex (FOSC), and 4 to the F. incarnatum-equisetii species complex (FIESC). Compared with identification based on sequence analysis, the species identification performed by TFTL was 100% accurate, whereas that performed by BPEI-OML was 50% accurate.

Sequence analysis of the 58 ITS consensus sequences (509–535 bp) identified 15 unique haplotypes, 8 of which comprised multiple isolates (Table 1). Nucleotide differences among the isolates ranged from 0% to 18% within and between species (Table 2). Figure 2 shows a maximum parsimony tree (MPT) rooted by the outgroup L. lecanii, a close relative to the Fusarium genus The MPT (Fig. 2) shows supported resolution of the isolates into their particular species complex as indicated by bootstrap values ranging from 74% to 99% for the four species complexes identified in this study. Four major species complexes were represented in the ocular infections (Table 3): F. solani (EU721674), F. oxysporum (EU721680), F. incarnatum-equisetii (FIESC; 5%), F. dimerum (FDSC; 2%), and one isolate (2%) that did not fit any species complex. The phylogenetic relationships between isolates within the FSSC were unresolved because of the lack of informative sites with this group (Table 2, Fig. 2).

The FSSC grouping was well supported by a terminal bootstrap value of 99%. The distance-based phylogenetic reconstruction also supported this grouping based on the small evolutionary distances within the FSSC. Isolates within this group had differences ranging from 0% to 4% and evolutionary distance less than 3.8% (Table 2). EU721706, EU721716, and EU721726, which share a unique sequence and were morphologically classified as F. incarnatum-equisetii, differed from members of the FOSC by less than 5%. This grouping was also supported by a terminal bootstrap value of 99%. The isolate EU721689, morphologically classified as F. incarnatum-equisetii by TFTL and as F. roseum by AOML, differed from members of the other species complexes by 14% to 18%. It was most similar to the FCSC (14% sequence variability) and least similar to FOSC (18% sequence variability), suggesting that it represents a new complex group. A database search of GenBank identified the isolate as Colletotrichum sp., Nectria sp., or Fusarium sp.

DISCUSSION

In this study, we examined the genetic diversity of Fusarium spp. isolates derived from patients with infectious keratitis diagnosed at the Bascom Palmer Eye Institute. Toward this end, we studied the feasibility of using the ITS region for the detection and identification of Fusarium spp. from ocular sources to the species level. In addition, we compared this molecular classification technique to classification based on examination of morphologic characteristics.

Fusarium spp. isolates causing ocular infections were classified within four main species complexes—FSSC, FOSC, FIESC, and FDSC—with a relative frequency similar to that previously reported.21,22 Of the isolates, 57% were represented by the three most common haplotypes (a, c, k). O’Donnell et al.21,22 genotyped 191 isolates using multilocus sequence typing (MLST) of the nuclear large rRNA subunit (LSU), translation elongation factor (EF-1α), and two contiguous regions of the RNA polymerase II second largest subunit (RPB2). They used isolates from ocular and environmental sources obtained from the Centers for Disease Control (CDC) investigation of a contact lens-associated Fusarium keratitis outbreak. Their phylogenetic study nested the isolates causing ocular infection into five different species complexes: FSSC (62%), FOSC (29%), GFSC (6%), Gibberella fujikuroi species complex, FIESC (4%), and FDSC (1.0%). No GFSC or FCSC isolates were identified in the present study, probably as a result of small sample size or differences in sampling region compared with other studies. Of note, only one case of FCSC-associated keratitis has been reported in the literature.34

The isolate EU721689 exhibited significant sequence variability (14%–18%) when compared to other isolates in our study (Table 2). This isolate may be representative of another Fusarium species complex still not defined or may represent an organism from another genus. However, both microbiology
laboratories morphologically classified this isolate as *Fusarium* spp. The clinical outcome was typical of an infection caused by a non-*F. solani* isolate, with good final visual acuity and without the need for surgical intervention. A database search of GenBank identified the isolate as *Colletotrichum* spp., *Nectria* spp., or *Fusarium* spp.

Taxonomic identification of *Fusarium* spp. has its limitations in a diagnostic setting. In this study, genus level taxonomic identification was 100% accurate at both BPEI-OML and TFTL, compared with genotypic classification. Species classification of the 30 selected isolates performed by TFTL was accurate and in concordance 100% of the time with the genotypic data. In contrast, a substantial discrepancy was observed in species assignment performed at BPEI-OML, where only 30 of the 58 isolates were assigned a species designation, which proved to have an accuracy of 50% when compared to the genotypic identification, because of low sensitivity for the *F. solani* isolates. Because nonreference clinical laboratories are not typically equipped to identify fungal organisms to the species level, this result is not altogether unexpected. Laboratory species designation based solely on microscopic and morphologic traits can be prone to misinterpretations because morphologic characteristics of certain fungal elements are not always clear. In fact, strains belonging to the same species may display different morphologic characteristics at different growth stages. In addition, micro- and macroscopic characteristics may be influenced by media, age of culture, and identification schema. Therefore, the success of this traditional method of identification relies on the taxonomic experience of the personnel who perform the tests.

Genus- and species-specific identification of fungi using microbiologic techniques is also time-consuming. Such techniques generally require approximately 3 and 7 days, respectively, even when undertaken at a fungal reference laboratory. In contrast, the use of PCR and sequence analysis is capable of identification of *Fusarium* isolates to the genus and species level in less than 24 hours. In this study, sequencing was undertaken by another institution which increased the total time required for species identification to 3 days.

The present study highlights two attractive features of the ITS region: the region aligned well across the spectrum of isolates represented in ocular infections; and the nucleotide variation provided strong bootstrap support to allow for the placement of 98% of the isolates within one of six monophyletic species complexes (Table 2). In addition, the ITS region lies within a multicopy gene, which is important in a clinical

**TABLE 3.** Genetic Identification Breakdown of the 58 Isolates and Comparison to the Reference Genotypic Classification for Medically Important *Fusarium* spp.

<table>
<thead>
<tr>
<th>Species Complex</th>
<th>Present Study</th>
<th>O’Donnell et al.²¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>(%)</td>
</tr>
<tr>
<td>FSSC</td>
<td>44 (75)</td>
<td>62</td>
</tr>
<tr>
<td>FOSC</td>
<td>9 (16)</td>
<td>29</td>
</tr>
<tr>
<td>FIESC</td>
<td>3 (5)</td>
<td>2</td>
</tr>
<tr>
<td>FDSC</td>
<td>1 (2)</td>
<td>1</td>
</tr>
<tr>
<td>FCSC</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>GFSC</td>
<td>—</td>
<td>6</td>
</tr>
<tr>
<td>Unidentified</td>
<td>1 (2)</td>
<td>—</td>
</tr>
</tbody>
</table>

Abbreviations are defined in Figure 2.
diagnostic setting, particularly in the field of ophthalmology, where sample size is very limited. The multiplex nature of the gene would theoretically result in increased assay sensitivity when applied within the context of a PCR-based diagnostic test. 11

Although the allelic variation within the ITS region allowed reliable placement of most isolates within one of the six species complex groups, the ability of this region to clearly differentiate distinct clades within each of these six species complexes is limited, particularly when compared with MLST. 21,22 Although this MLST technique described by O’Donnell et al. 21,22 is capable of more detailed classification, this level of identification may not be required in a clinical setting. When developing a cost-effective and efficient molecular-based analysis in a clinical setting, it is important to determine the level of identification that remains clinically relevant. Moreover, to what level do differences exist among the different species with regard to pathogenicity and antifungal susceptibility profiles that can potentially influence management decisions of the treating physician?

The Fusarium genus is largely resistant, both in vivo and in vitro, to the currently available antifungal agents. 22,24,35 However, previous studies have demonstrated antifungal MICs that are typically higher and more variable for Fusarium than for other pathogenic filamentous fungi; thus, it may be beneficial to establish susceptibility profiles among the different species as determined by molecular classification techniques. Repeatedly, broth microdilution studies based on NCCLS guidelines have demonstrated F. solani to carry higher levels of resistance to available antifungal agents (in particular amphotericin B and voriconazole) than non-F. solani species. 24,25,37 Similarly, O’Donnell et al. 25 studied the in vitro activity of 10 antifungals against 20 isolates representing 18 species that span the breadth of the FSSC phylogeny and found broad resistance across this complex without species-specific differences in susceptibility patterns.

In a recent report of 52 patients with Fusarium keratitis, differences in clinical characteristics among the different species complexes concluded that infections due to organisms within the FSSC, in contrast to non-FSSC organisms, were associated with worse final best corrected visual acuity, a longer resolution time, and higher penetrating keratoplasty rates among the FSSC, in contrast to non-FSSC organisms, were associated with worse final best corrected visual acuity, a longer resolution time, and higher penetrating keratoplasty rate (Oechsler RA et al. I OVS 2008;49:ARVO E-Abstract 2495). These findings suggest higher pathogenicity among the FSSC isolates in the setting of infectious keratitis.

Patient 9 was the only patient from which isolates with different haplotypes were recovered. The first isolate was from a corneal scraping of the right eye in April 2004, and the second isolate was from the patient’s right contact lens, also cultured in April 2004. This finding suggests that Fusarium isolates obtained from contact lenses or associated paraphernalia do not necessarily correlate with the organism causing the ocular infection. Of note, one case has been reported in the literature in which two different Fusarium genotypes were isolated from the same eye of a patient with infectious keratitis. 7 Although difficult, it may be clinically important to identify a dual infection, as antifungal susceptibility patterns are known to be widely variable among the different Fusarium isolates, 22,24,35 and the choice of antifungal medication may be influenced by such information.

Although in vitro and clinical correlation studies have demonstrated differences in susceptibility and pathogenicity profiles among the Fusarium species complexes, studies using an animal model of infectious keratitis are necessary to compare the pathogenic potentials as well as the in vivo susceptibility profiles of each species complex. Nonetheless, these existing reports encourage proper identification to the species complex level, which may aid the clinician by providing prognostic and therapeutic information that may, in fact, influence clinical decisions.

In summary, the ITS region provides a sufficient genetic scaffolding to detect and reliably differentiate Fusarium spp. isolates that cause ocular infections into one of six species complex groupings, a level of identification that appears to hold clinical relevance. In theory, the ITS region is an ideal candidate on which to base the development of a rapid molecular test for the diagnosis of infectious etiologies. When compared with MLSTs, the ITS region offers a less complex, more efficient, and more cost-effective means of differentiating ocular Fusarium isolates to a level that will influence clinical decision-making. Such DNA-based diagnostic tests show significant promise in allowing precise and rapid diagnosis of fungal ocular infections, and their design and implementation will certainly hasten the initiation of appropriate antimicrobial therapy and guide clinical management decisions, with the potential of decreasing the ocular morbidity associated with fungal ocular infections.

References

16. O’Donnell K, Sutton DA, Rinaldi MG, et al. Genetic diversity of human pathogenic members of the Fusarium oxysporum complex inferred from multilocus DNA sequence data and amplified fragment length polymorphism analysis: evidence for the recent dis...


