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# *Fusarium* Keratitis in Germany

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**ABSTRACT** *Fusarium* keratitis is a destructive eye infection that is difficult to treat and results in poor outcome. In tropical and subtropical areas, the infection is relatively common and associated with trauma or chronic eye diseases. However, in recent years, an increased incidence has been reported in temperate climate regions. At the German National Reference Center, we have observed a steady increase in case numbers since 2014. Here, we present the first German case series of eye infections with *Fusarium* species. We identified *Fusarium* isolates from the eye or eye-related material from 22 patients in 2014 and 2015. Thirteen isolates belonged to the *Fusarium solani* species complex (FSSC), 6 isolates belonged to the *Fusarium oxysporum* species complex (FOSC), and three isolates belonged to the *Fusarium fujikuroi* species complex (FFSC). FSSC was isolated in 13 of 15 (85%) definite infections and FOSC in 3 of 4 (75%) definite contaminations. Furthermore, diagnosis from contact lens swabs or a culture of contact lens solution turned out to be highly unreliable. FSSC isolates differed from FOSC and FFSC by a distinctly higher MIC for terbinafine. Outcome was often adverse, with 10 patients requiring keratoplasty or enucleation. The use of natamycin as the most effective agent against keratitis caused by filamentous fungi was rare in Germany, possibly due to restricted availability. Keratitis caused by *Fusarium* spp. (usually FSSC) appears to be a relevant clinical problem in Germany, with the use of contact lenses as the predominant risk factor. Its outcome is often adverse.

**KEYWORDS** fungal keratitis, contact lens, *Fusarium*, antifungal susceptibility testing

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Keratitis caused by *Fusarium* species is a sight-threatening disease often affecting otherwise-healthy patients. The infection is difficult to treat because *Fusarium* spp. are highly resistant to most antifungals. Thus, in many cases, corneal infection will progress to endophthalmitis (1), resulting in poor visual outcome and, in some cases, in enucleation (2, 3). Risk factors for the development of eye infections caused by *Fusarium* spp. include contact lens wear, trauma (4, 5), including surgery (2), and immunosuppressive disease or medication (6).

*Fusarium* eye infections are more common in tropical and subtropical countries. In these countries, defects in the epithelium of the cornea caused by trauma, often involving plant material, are the main risk factor for *Fusarium* keratitis (5, 7, 8). However, with the increasing use of contact lenses, *Fusarium* keratitis has also become a problem in urban areas with moderate climates. In 2005 to 2006, an international outbreak of contact lens-associated *Fusarium* keratitis was observed. This was a result of decreased

activity of the antimicrobial agent alexidine against *Fusarium* spp. after heating the cleaning solution ReNu with MoistureLoc (Bausch & Lomb) (9). The highest numbers of cases were noted in Hong Kong (10), Singapore (11), and the United States (12, 13), but European cases also associated with the ReNu WML solution have been reported (14–17). Independent of this outbreak, a general increase in fungal keratitis cases has been observed recently in countries with temperate climates, mostly due to an increase in keratitis caused by filamentous fungi (18), probably associated with the use of contact lenses.

Epidemiological analyses are hampered not only by a lack of clinical data but also by the fact that the taxonomy of the genus *Fusarium* is in a state of flux. Closely related species that share a similar morphology are combined in 20 species complexes (19). Eye infections are predominantly caused by members of the *Fusarium solani* species complex (FSSC) (20). Since morphological characteristics do not reliably differentiate the sibling species, molecular identification based on the translation elongation factor 1$\alpha$ (TEF-1 alpha) has been used as an appropriate method for species identification (20).

Resistance to most antifungals makes the treatment of *Fusarium* infections very difficult. They are intrinsically resistant against echinocandins (21, 22), and some species show high MICs for azoles, while differences in the response to azoles have been reported, depending on the taxa (23).

The National Reference Center for Invasive Fungal Infection (NRZMyk) serves as a national reference laboratory for the diagnosis of fungal infections in Germany. Between January 2014 and December 2015, the NRZMyk received 24 *Fusarium* isolates from 22 patients with ocular infections. The aim of the study was to perform a detailed analysis of these cases to address the following questions: what are the risk factors for acquiring *Fusarium* keratitis in Germany? Which *Fusarium* species cause these infections? Are there differences among the species concerning the *in vitro* antifungal susceptibility profile, response to treatment, and virulence?

## RESULTS

**Patient characteristics.** Twenty-four *Fusarium* strains from 22 patients (two cases had two identical isolates at different sampling times) isolated from the eye or related material were sent to the NRZMyk from 13 institutions all over Germany (Table 1). Most patients (18/22) were female, and the median age was 46 years (interquartile range [IQR], 26 to 56 years). The clinical specimen from which *Fusarium* was isolated was most commonly a corneal swab (9 patients) or contact lens/contact lens disinfectant solution (8 patients). The remaining patients had more invasive diagnostic procedures, with corneal scraping in one case, biopsy of the vitreous body in 2 cases, and anterior chamber puncture in one case. In one case, the exact origin of the specimen was not further specified.

Fifteen patients clearly had fungal keratitis or endophthalmitis and are therefore considered "cases" and described more thoroughly (Table 2). There were 4 patients with either no symptoms at all (one patient) or rapid improvement without antifungal treatment (3 patients). These were thus summarized as "contamination" (Table 3). In 3 patients, there was not sufficient clinical information to fully judge the clinical relevance of the isolation of *Fusarium* spp.; these are therefore termed "cases with unknown clinical relevance" (Table 4). However, all of these cases with unknown clinical relevance had *Fusarium* spp. isolated from a contact lens or contact lens disinfection solution only, whereas more representative material, like corneal swabs/scrapings, showed no evidence of *Fusarium* species. It is thus very likely that they also represent contaminations.

**Characteristics and clinical course of confirmed cases.** Among patients with fungal keratitis/endophthalmitis, 11 were female and 4 were male, and median age was 50 years (IQR, 26 to 58 years). *Fusarium* spp. were mostly isolated from the cornea (swabs/scrapings in 10 cases) but also from the anterior chamber (two cases) and the vitreous body (one case) (Table 2). In one case, the strain was grown from contact lens disinfectant solution, and in another case, the precise origin remains unknown. Ocular

**TABLE 1** *Fusarium* spp. detected in samples from the eye

| Taxon (no. of strains) | Clinical relevance | | | MICs (mg/liter) for[a]: | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Amphotericin B | | | Natamycin | | | Terbinafine | | | Voriconazole | | | Isavuconazole | | | | | |
| | None | Yes | Unknown | Range | GM | $MIC_{50}$ | Range | GM | $MIC_{50}$ | Range | GM | $MIC_{50}$ | Range | GM | $MIC_{50}$ | Range | GM | $MIC_{50}$ | ITZ | PCZ | CAS |
| FSSC (13) | | 13 | | 0.5–4 | 1.6 | 2 | 4–16 | 6.5 | 8 | 64 | 64 | 64 | 1–16 | 9.9 | 16 | 16 | 16 | 16 | 16 | 16 | 16 |
| *F. falciforme*[b] (1) | | 1 | | 2 | 2 | | | | | | 64 | | 16 | 16 | | | 16 | | 16 | 16 | 16 |
| *F. keratoplasticum*[b] (3) | | 3 | | 2–4 | 2.5 | 2 | 4–8 | 5.0 | 4 | 64 | 64 | 64 | 8–16 | 10.1 | 8 | 16 | 16 | 16 | 16 | 16 | 16 |
| *F. petroliphilum*[b] (6) | | 6 | | 0.5–2 | 1.3 | 1 | 4–8 | 7.13 | 8 | 64 | 64 | 64 | 4–16 | 11.3 | 16 | 16 | 16 | 16 | 16 | 16 | 16 |
| *F. solani*[b] (1) | | 1 | | 2 | 2 | | 16 | 16 | | | 64 | | 16 | 16 | | | 16 | | 16 | 16 | 16 |
| FSSC 9[c] (1) | | 1 | | 1 | 1 | | 4 | 4 | | | 64 | | 1 | 1 | | | 16 | | 16 | 16 | 16 |
| FSSC 25[c] (1) | | 1 | | 2 | 2 | | 4 | 4 | | | 64 | | 16 | 16 | | | 16 | | 16 | 16 | 16 |
| *F. oxysporum* SC (6) | 3 | | 3 | 0.5–2 | 1 | 1 | 4–8 | 4.5 | 4 | 2–8 | 3.6 | 2 | 4–16 | 5.0 | 4 | 8–16 | 14.3 | 16 | 16 | 16 | 16 |
| FFSC (3) | 1 | 2 | | 12 | 1.6 | 2 | 4–8 | 6.3 | 8 | 0.5–8 | 1.6 | 1 | 4–8 | 6.3 | 8 | 16 | 16 | 16 | 16 | 16 | 16 |
| *F. lactis* complex (1) | | 1 | | 2 | 2 | | 4 | 4 | | | 8 | | 8 | 8 | | | 16 | | 16 | 16 | 16 |
| *F. proliferatum* (2) | 1 | 1 | | 1–2 | 1.4 | 1 | 8 | 8 | 8 | 0.5–1 | 0.7 | 0.5 | 4–8 | 5.7 | 4 | 16 | 16 | 16 | 16 | 16 | 16 |

[a]ITZ, itraconazole; PCZ, posaconazole; CAS, caspofungin; GM, geometric mean; $MIC_{50}$, cumulative MIC for 50% of the isolates tested. High off-scale MICs/MECs were raised to the next highest concentration.
[b]These species were previously reported as FSSC 1 (*Fusarium petroliphilum*), FSSC 2 (*F. keratoplasticum*), FSSC 3+4 (*F. falciforme*), and FSSC 5 (*F. solani*).
[c]Unnamed but numbered phylogenetic species. FSSC, *Fusarium solani* species complex; FOSC, *Fusarium oxysporum* species complex; FFSC, *Fusarium fujikuroi* species complex.

**TABLE 2** Detailed characteristics of confirmed cases

| JMRZ no. | Isolate | Origin of isolate | Age (yr) | Sex | Symptoms | Contact lens? | Type of contact lens | Gardening/ livestock | Antifungal treatment | | | Outcome |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Topical | Invasive[a] | Systemic | |
| JMRC:NRZ:0012 | *Fusarium petroliphilum* | Corneal swab | 57 | Female | Endophthalmitis | Yes | Soft | Yes | Amphotericin B, voriconazole, natamycin | Voriconazole | Amphotericin B, voriconazole | Keratoplasty |
| JMRC:NRZ:0017 | *F. petroliphilum* | Anterior chamber | 56 | Male | Endophthalmitis | Yes | Unknown | Unknown | Voriconazole, amphotericin B, natamycin | Amphotericin B | Amphotericin B, voriconazole, posaconazole, terbinafin | Keratoplasty |
| JMRC:NRZ:0059 | *F. petroliphilum* | Corneal swab | 20 | Male | Endophthalmitis | Yes | Soft | Unknown | Yes, unspecified substances | Yes, unspecified substances | Yes, unspecified substances | Enucleation |
| JMRC:NRZ:0086 | *F. petroliphilum* | Corneal scraping | 50 | Male | Endophthalmitis | Yes | Soft | Yes | Voriconazole, amphotericin B | Voriconazole, amphotericin B | Voriconazole, amphotericin B | Keratoplasty, enucleation |
| JMRC:NRZ:0106[b] | *F. petroliphilum* | Corneal swab | 38 | Female | Keratitis | Yes | Soft | Unknown | Voriconazole, natamycin | Voriconazole, amphotericin B | Voriconazole, amphotericin B | Keratoplasty |
| JMRC:NRZ:0575 | *F. petroliphilum* | Corneal swab | 58 | Male | Endophthalmitis | Unknown | Unknown | Unknown | Voriconazole | Unknown | Amphotericin B | Keratoplasty |
| JMRC:NRZ:0049[c] | *Fusarium keratoplasticum* | Corneal swab | 60 | Female | Endophthalmitis | Yes | Soft | Unknown | Voriconazole, amphotericin B | Voriconazole | Voriconazole, posaconazole | Keratoplasty, enucleation |
| JMRC:NRZ:0131 | *F. keratoplasticum* | Corneal swab | 26 | Female | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown | Keratoplasty |
| JMRC:NRZ:0163; JMRC:NRZ:0164 | *F. keratoplasticum* | Contact lens; corneal swab | 77 | Female | Keratitis | Yes | Unknown | Unknown | Voriconazole, amphotericin B | None | Amphotericin B | Restored to normal |
| JMRC:NRZ:0138 | *Fusarium falciforme* | Corneal swab | 58 | Female | Keratitis | No | Unknown | Unknown | Amphotericin B, natamycin | Unknown | Voriconazole | Unknown |
| JMRC:NRZ:0205 | *Fusarium solani* | Vitreous body | 25 | Female | Endophthalmitis | Yes | Unknown | Unknown | Voriconazole | Amphotericin B | Amphotericin B | Keratoplasty |
| JMRC:NRZ:0233 | FSSC 9[d] | Unspecified | 27 | Female | Endophthalmitis | Yes | Soft | Yes | Natamycin, voriconazole | Amphotericin B, voriconazole | Voriconazole | Restored to normal |
| JMRC:NRZ:0061 | FSSC 25[d] | Corneal swab | 47 | Female | Endophthalmitis | Yes | Soft | Unknown | Voriconazole, amphotericin B | Voriconazole | Voriconazole | Keratoplasty |
| JMRC:NRZ:0548 | *Fusarium lactis complex* | Contact lens disinfectant solution | 23 | Female | Keratitis | Yes | Soft | Yes | Amphotericin B, fluconazole | None | None | Restored to normal |
| JMRC:NRZ:0202 | *Fusarium proliferatum* | Anterior chamber | 56 | Female | Endophthalmitis | Unknown | Unknown | Unknown | Amphotericin B, voriconazole | Unknown | Unknown | Unknown |

[a]Invasive antifungal treatment includes lavage of the anterior chamber and/or instillation into the vitreous body.
[b]Patient committed suicide during therapy.
[c]Case report published (3).
[d]Unnamed but numbered phylogenetic species.

**TABLE 3** Detailed characteristics of cases with contamination[a]

| JMRZ no. | Isolate | Origin of isolate | Age (yr) | Sex | Symptoms | Contact lens? | Type of contact lens | Gardening/ livestock | Antifungal treatment | | | Outcome | Eyesight preserved? |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Topical | Invasive | Systemic | | |
| JMRC:NRZ:0027 | *Fusarium oxysporum* complex | Swab from contact lens | 49 | Female | None | Yes | Soft | Unknown | No | No | No | Restored to normal | Unchanged[b] |
| JMRC:NRZ:0545 | *F. oxysporum* complex | Contact lens | 45 | Female | Keratitis | Yes | Soft | Yes | No | No | No | Restored to normal | Yes |
| JMRC:NRZ:0260 | *F. oxysporum* complex | Contact lens disinfectant solution | 41 | Female | Keratitis | Yes | Soft | Unknown | No | No | No | Restored to normal | Yes |
| JMRC:NRZ:0196 | *Fusarium proliferatum* | Contact lens | 19 | Female | Keratoconjunctivitis | Yes | Soft | Yes | No | No | No | Restored to normal | Yes |

[a]Contamination was assumed when the clinical conditions resolved without antifungal treatment.
[b]The eye had been blind before, so the reason for contact lens use remains unclear.

**TABLE 4** Available characteristics of cases with unknown clinical relevance

| JMRZ no. | Isolate | Origin of isolate | Age (yr) | Sex | Symptoms | Contact lens? | Type of contact lens? | Gardening/ livestock | Antifungal treatment | | | Outcome | Eyesight preserved? | Findings in corneal swab/scraping |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Topical | Invasive[a] | Systemic | | | |
| JMRC:NRZ:0204 | *Fusarium oxysporum* SC | Contact lens disinfectant solution | 55 | Female | Keratitis | Yes | Unknown | Unknown | Amphotericin B, voriconazole | Unknown | Unknown | Unknown | Unknown | *Candida* spp. |
| JMRC:NRZ:0198; JMRC:NRZ:0199 | *F. oxysporum* SC | Contact lens disinfectant solution (twice) | 26 | Female | Keratitis | Yes | Unknown | Unknown | Natamycin | Unknown | Unknown | Unknown | Unknown | *Staphylococcus aureus* in conjunctival swab |
| JMRC:NRZ:0189 | *F. oxysporum* SC | Contact lens | 30 | Female | Unknown | Yes | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown | Unknown | Sterile swab, *Staphylococcus epidermidis* in scraping |

[a]Invasive antifungal treatment includes lavage of the anterior chamber and/or instillation into the vitreous body. All patients had corneal swabs without evidence of *Fusarium* species complex.

**TABLE 5** Strains studied and their GenBank accession numbers and MLSTs

| Species complex[a] | Strain no. | Species | MLST | GenBank accession no. | | |
|---|---|---|---|---|---|---|
| | | | | TEF | RPB2 | ITS |
| FSSC | JMRC:NRZ:0017 | *F. petroliphilum* | FSSC 1-a | MF467469 | MF467494 | MF467472 |
| FSSC | JMRC:NRZ:0086 | *F. petroliphilum* | FSSC 1-a | MF467468 | MF467496 | MF467471 |
| FSSC | JMRC:NRZ:0575 | *F. petroliphilum* | FSSC 1-a | MF467467 | MF467495 | MF467473 |
| FSSC | JMRC:NRZ:0012 | *F. petroliphilum* | FSSC 1-b | MF467470 | MF467492 | MF467475 |
| FSSC | JMRC:NRZ:0059 | *F. petroliphilum* | FSSC 1-b | MF467466 | MF467493 | MF467474 |
| FSSC | JMRC:NRZ:0106 | *F. petroliphilum* | FSSC 1-b | MF467465 | MF467491 | MF467476 |
| FSSC | JMRC:NRZ:0131 | *F. keratoplasticum* | FSSC 2-d | MF467461 | MF467487 | MF467483 |
| FSSC | JMRC:NRZ:0164 | *F. keratoplasticum* | FSSC 2-ii | MF467460 | MF467486 | MF467481 |
| FSSC | JMRC:NRZ:0049 | *F. keratoplasticum* | FSSC 2-unique | MF467459 | MF467485 | MF467482 |
| FSSC | JMRC:NRZ:0138 | *F. falciforme* | FSSC 3+4-b | MF467462 | MF467484 | MF467480 |
| FSSC | JMRC:NRZ:0205 | *F. solani* | FSSC 5-c | MF467463 | MF467488 | MF467479 |
| FSSC | JMRC:NRZ:0233 | FSSC 9 | FSSC 9-unique | MF467464 | MF467489 | MF467478 |
| FSSC | JMRC:NRZ:0061 | FSSC 25 | FSSC 25-unique | MF467458 | MF467490 | MF467477 |
| FOSC | JMRC:NRZ:0027 | *F. oxysporum* SC | | MF467452 | | |
| FOSC | JMRC:NRZ:0189 | *F. oxysporum* SC | | MF467453 | | |
| FOSC | JMRC:NRZ:0198 | *F. oxysporum* SC | | MF467455 | | |
| FOSC | JMRC:NRZ:0204 | *F. oxysporum* SC | | MF467456 | | |
| FOSC | JMRC:NRZ:0260 | *F. oxysporum* SC | | MF467454 | | |
| FOSC | JMRC:NRZ:0545 | *F. oxysporum* SC | | MF467457 | | |
| FFSC | JMRC:NRZ:0196 | *F. proliferatum* | | MF467449 | | |
| FFSC | JMRC:NRZ:0202 | *F. proliferatum* | | MF467450 | | |
| FFSC | JMRC:NRZ:0548 | *F. lactis* complex | | MF467451 | | |

[a]FSSC, *Fusarium solani* species complex; FOSC, *Fusarium oxysporum* species complex; FFSC, *Fusarium fujikuroi* species complex.

involvement was limited to keratitis in 4 patients but had extended to intraocular structures (endophthalmitis) in 10 patients (unknown in 1 patient). None of the patients had reported major trauma or immunosuppression (including the use of steroids). Eleven patients used contact lenses; however, in one case of *Fusarium falciforme* infection, there was no history of contact lens use, and in 3 cases, no information was available. Of those with contact lens use, at least 7 wore soft contact lenses (unknown for the remaining patients). Six patients reported working in the garden or with livestock at the time of infection (unknown in the remaining 9 cases).

At least 14 patients received topical antifungal agents (unknown in one case): five patients received amphotericin B and voriconazole; two patients received amphotericin B, voriconazole, and natamycin; two patients received voriconazole and natamycin; two patients received voriconazole only; and one patient each received voriconazole and natamycin, amphotericin B only, and unspecified antifungal substances. Eight patients had more invasive antifungal treatment involving instillation of amphotericin B or voriconazole into the anterior chamber or the vitreous body. Information on outcomes is available for 13 patients: nine patients received at least one keratoplasty, whereas three patients resolved their infection without operation. Two of the patients with keratoplasty and an additional patient without known keratoplasty underwent enucleation. In contrast, 7 patients recovered some visual acuity. For four patients, information on the recovery of eyesight was not available.

***Fusarium* species involved in eye infections and multilocus sequence typing of FSSC.** Species of the FSSC, especially *F. petroliphilum* and *F. keratoplasticum*, dominated in eye infections (Table 1). Among the confirmed clinical cases, FSSC accounts for the vast majority (13/15 [87%]). In contrast, species isolated from cases with unknown clinical relevance and cases with contamination mostly belonged to the *Fusarium oxysporum* complex (Table 1). Two strains of the FSSC were assigned to the still-unnamed phylogenetic species 9 and 25. *Fusarium proliferatum* and *F. lactis* were representatives of the FFSC causing keratitis. The sequence types of the FSSC species are listed in Table 5. Three out of the nine sequence types detected in this study have not been published before.

**Antifungal susceptibility profiles of the *Fusarium* species.** The ranges of the MICs, geometric means (GMs), and the cumulative MICs for 50% of the isolates tested

($MIC_{50}$) of 22 studied *Fusarium* strains for eight antifungal agents are listed in Table 1. The MICs of the quality control strains were in the expected ranges in all batches tested. The susceptibilities to amphotericin B, natamycin, isavuconazole, itraconazole, posaconazole, and caspofungin did not differ between the species complexes. The lowest MICs were reached by amphotericin B, with a total GM MIC of 1.41 mg/liter. With the exception of the *F. solani sensu stricto* strain JMRC:NRZ:0205, the MICs for natamycin ranged between 4 and 8 mg/liter. All isolates showed MICs of >8 mg/liter for caspofungin, itraconazole, and posaconazole. The isavuconazole MIC was found to be 8 mg/liter for only one strain; the remaining strains had MICs greater than 8 mg/liter. For voriconazole, more pronounced differences among the different species were found: in the FSSC, 8/13 (62%) of isolates showed MICs of >8 mg/liter, while for FOSC, only 1/6 (17%) and none of the FFSC strains had MICs of >8 mg/liter, which was also underlined by the higher voriconazole GM of the FSSC (9.9 mg/liter) than the GMs of the FOSC (5.0 mg/liter) and the FFSC (6.3 mg/liter). In our experimental setting, all strains of the FSSC showed MICs for terbinafine of >32 mg/liter. In contrast, all members of the FOSC and the FFSC tested showed MICs for terbinafine of ≤8 mg/liter (Table 1).

## DISCUSSION

Fifteen confirmed cases of *Fusarium* keratitis or endophthalmitis within 2 years diagnosed at the NRZMyk show that eye infections by *Fusarium* species are a rare but serious cause of ocular infection in Germany. A British study found a distinctly rising number of *Fusarium* keratitis cases after 2007 (18). Unfortunately, despite a clear increase in yearly cases since 2014, our data do not allow conclusions on a potential rise in Germany, as the NRZMyk was only established in 2014 and has generally experienced rising numbers of samples submitted for diagnostic work-up.

***Fusarium* species complexes, species, and sequence types involved in eye infections.** In our study, species of the FSSC are the dominating etiological agents of eye infections, accounting for the vast majority of definite clinical cases. This is in line with data found in other countries, e.g., Tunisia (66% [5]), India (75.7% [24]), and Mexico (80.9% [25]), and in contact lens-associated outbreak cases in the United States (77% [12]). Only one French study reported almost equal proportions of FSSC (47%) and FOSC (41%) (15).

There are only a few studies (12, 26, 27) with a focus on *Fusarium* eye infections that identified the strains to the species level or sequence type. In agreement with our findings, *F. petroliphilum* and *F. keratoplasticum* (corresponding to the sequence type/phylogenetic species 1 and 2 of Chang et al. [12] and O'Donnell et al. [26]) were the dominating species infecting the human eye in the aforementioned studies.

Oechsler et al. (28) reported that infections with FSSC required a significantly longer treatment course and a higher necessity for a therapeutic penetrating keratoplasty, and they were associated with a poorer outcome than infections by non-*solani Fusarium* species. We found no definite clinical case caused by the FOSC in this study, while the FOSC was involved in 18% of U.S. outbreak cases (12). In the three cases with uncertain clinical relevance and evidence of FOSC, the fungus was isolated only from the contact lens or contact lens disinfectant solution, whereas no fungus could be isolated from corneal swabs. It is unclear whether the contact lens and/or fluid were contaminated or if the isolation of FOSC from clinical specimens was not successful because the vitality of the fungus was reduced by antifungal treatment. However, these results and the three contaminations caused by FOSC suggest that the presence of this species complex does not necessarily result in an infection, which could be due to a reduced ability of spore attachment and penetration. Experimental evidence suggests that FOSC has a lower pathogenic potential than FSSC (29–31).

*Fusarium lactis* being a cause of keratitis is a new finding in our report. This species has been considered to be a specific pathogen of figs geographically restricted to California (61). In a survey on dried figs in Apulia (Italy), only the fig-specific *Fusarium ramigenum* was isolated, not *F. lactis* (33). However, a second isolate of *F. lactis* from

corneal scrapings was received by the NRZMyk in 2016 (JMRC:NRZ:0506, our unpublished data), supporting the occurrence of this species in clinical specimens in Germany.

The number of FSSC sequence types (STs) determined in this study is too low to detect clear differences in diversity or virulence, but some similarities to the isolates involved in the keratitis outbreak of 2005 and 2006 (13) become apparent. FSSC 1-a was a dominating ST of *F. petroliphilum* in the outbreak and also frequent among our isolates (50%). In agreement with O'Donnell et al. (13), the diversity of STs was higher in *F. keratoplasticum* (3 STs in 3 isolates) than in *F. petroliphilum* (2 STs in 6 isolates). Similarly to the findings of O'Donnell et al. (13), we found only FSSC 5-c of. *F. solani* to infect the eye.

***In vitro* antifungal susceptibility of *Fusarium* species infecting the eye.** To date, no clinical breakpoints have been established for *Fusarium* species. Recently, CLSI epidemiological cutoff values (ECVs) were determined for the FSSC and the FOSC and four antifungals (amphotericin B, voriconazole, posaconazole, and itraconazole) (34). The CLSI protocol applies a distinctly lower final inoculum of 0.4 to $5 \times 10^4$ CFU per ml (33), while the EUCAST protocol uses a final inoculum between 1 and $2.5 \times 10^5$ CFU/ml (35). These differences in the inoculum MICs of the FSSC strains tested in this study are similar to those obtained by Espinel-Ingroff et al. (34). For the FOSC, only the MICs determined for posaconazole (>8 mg/liter) exceeded the CLSI ECV of 8 mg/liter.

Our results confirm amphotericin B to be the antifungal agent with the lowest MICs for *Fusarium* spp., which has been shown in several studies (23, 34, 36). The MICs obtained for natamycin (which has a polyene structure similar to amphotericin B) are comparatively high but in line with the MICs reported by other authors (4 to >8 mg/liter [37, 38]). Lalitha et al. (37) considered isolates with MICs of ≤16 mg/liter to be susceptible to natamycin, because these levels are reached in the eye during standard therapy. If the typical prescription dose is considered, natamycin was found to be more effective than amphotericin B, which may be a result of differing drug penetration, since natamycin, due to its smaller molecular size, more easily penetrates the eye (39). The intrinsic resistance of *Fusarium* to echinocandins has been shown in other studies (21, 22) and was confirmed by the high MICs recorded for caspofungin in this study.

The susceptibility findings of *Fusarium* strains against isavuconazole, itraconazole, and voriconazole were in agreement with former findings (23, 32, 34, 38, 40, 41). Only the high MICs for posaconazole (>8 mg/liter) obtained for all isolates included in the present study differ from the published values that are, in general, slightly lower for the FOSC and the FFSC (23, 34, 36, 38, 41).

In our setting, all isolates of the FSSC had MICs for terbinafine of >32 mg/liter, while all members of the FOSC and the FFSC tested showed distinctly lower MICs (Table 1). This was a clear-cut delimitation of the FSSC, allowing the assignment of a *Fusarium* strain to the FSSC already by its susceptibility profile. To date, microdilution tests of more than 60 clinical *Fusarium* strains have been performed by the NRZMyk, and not a single strain belonging to a non-*solani Fusarium* species complex had a terbinafine MIC of 16 mg/liter or higher (data not shown). Using the same inoculum, Alastruey-Izquierdo et al. (23) found similar terbinafine MICs for *Fusarium solani* but also for single isolates of other species complexes. The distinctly lower terbinafine MICs obtained for the FSSC by Homa et al. (24) might be due to the lower CLSI inoculum used in that study.

There is no doubt that identification to the species level is required for an understanding of the epidemiology of *Fusarium* infections. However, from the clinical point of view, the distinction within a species complex is not necessary, since the susceptibility profiles within FSSC or within FOSC are similar. The identification of a pathogenic strain to the species complex is likely to be sufficient to make a treatment decision.

**Risk factors.** In contrast to studies from countries with a tropical climate, we identified the use of soft contact lenses as the main and possibly most important risk factor. Other than in previous reports, we were not able to identify any evidence of major trauma (4, 5, 42) or surgery (2, 43) or underlying immunosuppression (6) in any

of our patients. Wearing soft contact lenses is a well-known risk factor in temperate climates (44), and cases with a severe course have been described (3, 45). The risk appears to be related to the contact lens disinfectant solution used (10, 11), as it has been repeatedly shown that some disinfectant solutions are not effective against *Fusarium* spp. (46, 47), especially when heated (9). According to Ahearn et al. (48), the increase in the incidence of mycotic keratitis since 2006 might also be connected with marketing of a "no-rub" multipurpose contact lens solutions and the higher frequency of silicone hydrogel lens use. In addition, overnight use and poor lens care, as well as certain practices, such as refilling bottles of disinfectant solution, may contribute to an increased risk of fungal keratitis. It should be noted that isolation of *Fusarium* spp. solely from the contact lens or the used disinfection fluid does not confirm fungal keratitis. These isolations are often not linked to clinical infection but represent contaminations. Therefore, sampling from the infected cornea is strongly recommended for diagnostic purposes. Of note, we found a striking predominance of female patients in our population. This might be due to the fact that women are more likely to wear contact lenses (49). In addition, the use of cosmetics may increase the risk of infection, since they may be heavily contaminated (50).

**Therapy and outcome.** Our data show that invasive treatment of *Fusarium* keratitis and endophthalmitis is frequently necessary. The outcome can be disastrous, with a rate of 3/13 enucleations in confirmed clinical cases with known outcome. The well-known and sometimes long delay from clinical manifestation to establishment of the correct diagnosis is known to be a risk factor for a poor outcome. The use of steroids during this period mitigates any local inflammatory response to the infection and thus also contributes to a late diagnosis and poor outcomes, e.g., due to intraocular spread and subsequent need of enucleation.

Natamycin (51) and voriconazole (52) may be effective, but our data are not representative of a decision concerning the most effective treatment. In the literature, good efficacy of natamycin in eye infections caused by *Fusarium* spp. has been reported (7, 42, 53). Natamycin was rarely used in our patient population ($n = 5$), but it may have been effective, since three of four patients treated with natamycin and who had a known outcome preserved at least some eye sight. Of note, natamycin is commonly used as a 5% solution, but this formulation is currently not commercially available in Germany. Therefore, natamycin eye drops can only be obtained by import or compounded specifically by a local pharmacist. With increasing frequency of *Fusarium* eye infection, clinicians should be aware of this additional therapeutic option. In addition to local treatment, more invasive management, including corneal transplantation (54) and even enucleation (7% of enucleations were due to *Fusarium* in an eye hospital in Thailand [8]), has been reported. Long duration of antifungal treatment seems to be required due to common recurrence of infection (53, 54).

In conclusion, this first report of eye infections caused by *Fusarium* spp. in Germany reveals the use of soft contact lenses to be the most important risk factor. Definite and often very serious infection is commonly caused by FSSC, and the MIC of terbinafine allows a simple and early differentiation of FSSC and non-*solani Fusarium* species. Therapy remains difficult, with a frequently adverse outcome. Given the severity of the disease, the diagnosis should be made as early as possible, and the therapeutic arsenal should include natamycin, although this is not commercially available in Germany. Since this type of ocular infection is relatively rare, establishing a collaborative approach of ophthalmic centers and microbiologists involved in the treatment of this difficult disease is mandatory to obtain more extensive information about the clinical course and to correlate this with microbiological findings. A registry-type approach would be an appropriate way to collect anonymized data from the centers involved.

## MATERIALS AND METHODS

**Isolates.** All *Fusarium* isolates from patients with suspicion of fungal keratitis which were received by the NRZMyk between January 2014 and December 2015 were included in the study. The isolates were either grown from the infected eye (corneal swabs or scrapings or vitreous aspirates) or from material related to the eye (contact lens, disinfectant solution, or contact lenses container). All

isolates have been deposited in the Jena Microbial Resource Collection (JMRC) and are listed in Tables 2 to 5.

**Patients.** The study was approved by the local ethics committee (no. 4455-06/15). Clinical information provided by the microbiologist or treating clinician was documented in anonymized form. A subgroup of six patients gave written informed consent for personal interviews to collect more detailed data. Of these patients, information was documented in pseudonymized form. For patient characteristics, basic descriptive statistics, such as percentage or median and interquartile range (IQR), are used. Because of the low case number, no test for differences was applied.

**Molecular species identification and multilocus sequence typing of the FSSC.** Genomic DNA was extracted from 2- to 5-day-old cultures grown on 4% malt extract agar (MEA; Difco), according to the protocol described by Möller et al. (62), with diverse modifications. Briefly, fungal material was transferred to a tube containing acid-washed glass beads and 1 ml of lysis buffer (50 mM Tris, 50 mM sodium EDTA, 3% [wt/vol] sodium dodecyl sulfate [SDS] [pH 8]). The samples were homogenized for 5 min at maximum speed using a vortex adapter, followed by 1 h of incubation in a thermomixer at 68°C. Thereafter, the tubes were spun for 10 min at 16,000 relative centrifugal force (RCF), and the supernatant was transferred to a new 2-ml tube. An equal volume of a mixture of phenol, chloroform, and isoamyl alcohol (25:24:1 [vol/vol/vol] [pH 7.5 to 8.0]) was added. The samples were mixed by turning and spun for 10 min at 16,000 RCF. The upper (aqueous) phase was transferred to a new tube, and the step was repeated. Then, a 0.5 volume of 99.9% ethanol was added to precipitate the DNA. After incubation for at least 10 min, the DNA was pelleted at 16,000 RCF for 10 min. The supernatant was decanted, and the DNA pellet was washed twice with 200 ml of 70% ethanol, dried, resuspended in 50 $\mu$l of distilled water, and stored at −20°C.

For species identification, a fragment of the translation elongation factor-1$\alpha$ (TEF-1 alpha) gene was amplified by PCR and sequenced using the primers EF-1 (5′-ATGGGTAAGGAGGACAAGAC-3′) and EF-2 (5′-GGAAGTACCAGTGATCATGTT-3′) (55). For the FSSC, sequence types were identified based on TEF, the second largest subunit of RNA polymerase II gene (RPB2), and the internal transcribed spacer region (ITS). The RPB2 was amplified and sequenced using the primers 5F2 (5′-GGGGWGAYCAGAAGAAGGC-3′) and 7cR (5′-CCCATRGCTTGYTTRCCCAT-3′) (56). For the ITS, the primers V9G (5′-TTACGTCCCTGCCCTTTGTA-3′) (57) and LR3 (5′-CCGTGTTTCAAGACGGG-3′) (58) were used for amplification, and ITS1 (5′-TCCGTAGGT GAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (59) were used for sequencing. The 50-$\mu$l PCR mixture contained a 0.2 $\mu$M concentration of each primer, 10 $\mu$l of 5× MyTaq reaction buffer (including 5 mM deoxynucleoside triphosphates and 15 mM $MgCl_2$; Bioline GmbH, Luckenwalde, Germany), 1 U of MyTaq DNA polymerase (Bioline GmbH), and approximately 100 ng of DNA. PCRs were conducted on a TProfessional Trio PCR thermocycler (Biometra GmbH, Göttingen, Germany). Amplification of TEF and ITS had the following PCR profile: one initial cycle at 95°C for 8 min, followed by 40 cycles of 45 s at 95°C, 45 s at 55°C, and 90 s at 72°C, and one final cycle of 10 min at 72°C. For the RPB2 a touchdown PCR profile was used, with 5 cycles of 45 s at 48°C, followed by 5 cycles of 45 s at 50°C and 30 cycles of 45 s at 52°C.

Consensus sequences were constructed by means of the SeqMan program version 11.0.0 (DNAStar; Lasergene) and aligned using the program Se-Al version 2.0a11 (http://tree.bio.ed.ac.uk/software/seal/). Species were identified by using the BLAST tool of GenBank (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch). Sequence types within the FSSC were determined using the *Fusarium* MLST database of the Westerdijk Institute (http://www.westerdijkinstitute.nl/Fusarium/Biolomicsid.aspx). In cases where no 100% identical match was found using the *Fusarium* MLST database, a BLAST search was run for each sequence to find a previously described sequence type. If a 100% match in the *Fusarium* MLST database was based on only one or two loci, the sequence of the lacking locus of the 100% matching strain was searched in GenBank (https://www.ncbi.nlm.nih.gov/) for comparison.

***In vitro* antifungal susceptibility testing.** The *in vitro* antifungal susceptibilities of 22 isolates were determined by broth microdilution technique according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) standard methodology (35). Pure powders of known potency of the following antifungals were used: amphotericin B (AMB; European Pharmacopoeia, Strasbourg, France), natamycin (NAT; ChemicalPoint, Deisenhofen, Germany), terbinafine (TBF; Novartis Pharma AG, Cork, Ireland), voriconazole (VCZ; Pfizer, Inc., Peapack, NJ, USA), itraconazole (ITZ; Janssen-Cilag GmbH, Neuss, Germany), posaconazole (PCZ; MSD, Rahway, NJ, USA), isavuconazole (ISA; Basilea Pharmaceutica International Ltd., Basel, Switzerland), and caspofungin (CAS; MSD). Microplates containing each antifungal drug in one row were prepared by batch and stored frozen at −80°C for <6 months. *Fusarium* isolates were grown on MEA for 2 to 5 days at 30°C. Spore suspensions were counted with a hemocytometer. The final inoculum was $2 \times 10^5$ spores/ml. MIC endpoints were determined visually using a mirror after 48 h of incubation at 35°C and defined as a 100% reduction in growth in comparison to the drug-free wells. For caspofungin, minimum effective concentrations (MECs) were determined by reading the microplates with the aid of an inverted microscope. Two reference strains, *Aspergillus fumigatus* ATCC 204305 and *Candida parapsilosis* ATCC 22019, which were recommended by EUCAST (35, 60) for antifungal susceptibility testing using amphotericin B, itraconazole, posaconazole, voriconazole, and caspofungin, were included as quality control in each set of tests. In order to allow the calculation of geometric means, high off-scale MICs/MECs were raised to the next higher concentration.

**Accession number(s).** The sequences generated in this study were deposited in GenBank (https://www.ncbi.nlm.nih.gov/nucleotide/) under accession numbers MF467449 to MF467496 (see Table 5).

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