51 *Fusarium*

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51.1 INTRODUCTION

Fusarium species are widely distributed filamentous fungi that commonly occur in soil, water environments, and plants. The genus includes both plant pathogenic and soil saprophytic representatives.[1] During the past decades, *Fusarium* species have emerged as increasingly important causal agents for opportunistic infections in human population.[2]

51.1.1 Classification

Fusarium, first described by Link in 1809,[3] is a genus classified under the order Hypocreales, class hyphomycetes (Ascomycetes). Formerly included in the Deuteromycetes, the genus contains about 100 recognized species,[4] many of which can induce diseases in several agriculturally important crops and cause diseases in humans and domestic animals.[5–7] *Fusarium* spp. are ubiquitous filamentous fungi in all major geographic regions of the world and are routinely isolated from environmental sources such as soil, plant roots, plant debris, and water systems.[2,8] Most of the species of the genus have both an anamorphic (asexual) and teleomorphic (sexual) life cycle and exist in soils as saprophytes or "chlamydospores."[4]

As a plant pathogen, *Fusarium* has been reported as the causal agent of major devastating diseases in most economically important plants, e.g., banana,[9,10] wheat, and barley.[11]

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In humans, *Fusarium* cause four patterns of invasive infections predominantly in immunocompromised patients[2,12–14]: refractory fever of unknown origin, sinopulmonary infection, disseminated infection, and a variety of focal single-organ infections[15] such as ocular infections, usually keratitis or endophthalmitis.[16–18] The mortality rate is greater than 70% in systemic infections.[9] In addition, an array of secondary metabolites is produced by *Fusarium* spp., which are associated with cancer and other growth defects in humans and animals. Interestingly, some of these secondary metabolites are used commercially either directly or as the starting material for chemical synthesis of plant and animal growth promoters in both first-world and second-world settings, and the mycotoxins produced by some of these fungi were reported to be used as biological weapons.[19–23] Few species such as *F. pallidoroseum* can be directly used as a mycoherbicide against water hyacinth.[24]

The *Fusarium* classification system is developed mainly based on the morphology of the conidia produced by the representatives of the genus. Macroscopic and microscopic features, such as color of the colony; length and shape of the macroconidia; the number, shape, and arrangement of microconidia; and the presence or absence of chlamydospores are the key features for the differentiation of *Fusarium* spp., and the main taxonomic systems have organized sections from the species sharing common morphological characteristics (Figures 51.1 through 51.3). However, speciation may be

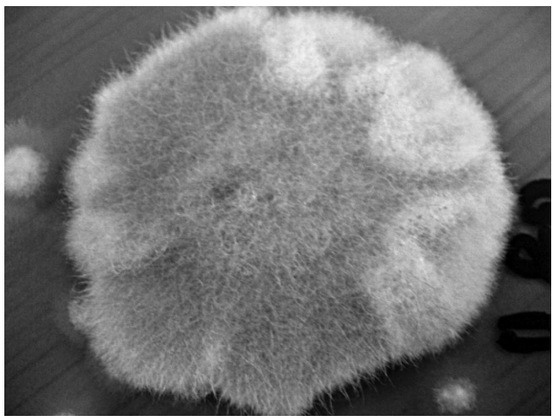

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FIGURE 51.1 Colony morphology of *Fusarium* (white to violet pigment) from patients with *Fusarium* keratitis on PDA.

FIGURE 51.2 Differential interference contrast microphotograph showing chlamydospores produced by *Fusarium solani* (magnification 40×).

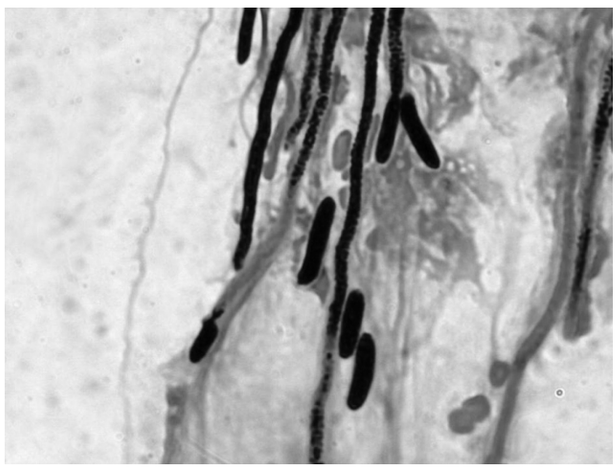

FIGURE 51.3 Gram spear showing macroconidia from corneal scarping of patients with *Fusarium* keratitis.

difficult due to the variability between isolates and because the features that are required are not always well developed. This would best describe why although more than 100 species have been recognized so far based on the various identification concepts, only 70 species are well described.[25]

The morphologically similar *Fusarium* species are grouped together as species complexes (SCs). For instance, the *Fusarium solani* species complex (FSSC) includes morphologically identical isolates. Further genotypic characterization of isolates from this complex is laborious and usually not routinely performed in clinical laboratories. Members of this SC are usually reported in the literature as *Fusarium solani*.[26] During the past few decades, there are controversies amongst researchers that the taxonomic system based on the sections sharing common morphological characteristics is of poor reliability compared to advanced molecular investigation methods such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), and DNA sequencing.[9] For instance, O'Donnell et al. reported that the *Gibberella fujikuroi* species complex (GFSC) revealed 45 species using molecular methods for several gene targets, of which 23 were never reported before.[27] From the clinical point of view, identification at the species level is important for epidemiological studies and may also be very important because some new antifungal agents exhibit variable activities against *Fusarium* isolates belonging to different species.[28,29]

Together, in spite of the key work done by Wollenweber and Reinking[30] for developing a main taxonomic system, and the contributions during the last few decades by a significant number of scientists in *Fusarium* taxonomy, the systematic classification of *Fusarium* species has still a number of open question marks that need to be solved.[9]

51.1.2 Epidemiology

A variety of human and animal infections due to *Fusarium* spp. are widely reported from all over the world as the genus does not require any strict environmental conditions for survival. For instance, *Fusarium* can be recovered from diverse sources including soil, decomposing organic matter, plant roots, woody plants, trees, agricultural, and non-agricultural substrates and are found in various geographic regions with hot, temperate, or cool climates. *Fusarium* infections are being increasingly recognized as life-threatening mycotic infections worldwide.[31–33] The first documented case of disseminated invasive *Fusarium* infection was reported in 1973 in a child with acute leukaemia.[34] Infections caused by *Fusarium* species in different organs are collectively known as fusariosis. The epidemiological distribution of fusariosis is controversial,[35] as 85% of the cases until the year 2000 were reported from Western and Mediterranean countries such as United States, France, Italy, and Brazil,[35] and a large number of institutions in the world have only occasionally or have never documented invasive *Fusarium* infections.[35] The distribution of these cases only among such geographical areas cannot yet be ruled out. Among the well-described

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70 *Fusarium* spp., the taxons implicated in almost half (50%) of the disease cases include *F. solani*, followed by *F. oxysporum* (14%), *F. verticillioides* (11%), *F. moniliforme* (10%), and *F. proliferatum* (5%). Further common human pathogens are *F. dimerum*, *F. chlamydosporum*, *F. nygamai*, *F. napiforme*, *F. semitectum*, and *F. equiseti*.[35]

It is likely that *Fusarium* spp. colonize patients prior to hospital admission and the subsequent immunosuppression and neutropenia could then result in a variety of infections.[31,36] Although skin, blood, lung, and sinus infections are most common, other body organ systems can also be affected. AQ5 *Fusarium* have been documented as etiological agents in localized tissue infections, including keratitis, endophthalmitis, septic arthritis, cystitis, peritonitis, brain abscesses, and breast abscess.[37–39] *F. solani* breast abscess was reported from India in an uncontrolled diabetic patient who was a paddy grower.[38] Disseminated fusariosis with heavy involvement of liver and lungs was reported in a healthy farmer.[40] Although infrequent, post-transplant invasive fusariosis is reported in Asian countries like India.[41] In systemic fusariosis, *Fusarium solani* is the predominantly isolated taxon, followed by *F. moniliforme*, *F. oxysporum*, and *F. proliferatum*.[31]

Fusarium species are isolated and reported in large numbers from eye infections in developing countries such as India.[42] Based on the reported case numbers, keratomycosis is the most frequent human infection caused by *Fusarium*. Most of the studies about fungal keratitis report the occurrence of *Fusarium* at the genus level only; however, identification of the *Fusarium* isolates at the species level would be of great importance as it is gaining consequences on clinical outcome.[43]

The literature provides a large amount of data about the epidemiology of *Fusarium* keratitis. The incidence and epidemiological pattern of *Fusarium* spp. among culture-proven cases of keratomycosis is different from country to country (Tables 51.1 and 51.2).[2,5,14,44–107] According to a series of retrospective studies, *Fusarium* spp. can be the predominant causal agents of keratomycosis[5,14,45,46,53–55,58–62,65–67,70,73,76,79,80,82,86,87,91,92,97,100] besides *Aspergillus* spp.,[47,51,56,71,83,84,88,94–96,98,99,102–104,106] *Candida* spp.,[44,68,78,83] and *Acremonium* spp.[74] Tropical and subtropical countries are the most affected, indicating that climate plays an important role in determining the predominance of certain species in fungal keratitis. Certain regions of different continents, e.g., southern Florida, Ghana, and southern India, have similar climates that are favoring the predominance of *Fusarium* spp.[55,79,86,100] The incidence of *Fusarium* spp. in keratomycosis may also vary with climatic conditions within a single country, e.g., between different parts of China,[59,60,62,63] Ghana,[55] and India (Table 51.2).[55,86,87,88,94,98–100,103,104] Keratitis caused by *Fusarium* is less frequent in regions with temperate climates like European countries: only four cases have been reported from Paris, France, in 8 years,[68] and only a single case has been diagnosed in Hungary.[108] The most frequent taxon of *Fusarium* reported from corneal infections is the FSSC.[14,58,63,64,79,86,87,109]

Fusarium keratitis may occur as a mixed infection with bacteria, mainly *Streptococcus* and *Staphylococcus* spp.[73,74,86,91,109] or herpes simplex virus.[109]

In the retrospective studies available, the proportion of farmers and agricultural workers among the affected patients was relatively high (16%–86%). Male patients were generally more frequent than females: male:female ratios were between 1.4:1 and 3.5:1, with the exception of a study from Nepal, where both sexes were equally affected.[56] The average age of the patients in the studies ranged from 35.8 to 59 years.

Among the factors predisposing for keratomycosis, corneal trauma is considered the most common, with an incidence between 31.6% and 89.9%, apart from a study from Pennsylvania, in which only 8.3% of the patients reported a recent trauma.[78] The reported injuries were caused by different traumatizing agents including plant material (corn stalks, grass, ground nuts, hay, kernel, onions, paddy, palm leaf, sugar cane, thorn, tree branch, and vegetable matter), animal matter (cat scratch, cow dung, cow's tail, hair, hen peck, and insects), chemical gas, dust, fingernails, electric welding light, glass, heat injury, metal objects, mud, soil, stones, or physical violence. In Tanzania, positive correlation was found with HIV carriage: 81.2% of patients with fungal keratitis were HIV positive.[14] Further predisposing factors include preexisting ocular diseases (e.g., atopic conjunctivitis, chronic dacryocystitis, dry eyes, lagophthalmos, recurrent corneal erosion, corneal scaring, or ulcer), the use of topical corticosteroids, previous eye surgery, systemic diseases (e.g., diabetes mellitus, leprosy, or rheumatoid arthritis), as well as contact lens wear. During the past decade, the epidemiology of contact lens solution-related *Fusarium* keratitis has been studied in detail.

Risk factors associated with microbial keratitis in contact lens wearers include continuous overnight wear, lower socioeconomic class, smoking, hypoxia, poor lens hygiene practice, blepharitis, diabetes mellitus, epithelial trauma, and steroid use, specifically in daily wear lenses.[110] Contact lens wearers have an elevated risk for fungal keratitis, but the incidence of *Fusarium* keratitis is quite rare among this group of patients. There has been no report until the beginning of 2006 on the association of multipurpose contact lens solutions with fungal keratitis. Before June 2005, the number of cases with contact lens-related fungal keratitis was about 3 per year in Hong Kong. In late August 2005, the Centre for Health Protection (CHP), Department of Health of Hong Kong, initiated an investigation because of the sudden increase in the number of cases of contact lens-related microbial keratitis. The initial investigation suggested that many fungal keratitis cases were caused by *Fusarium* spp. Great majority of the patients were disposable contact lens users and had reported of using a commercial multipurpose contact lens solution, namely Bausch&Lomb ReNu. Up until May 31, 2006, a total of 33 cases of contact lens-related *Fusarium* keratitis were reported to the CHP. Sixty-four percent (21/33) were female, and the age range of all cases was 16–51 years (mean 28 years). In a retrospective unmatched case–control study, Ma et al.[111] focused on the risk factors playing a role in the development of *Fusarium* keratitis among disposable soft contact lens users. They successfully interviewed 32 patients, and they choose 24

TABLE 51.1
Incidence of *Fusarium* spp. among Culture-Proven Cases of Fungal Keratitis (Based on Literature Search)

Country Studied	Period of Study	Number of Fungal Ulcers	Number of *Fusarium* Isolated	Reference
Australia	July 1996–May 2004	35	5 (14.3%)	[44]
Australia	1998–2008	16	8 (50.0%)	[45]
Australia	October 1999–September 2004	13	7 (53.8%)	[46]
Bangladesh	11 months	51	10 (19.6%)	[47]
Bangladesh	1987	7	1 (14.0%)	[48]
Bangladesh	1991	107	NA (28%)	[49]
Bangladesh	Unknown	63	22 (34.9%)	[50]
Brazil	1975–2003	265	137 (58.8%)	[51]
Brazil	1983–1997	49	12 (32.0%)	[52]
Brazil	January 1994–December 1999	20	12 (60.0%)	[53]
Brazil	2000–2004	66	44 (66.7%)	[54]
Ghana, Accra (south)	June 1999–May 2001	43	27 (63.0%)	[55]
Nepal	1985–1987	68	8 (11.8%)	[56]
Nepal	August 1998–July 2001	145	45 (22.0%)	[57]
Thailand (central)	January 1988–December 2000	35	12 (34.3%)	[5]
Nigeria	1974–1977	42	14 (33.3%)	[58]
Tanzania	October 1994–October 1995	32	24 (75.0%)	[14]
China, Beijing (north)	January1995–October 2000	498	321 (64.5%)	[59]
China, Zhengzhou (central)	January 1975–June 1997	615	NA (65.0%)	[60]
China, Zhengzhou (central)	January 2000–March 2009	1458	076 (73.8%)	[61]
China, Quingdao (north)	January 1996–December 1999	97	63 (64.9%)	[62]
China, North	January 1999–December 2004	596	437 (73.3%)	[63]
China, North	January 2001–December 2006	549	426 (77.6%)	[64]
China	1989–2000	775	455 (58.7%)	[65]
China	January 2001–December 2004	681	394 (57.9%)	[66]
China, Zhejiang	September 2002–July 2004	61	33 (54.1%)	[67]
France, Paris	January 1993–January 2001	19	4 (21.1%)	[68]
Iran	May 2004–March 2005	7	1 (14.3%)	[69]
Iran	1998–1999	29	10 (34.5%)	[70]
Iran	1982–2001	27	2 (7.4%)	[71]
Malaysia	January 2004–April 2005	4	2 (50.0%)	[72]
Paraguay	April 1988–April 1989	26	11 (42.3%)	[73]
Paraguay	1988–2001	136	41 (15.0%)	[74]
Paraguay (children)	1988–2002	35	5 (14.3%)	[75]
Singapore	January 1991–December 1995	29	15 (52.0%)	[76]
Thailand	January 2001–December 2004	49	13 (26.5%)	[77]
Pennsylvania, USA	January 1991–March 1999	24	6 (25.0%)	[78]
South Florida, USA	January 1982–January 1992	127	79 (62.2%)	[79]
South Florida, USA	January 1969–December 1977	133	82 (61.6%)	[80]
Florida, USA	January 2004–December 2005	122	66 (54.1%)	[81]
Florida, USA	January 1999–June 2006	59	24 (40.7%)	[82]
Minneapolis, USA	January 1971–January 1981	19	3 (15.7%)	[83]
Massachusetts, USA	January 2004–November 2007	46	19 (41.3%)	[84]

from them based on their case criteria. The mean age was 30.3 and 29.2% (7/24) were male. Twenty-three of 24 used Bausch&Lomb contact lens solution and 21 could specify it as ReNu MoistureLoc. Twenty-one of the 24 stored their solutions in the bathroom or kitchen. Based on these data it can be stated that ReNu MoistureLoc was strongly connected to the cases of *Fusarium* keratitis in Hong Kong. However, poor lens hygiene practice was also characteristic among the patients. In late February 2006, the Bausch&Lomb Company voluntarily stopped sales of ReNu solution from the markets in Hong Kong. In Singapore, Wong et al.[76] conducted a retrospective case series study on fungal keratitis in the period 1991–1995 at the Singapore National Eye Centre. They identified 29 cases of fungal keratitis. Among these cases,

TABLE 51.2
Incidence of *Fusarium* spp. in India among Culture-Proven Cases of Fungal Keratitis (Based on Literature Search)

State Studied	Period of Study	Number of Fungal Ulcers	Number of *Fusarium* Isolated	Reference
Tamilnadu				
Coimbatore (children)	February 1997–January 2004	37	17 (16.8%)	[85]
Madurai (south)	January 1994–March 1994	155	73 (47.1%)	[86]
Tiruchirapalli (south)	June 1999–May 2001	353	141 (39.9%)	[55]
Tiruchirapalli (south)	July 1985–November 1985	40	19 (47.5%)	[87]
Madras (south)	1980–1982	68	8 (11.8%)	[88]
Vellore	NA	7	3 (42.8%)	[89]
Chidambaram	July 2002–June 2005	230	74 (32.0%)	[90]
Tirunelveli	September 1999–March 2001	554	254 (45.8%)	[91]
Tirunelveli	September 1999–August 2002	1100	471 (42.8%)	[92]
Tirunelveli	September 1999–August 2002	1138	511 (41.6%)	[93]
Rest of India				
New Delhi (north)	January 1999–December 2001	215	23 (10.7%)	[94]
New Delhi	January 2000–December 2004	77	6 (7.8%)	[95]
New Delhi	January 1999–June 2001	191	24 (12.5%)	[96]
New Delhi	NA	31	10 (32.3%)	[97]
Chandigarh (north)	6 years	61	10 (16.4%)	[98]
Chandigarh (north)	January 1999–December 2003	34	8 (23.5%)	[99]
Hyderabad (south)	January 1991–December 2000	1360	506 (37.2%)	[100]
Hyderabad	January 1991–December 1996	557	210 (37.6%)	[101]
Hyderabad	February 1991–June 1995	21	3 (4.1%)	[102]
Patna (east)	2 years	76	6 (7.8%)	[103]
Mumbai (west)	1988–1996	387	33 (8.5%)	[104]
Goa	February 1993–January 1994	16	2 (12.5%)	[105]
Kolkata	January 2001–December 2003	623	132 (21.2%)	[106]
Aurangabad	NA	12	4 (33.3%)	[107]

15 were due to *Fusarium* species (Table 51.1). Twelve further cases were observed in this institute from 2001 to 2004 (unpublished data). Since March 1, 2005, there was a significant increase of *Fusarium*-related eye infections among contact lens users. By May 2006, there were 66 reported cases nationwide, and 12 of these cases occurred between March and May, 2006. In a case study, Khor et al.[17] interviewed these patients. The patients were equally distributed by sex and the mean age was 27.1 years. Except form one, the patients wore disposable contact lenses. A total of 62 patients reported using multipurpose solutions from the same brand (ReNu, Bausch&Lomb, Rochester, NY). Forty-two patients (63.6%) reported using ReNu with MoistureLoc, 6 (9.1%) reported using ReNu MultiPlus, and 11 (16.7%) reported using an unspecified ReNu multipurpose cleaning solution. Based on the interview with the patients, several risk factors have been revealed. Isolates derived from these cases showed 100% identity with *Fusarium solani* CBS490.63 based on the 28S rRNA gene sequence. The main risk factors were the extended use of contact lenses after the planned replacement date (43.9%), the overnight use of daily wear contact lenses (19.7%), and swimming with contact lenses (30.3%) with or without the use of goggles. A case study—based on 61 cases and 367 controls—carried out by Saw et al.[112] showed that the risk of *Fusarium* keratitis was much higher in cases compared with control group for ReNu with MoistureLoc than for ReNu MultiPlus. Soft monthly disposable contact lenses also increased the risk of *Fusarium* keratitis. They revealed that the use of contact lenses past the replacement date increased the risk of *Fusarium* keratitis. Other factors such as washing of hands before replacing the lens, leaving the cap of the solution bottle or the lens case open, or outdoor activity were not significantly associated with *Fusarium* keratitis. On February 17, a news release warned contact lens wearers about the increasing incidence of fungal keratitis. On that same day, Bausch&Lomb voluntarily stopped all sales of ReNu products in Singapore. As a result of actions taken by the Ministry of Health and Bausch&Lomb, the number of cases appeared to be decreasing after March.

The *Fusarium* isolates responsible for the cases of contact lens-related microbial keratitis[17,112,113] showed distinct genotypes. This observation suggested that it is unlikely that common or clonal strains were the cause of the infections. The analysis of the *F. solani* and *F. oxysporum* isolates

showed that these strains are capable of forming biofilms on soft contact lenses under permissive conditions, but not in the presence of, or after recommended 4 h treatment with, MoistureLoc solution. These studies also concluded that there are no significant differences between the effectiveness of a freshly opened or an aged MoistureLoc solution if the conditions of usage are adequate.

Investigation carried out by both the U.S. Food and Drug Administration (FDA) and Bausch&Lomb found no point source of contamination in the factory. No contamination was found in any bottles, and all the tested products were found stable and effective. However, an analysis by Bausch&Lomb showed that the solution can induce a breach in the corneal epithelium that can lead the entry of *Fusarium* into the cornea. Bausch&Lomb concluded that ReNu with MoistureLoc's formulation could create biofilms that shield the fungus from the sterilizing agent.[114,115] In the review of Epstein,[116] the five principal elements of the *Fusarium* outbreak were specified as the decrease of the antimicrobial effect due to biocide uptake by soft contact lenses, selection of *Fusarium* due to persistence, the supported growth of microbes by MoistureLoc biofilm-forming capabilities and the nutritive properties of cellulose, chemical trauma (corneal staining) upon lens insertion due to biocide release, and the blocking of host inflammatory response by lens. In 2006, the U.S. FDA initiated an inspection against Bausch&Lomb's manufacturing site in Greenville, South Carolina. They found that the company failed to regulate the storage and transport temperatures in and beyond the plant. Bullock et al.[117] demonstrated the *in vitro* loss of antimicrobial activity of ReNu MoistureLoc against clinical isolates of *Fusarium* when the product was exposed for a prolonged time (4 week) to a temperature of 60°C. With this study, they simulated the possible conditions to which some of the manufacturer's bottles may have been exposed during storage and transport, or even, perhaps, after purchase. They also concluded that ReNu MoistureLoc was effective against *Fusarium* isolates after a 10 min boiling. This suggests that the disinfectant alexidine inactivation is both time and temperature dependent.

Fusarium was also reported to be the cause of mycotic otitis of the external ear in Gabon, Central Africa,[118] and was also found to germinate in the middle ear of agricultural workers.[119] Four of five cases of *Fusarium* osteomyelitis were reported in healthy individuals following surgery or trauma.[120] Skin lesions are present in 80% of the cases of disseminated infection and they may be primary or metastatic. They are important for early diagnosis because of accessibility for biopsy and culture. Typical skin lesions are painful, erythematous, subcutaneous nodules, and plaques, which later undergo central necrosis.[121]

Fusarium species can cause localized infections of the nails and skin.[122] *Fusarium* can also cause eumycetoma[123] and infection in burn wounds, colonization in burn wounds,[124] granulomas, ulcers, and panniculitis.[2] *Fusarium oxysporum* mainly causes infection in fingernail and toenail[125–128]; however, *F. solani* is more frequently isolated from toenails only.[35,128,129] More than 50% of the fusarial infections is involving the legs.[15] Outbreaks of nosocomial fusariosis have also been reported. *Fusarium* spp., in hospital water distribution system, may result in disseminated fusariosis in immunosuppressed patients.[130]

The possible association of the fusariosis of hospitalized patients with the colonization of a hospital water system by *Fusarium* was reported by Anaissie et al.[131] The authors applied molecular typing methods, including RAPD, RFLP, and interrepeat (IR) PCR, and demonstrated that two patients were infected by *F. solani* strains with genotypes identical to that of certain environmental isolates, while the isolates deriving from six patients matched the isolate of another patient. In a similar study, there was no identity of RAPD patterns between clinical isolates and strains isolated from water samples from the hospital environment, suggesting that the most likely source of fusariosis was the external environment, rather than the hospital water system.[132] In the study by Anaissie et al.,[131] the two clinical isolates matching environmental strains were collected several months before the environmental isolates; therefore it cannot be excluded that the patients may have contaminated the water systems.[132]

Fusarium spp. may also exist in the soil of potted plants in hospitals. These plants constitute a hazardous mycotic reservoir for nosocomial fusariosis.[133]

The reported major risk factors for invasive fusariosis include acute leukemia, bone marrow transplantation, immunosuppressed state, particularly neutropenia, and the use of corticosteroids.[134,135] The modes of entry are the respiratory tract, the skin, and injuries of the corneal epithelium in the case of mycotic keratitis. Trauma remains the major predisposing factor for the development of cutaneous and corneal infections caused by *Fusarium* in immunocompetent hosts.[37] Although many studies reported that skin (vascular catheters, periungual regions, or burns) is the main portal of entry in hematologic cancer with neutropenic patients, other portals of entries such as inhalation into the lungs or upper airways have also been documented.[136] Intake of contaminated grain foods involves gastrointestinal route of infection.[137] In particular, fusariosis in patients with cancer is predominantly a community-acquired infection, usually transmitted via the airborne route from the outdoor environment.[131]

Fusarium spp. are the only opportunistic molds that can be easily recovered from the blood stream.[128] Histopathologically, *Fusarium* infection may mimic any other mycoses exhibiting moniliaceous, septate branching, or non-branching hyphae. Because of these morphological similarities, identification of the fungus obtained from cultures is required to establish fusarial aetiology.[138]

Seasonal variation has also been observed in fusariosis. Among patients living in rural areas, most infections occurred between June and September. Fifty percent of the eye infections and pulmonary infections peaked during August[15,31] and 62% of the fusariosis occurred during summer months, June through August.[139] Fusariosis occurs more commonly in males in all age group ranging from 2 to 78.[31] *Fusarium* can also be isolated from the conjunctival sac and from pharynx as a normal flora.[140]

51.1.3 Clinical Features and Pathogenesis

The clinical features and manifestations of severe invasive fusariosis in immunosuppressed patients mimic those seen in patients with aspergillosis and are always nonspecific.[141] This can lead to inappropriate treatment regimen by the clinicians.[142] Also, nosocomial fusariosis has a longer latency period than community-acquired infection[131]; therefore the progression of disease may be unnoticed. However, the most common findings at the initial presentation are persistent fever, sinusitis, or skin lesions with a black necrotic center.[31]

The neutropenic patients, especially those with acute leukemia, allogeneic bone marrow transplant recipients, as well as the patients with extensive burns or with chronic renal failure[15] are at increased risk of disseminated fusariosis during hospitalization.[122,143–146] In particular, patients with hematological malignancies account for approximately 90% of the reported cases of disseminated fusariosis.[147] Infection typically occurs during a prolonged period of neutropenia, lasting up to 65 days.[31,128,144,148] In neutropenic cancer patients, disseminated infections by *Fusarium* spp. usually present as persistent fever, fungemia, myalgia, and unresponsiveness to a wide spectrum of antibiotics. In immunocompromised patients, it may also cause eumycetoma, cause onychomycosis, or harmlessly colonize the ulcers.[39,138] Upon recovery from myleosuppression, the infection may either resolve completely or become chronic and localized to sinuses, lungs, eye, brain, joint, or muscle[149] with the potential for relapse and dissemination upon reinstitution of cytotoxic chemotherapy.[128] Other presentations of invasive fusariosis in compromised hosts include osteomyelitis, septic arthritis, myositis, foot abscesses, endocarditis, myocarditis, external otitis, peritonitis, brain abscesses, cystitis, meningoencephalitis, and chronic hepatic infection.[2,32,134,120,150–156]

As mentioned previously, infections caused by this fungus can mimic aspergillosis.[122] However, some distinct differences can be seen, such as an increased incidence of skin and subcutaneous lesions and a positive blood culture, mainly in the first days of fever, for patients with disseminated fusariosis.[136,143] In contrast to invasive aspergillosis, the blood cultures are positive in disseminated fusariosis in up to 50%–70% of the cases.[32,145,157] Skin lesions, the hallmarks of disseminated fusariosis, occur in 60%–90% of the cases, compared with a rare occurrence (10%) of such lesions in disseminated aspergillosis.[2,158,159] Patients with neutropenia have a higher rate of disseminated skin lesions compared with nonneutropenic immunocompromised patients.[37] Subcutaneous nodules, palpable and nonpalpable purpura, red or gray macules, red or gray papules, macules or papules with progressive central necrosis with central, flaccid pustules, vesicles, and hemorrhagic bullae are types of lesions seen in patients with disseminated fusariosis.[2,148,159] These skin lesions can involve any site, with predominance on the extremities.[37,159] The lesions, especially the subcutaneous nodules, are often tender[159] and most patients have lesions at various stages of evolution.[31,159] The number of disseminated skin lesions is variable and ranges between 4 and more than 30.[159] Skin lesions are important potential sources of diagnostic tissue in some patients with fusariosis.[37]

When four different taxa of *Fusarium* were tested in murine model, *F. solani* was found to be the most virulent species.[39,160] The production of secondary metabolites, mycotoxins are mainly associated with the pathogenesis.[2] Common mycotoxins produced by *Fusarium* spp. include moniliformin, zearalenone, trichothecenes (deoxynivalenol, nivalenol, and diacetoxyscirpenol), fumonisins, T-2 toxin, and fusaric acid.[161] Among these, trichothecenes, zearelenones, and fumonisins are the most notorious.[162] Ingestion of grains contaminated with these toxins may give rise to allergic symptoms or be carcinogenic in long-term consumption. They are known to cause myelosuppression through toxin production.[126] Furthermore, exposure to fumonisin may lead to human birth defects.[163] The best example is alimentary toxic aleukia, which has been associated with the ingestion of overwintered cereal grains colonized by the toxigenic *F. sporotrichioides* and *F. poae*.[164] The toxin produced by these organisms, called T-2 toxin, is considered as the principal component responsible for the acquisition of alimentary toxic aleukia. The first recognized trichothecene mycotoxicosis was alimentary toxic aleukia in the USSR in 1932 and the mortality rate was 60%.[165] Common manifestations of trichothecene toxicity are depression of immune responses and nausea. *In vitro*, they impair cellular immunity and decrease the humoral response to T-dependent antigens.[166] Experimental injection of T-2 toxin resulted in cardiomyopathy.[167] Zearalenone is mainly produced by *F. graminearum*, and it produces estrogenic effects such as infertility, vulval edema, vaginal prolapsed, and mammary hypertrophy in females and feminization of males—atrophy of testes and enlargement of mammary glands. *F. moniliforme* and *F. proliferatum* produce fumonisin toxin in maize which may cause esophageal cancer.[168] In India, a single outbreak of acute foodborne disease possibly caused by fumonisin B_1 has been reported. The main features of the disease were transient abdominal pain, borborygmus, and diarrhea.[169] Studies on reduction or elimination of *Fusarium* spp. and mycotoxins from contaminated agricultural and food commodities are in progress.[170–172] As a saprophytic nature of the genus, it can easily invade in the neutropenic patient and develop invasive fusariosis. In addition, prolonged usage of corticosteroids and antibiotics in patients with organ transplantation, silastic catheters, and infections of central venous catheters (CVCs), continuous ambulatory peritoneal dialysis (CAPD) catheters, and contact lenses have been reported.[2]

Keratomycosis, one of the most frequent human infections caused by *Fusarium*, is a suppurative, usually ulcerative corneal disease, most frequently occurring as a localized infection.[173] Infection is exogenous in most of the cases, the pathogen is entering through the epithelium of the cornea. Patients usually present with photophobia and discharge from the eyes. A persistent infiltrate is often present at the site of superficial injury, which gradually increases in size and density. The cornea becomes slightly thickened, and satellite lesions may develop peripheral to the focal area of

infiltrations. The signs of inflammation are minimal in comparison with bacterial keratitis.

Fusarium species are often resistant to most of the antifungal agents, and *F. solani* is the most resistant species within the genus.[29,174,175] *In vitro* resistance has been documented against the antifungal agents miconazole, ketoconazole, 5-fluorocytosine, fluconazole, itraconazole, and nikkomycin Z.[28,122,128,176–181] However, amphotericin B (AMB) has been shown to be the mainstay in the treatment of fusariosis.[182] In view of the inherent resistance of *Fusarium* spp. to most antifungal agents, AMB-based combination regimens have also been suggested or used for fusariosis.[128] Natamycin is also active against *Fusarium* spp. both *in vitro* and *in vivo*,[25,26,123,124] and in combination with AMB it has been the mainstay of treatment for *Fusarium* keratitis.[123] However, its toxicity precludes its systemic use in clinical practice although topical application in powder form could halt the progression of fusariosis in pediatric patients with severe burns.[125] Ketoconazole, miconazole, fluconazole, flucytosine, and itraconazole have no *in vitro* activity against *Fusarium* spp.[29,174] However, the newer broad-spectrum triazoles, voriconazole (VRC), posaconazole, and ravuconazole have variable *in vitro* activities against *Fusarium* spp. and show promise for the management of fusariosis.[29,126–129,174,175,178,183–187] Posaconazole, a broad-spectrum triazole, is active against *Fusarium* species both *in vitro* and in animal models.[29,174] Overall, due to the inherent resistance of *Fusarium* spp. against most of the current group of antifungal agents and the profound solid state of immunosuppression in patients who typically develop fusariosis, the currently available therapeutic strategies for invasive fusariosis particularly in heavily immunocompromised patients have less solutions.[2,5–9,25]

51.1.4 Diagnosis

51.1.4.1 Conventional Techniques

Identification of *Fusarium* species is traditionally carried out by the investigation of macro- and micromorphology. The most commonly used macromorphological characters are the colony morphology on the upper and lower surface of the medium, the linear or radial growth rates, and the pigmentation.[2,188,189] These characters are useful for describing a species under standard environmental conditions (e.g., light, temperature, and substrates)[2,190]; however, they should be applied with caution as they may vary between the isolates of a certain species, especially among clinically important ones.[2]

The micromorphological characters of the three types of spores (macroconidia, microconidia, and chlamydospores) are more suitable criteria for the differentiation of *Fusarium* species.[2,189] The absence or presence of one type of them is a key characteristic. The shape of macroconidia (which are formed in sporodochia) is the main distinguishing character, as it is relatively consistent and stable on natural substrates and under standard environmental conditions, in contrast to the size of macroconidia, which is varying within an individual species.[2,189,191] Macroconidia vary in shape from short and squat to highly curved and elongate with needle-like spores.[189] Further key macroconidial characters are the number of septa, and the shape of the apical and basal cells: the apical cell of the macroconidium may be rounded, needle-like, or whip-like and the basal cell may be barely notched or resemble an upside-down foot.[189] The presence or absence of microconidia with various shapes and sizes (which are formed in aerial mycelium) is a primary distinguishing character in *Fusarium* taxonomy. They may be produced singly, in false heads only, or in false heads and chains.[2,189] Their shapes may be napiform, oval, pyriform, clavate, fusiform, or globose.[2] The microconidiophores can be monophialidic only or both mono- and polyphialidic.[2,189] Monophialides have a single opening in the conidiogenous cell, while polyphialides have two or more.[189] Chlamydospores are more common in the older cultures, their presence or absence is a distinguishing character. These thick-walled, verrucose, light-colored spores filled with lipid-like material can be formed singly, in pairs, in clumps, or in chains in the hyphae above or below the agar surface.[2,189] Their outer wall can be smooth or rough.[2] Pseudochlamydospores were described by Marasas et al.[164] in the case of *F. andiyazi*. They are thin and smooth walled and can be found singly or in short chains in the hyphae.[164]

A lot of secondary characters can be used in *Fusarium* identification, e.g., the presence or absence of sporodochia, sclerotia, and stroma[2]; toxins and other metabolites[192]; or odor.[189] Their usage is limited, as these data are not commonly available for routine diagnoses.[189]

Beyond morphology, the studies of sexual cross-fertility can be used for the identification of *Fusarium* species.[189,193] Due to the disadvantages of this technique (that it is slow and needs adequate incubator space), it is not recommended for the purposes of clinical diagnosis.

51.1.4.2 Molecular Techniques

Molecular techniques may help us to avoid the misidentification of *Fusarium* based on their macro- and microscopic observation. The main advantage of the identification by molecular techniques is that they do not require viable organism or sporulation; therefore, they enable a rapid diagnosis.[194]

Several methods are available for molecular identification of *Fusarium* species, and they have been continuously improving by new methods. Fingerprint techniques, like RFLP, AFLP, etc., are useful to identify an unknown strain, if a set of reference strains are available.[25,189] Their advantages are that DNA sequencing is not required and these methods are also appropriate to reveal the evolutionary distance between the investigated isolates.[25,189] DNA sequences of the internal transcribed spacer (ITS) regions of the ribosomal RNA gene cluster are not very useful in distinguishing *Fusarium* species.[195] The sequencing of partial fragments from other amplified genes and their comparison with similar sequences available in the GenBank database is a commonly followed strategy for *Fusarium* identification.[25,189,196] The most frequently used targets are the β-tubulin gene, the translation elongation factor 1α gene (*tef1*), and the histon

gene.[25,189,196] For rapid group- or species-level identification, specific primer pairs for PCR are described in the literature.[197,198] Real-time PCR detection protocols were evaluated for fast detection and identification based on the differences between the tef-encoding genes[199] and the genes involved in mycotoxin biosynthesis; however, this approach is not yet available for all species.[200–204] A DNA microarray was also developed for the easy and fast detection and identification of the fungal genus *Fusarium* based on the recent phylogenetic analyses of *tef1*.[205] Investigation of the intergenic spacer (IGS) regions with molecular methods is useful to resolve the identified species into various subgroups.[206]

51.2 METHODS

51.2.1 Sample Preparation

In the case of filamentous fungi, conventional DNA extraction methods are well suited for the preparation of total genomic DNA.[207] Disadvantages of these techniques are that they are slow, and relatively high amount of sample is needed. In the studies reviewed in the previous sections, commercial DNA purification kits were used for rapid and safe sample preparation.

Media and culture conditions: The following three media are preferred for morphological identification of *Fusarium* species: potato dextrose agar medium (PDA, 50% potato filtrate obtained by boiling 300 g diced potato in 500 mL water and filtering, 20 g sucrose) for the investigation of colony morphology and pigmentation; and for measuring of the growth rates[189]; carnation leaf-piece agar medium (CLA; sterile 3–5 mm carnation leaf pieces in agar) for the investigation of macrocinidia[208]; and "Spezieller Nährstoffarmer agar" (SNA, 1 g KH_2PO_4, 1 g KNO_3, 0.5 g $MgSO_4 \times 7\ H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose) for the investigation of microconidia and chlamydospores[209] (all media are given for 1 L and contain 20 g agar). After incubation at 20°C–25°C for 7–10 days in the presence of light, particularly some exposure to UV (black) light ($\lambda = 310–360$ nm), *Fusarium* isolates grow well and form conidia.[189] Artificial daylight (12 h light/12 h dark) is often used, although total darkness or continuous light is necessary for the evaluation of critical diagnostic characters of some species.[209] In the case of PDA cultures, the presence of light is not essential, but it is increasing the production and total amount of pigment. On the other hand, light is important for CLA cultures as it increases the production of sporodochia.[189] Genomic DNA can be extracted from mycelia grown either on solid or in liquid malt extract medium after incubation at 20°C–25°C for 7 days.

Microscopy sample preparation: Microscopy samples can be made from colonies grown on PDA, CLA, and SNA after incubation at 20°C–25°C for 7–10 days in the presence of light.

Genomic DNA preparation: Genomic DNA can be extracted from mycelia grown either on solid or in liquid PDA medium after incubation at 20°C–25°C for 5–7 days. Beyond conventional DNA preparation methods,[25,194] commercial DNA purification kits can be used for rapid sample preparation.

51.2.2 Detection Procedures

51.2.2.1 Morphological Identification of *Fusarium* Species

The steps of identification based on the macro- and micromorphological characters were described by Summerell et al.[189] and Leslie and Summerell.[25]

Procedure

1. Plate the strain onto PDA, CLA, and SNA medium and incubate it at 20°C–25°C for 7–10 days in the presence of light.
2. Observe the colony morphology, the growth rates, the presence of sporodochia, and the color of the culture and of the produced pigments in PDA.
3. Examine under light microscope the shape and size of macroconidia and chlamydospore production on CLA.
4. Investigate under light microscope the shape, size, and formation of microconidia, their conidiogenous cells, and chlamydospore production on SNA.
5. Examine the growth rates based on linear growth in a race tube as described by Ryan et al.,[211] and/or in a Petri-dish as described by Burgess et al.[212]
6. Organize the data with the use of a recording sheet.[25,212]
7. Make the identification using a manual. The four most commonly used manuals for morphology identification were written by Gerlach and Nirenberg,[213] Nelson et al.,[191] Burgess et al.,[212] and Leslie and Summerell.[25]

The main macromorphological characteristic features are[25,191,212,213]

a. Abundance and color of aerial mycelium
b. Pigmentation
c. Growth rate

The main micromorphological characteristic features are[25,191,212,213]

d. Macroconidia: size, shape, number of septa, shape of apical and basal cell
e. Microconidia: size, shape, number of cells, formation, nature of conidiogenous cells, and conidiophores
f. Chlamydospores: presence or absence, formation

51.2.2.2 Molecular Identification of *Fusarium* Species

51.2.2.2.1 Genus-Specific Identification

51.2.2.2.1.1 PCR Protocol of Hue et al.[197] PCR methods for rapid and reliable identification of *Fusarium* species isolated from clinical samples were evaluated by Hue et al.[197] and Hennequin et al.[214] Hue et al.[197] described a method appropriate for the preparation of PCR templates from *Fusarium*-containing blood and performed PCR with primers designed for the ribosomal RNA genes (rDNAs) based on a large number of isolates belonging to the genus *Fusarium*. AQ8

Primers P28SL (5′-ACA AAT TAC AAC TCG GGC CCG AGA-3′) and P58SL (5′-AGT ATT CTG GCG GGC ATG CCT GT-3′) amplified a fragment of 329 bp containing ITS2 and a portion of 5.8S and 28S rDNA. To avoid false-negative results, a positive internal control was used from a part of λ phage DNA. It was amplified by two primers (C1, 5′-ACA AAT TAC AAC TCG GGC CCG AGA CCA CAG CGC-3′ and C2, 5′-AGT ATT CTG GCG GGC ATG CCT GTG TAC AAC TGG-3′), whose 3′ ends correspond to the λ DNA while the 5′ ends correspond to the primers used in the PCR amplification. The PCR reaction resulted in a 517 bp fragment which contains the *Fusarium* sequence at the ends, than it was amplified with the *Fusarium* primers.

Procedure[197]:

1. Lyze the cells with a commercial genomic blood DNA purification kit. Mix it with 200 mL of enzyme buffer (0.9 M sorbitol, 0.1 M Tris, 0.1 M EDTA) and 20 mL of lyticase and incubate the samples at 37°C for 90 min. Treat the sample with proteinase K (10 mL, 20 mg/mL) and incubate it at 55°C for 30 min. Add 5 µL of RNase and incubate the sample at 37°C for 30 min. Extract the DNA with a commercial genomic blood DNA purification kit.
2. Prepare the reaction mixture according to the manufacturer's instructions. Use 1 ng of template DNA per reaction mixture.
3. Perform the first PCR in a thermocycler using the following reaction conditions: 94°C for 5 min; 40 cycles of 94°C, 68°C, and 72°C for 1 min each; and a final 72°C for 10 min. Include the positive internal control.
4. Visualize the PCR products after electrophoresis on 2% agarose gel stained with ethidium bromide and run in 1 × TAE (Tris-acetate-EDTA) buffer.

51.2.2.2.1.2 PCR Protocol of Hennequin et al.[214] Hennequin et al.[214] designed a primer pair based on the 28S rDNA sequences of *Fusarium* species associated with human infections. In contrast to the DNA from unrelated genera, the primers Fus1 (5′-TGA AAT CTG GCT CTC GGG-3′) and Fus2 (5′-CAT GCG CGA ACC TCA GTC-3′) amplified a 480 bp fragment from the DNA extracts of *Fusarium* strains and of the members of related genera *Acremonium* and *Cylindrocarpon*.

Procedure[214]:

1. Prepare the reaction mixture in a final volume of 50 µL containing 5 µL genomic DNA template, 10 mM Tris–HCl, 50 mM KCl, 2.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 5 µL of DNA, 0.25 µM of each primers, 0.2 mM of each deoxynucleoside triphosphates (dNTPs), and 0.3 U of Taq polymerase.
2. Perform the PCR in a thermocycler using the following reaction conditions: 94°C for 10 min; 35 cycles of 94°C for 30 s, 64°C for 90 s, and 72°C for 90 s; and a final 72°C for 10 min.
3. Visualize the PCR products after electrophoresis on 1% agarose gels stained with ethidium bromide and run in 1 × TAE buffer.

51.2.2.2.2 Species-Specific Identification

Fusarium species can be identified at the species level by sequencing of amplified gene fragments and comparing the sequences with similar ones available in sequence databases (FUSARIUM-ID, GenBank, EMBL, and DDBJ) using a Basic Local Alignment Search Tool (BLAST) search.

51.2.2.2.2.1 β-Tubulin Gene PCR Protocol 1[206] Yli-Mattila et al.[206] used a universal forward primer (T1: 5′-ATG CGT GAG ATT GTA AGT-3′)[215] and a specific reverse primer (tub-conrev T22: 5′-TGA CCG AAA ACG AAG TTG TC-3′) for amplifying a fragment with the first two introns of the β-tubulin-encoding gene.[206]

1. Prepare the reaction mixture containing 5–50 ng genomic DNA, 2.5 mM $MgCl_2$, 10 mM Tris–HCl (pH 8.8), 50 mM KCl, 0.1% (w/v) Triton X-100, 0.4 µM of each primer, 0.12 mM of each dNTP, 3 U of Taq polymerase.
2. Perform the PCR in a thermocycler using the following reaction conditions: 1 min at 94°C; 30 cycles of 94°C, 51°C, and 74°C for 1 min each; and a final 74°C for 7 min.
3. Visualize the PCR products after electrophoresis on 1% agarose gels stained with ethidium bromide and run in 1 × TAE buffer.
4. Purify the PCR products with a commercial PCR DNA purification kit.
5. After the sequencing of the PCR product, compare it with the β-tubulin sequences available in the GenBank database using a BLAST search.

Amplification of a partial β-tubulin gene fragment was carried out by Chung et al.[216] using a specific primer pair, FU-tubulin3 (5′-CGA GCC CGG TAC CAT GGA CG-3′) and FU-tubulin2 (5′-GGT CGC CGT AAG AGG GGT TGG-3′).

51.2.2.2.2.2 β-Tubulin Gene PCR Protocol 2[216]

1. Prepare the reaction mixture in a final volume of 20 µL containing 5–50 ng genomic DNA, 0.2 µM of each primer, 0.25 mM of each dNTP, 1 U of Ex Taq polymerase and Ex Taq reaction buffer (containing 2 mM $MgCl_2$).
2. Perform the PCR in a thermocycler using the following reaction conditions: 94°C for 3 min; 35 cycles of 94°C for 1 min, 60°C for 30 s, and 72°C for 2 min; and a final 72°C for 5 min.
3. Visualize the PCR products after electrophoresis on 1% agarose gels stained with ethidium bromide and run in 1 × TAE buffer.

4. Purify the PCR products with a commercial PCR DNA purification kit.
5. After the sequencing of the PCR product, compare it with the β-tubulin sequences available in the GenBank database using a BLAST search.

51.2.2.2.2.3 Translation Elongation Factor 1α Gene (tef1) PCR The *tef1* gene has high phylogenetic utility at the species level in *Fusarium*.[196] Several universal primers have been designed that work across the phylogenetic breadth of the genus.[196,217] Geiser et al.[196] created the first generation of a database (FUSARIUM-ID v.1.0, http://fcgp.fusariumdb.org/intro.php), which contains more than 400 sequences representing a phylogenetically diverse selection of *tef1* sequences from the *Fusarium* genus.[217]

Procedure[196,217]

1. Perform a standard PCR protocol with an annealing temperature of 53°C to amplify the *tef1* gene region using forward primer ef1 (5′-ATG GGT AAG GA(A/G) GAC AAG AC-3′) and reverse primer ef2 (5′-GGA (G/A)GT ACC AGT (G/C)AT CAT GTT-3′).
2. Visualize the ~700 bp PCR products after electrophoresis on 1% agarose gel stained with ethidium bromide and run in 1 × TAE buffer.
3. Purify the PCR products with a commercial PCR DNA purification kit.
4. After the sequencing of the PCR product, compare it with the *tef1* sequences available in the *Fusarium* database (http://isolate.fusariumdb.org/) using a BLAST search.

51.2.2.2.2.4 Histon-Encoding Gene PCR Steenkamp et al.[218] performed PCR for amplification of a histone H3 gene fragment ranging from 519 to 527 bp with the primers H3-1a (5′-ACT AAG CAG ACC GCC CGC AGG-3′) and H3-1b (5′-GCG GGC GAG CTG GAT GTC CTT-3′).[219]

Procedure[218]

1. Prepare the reaction mixture containing 0.25 ng genomic DNA/0.05 U Taq polymerase, 2.5 mM $MgCl_2$, 1 × reaction buffer, 0.2 µM of each primer, 0.25 mM of each dNTPs.
2. Perform the PCR in a thermocycler using the following reaction conditions: 92°C for 1 min; 30 cycles of 92°C, 68°C, and 72°C for 1 min each; and a final 72°C for 5 min.
3. Visualize the PCR products after electrophoresis on 1% agarose gel stained with ethidium bromide and run in 1 × TAE buffer.
4. Purify the PCR products with a commercial PCR DNA purification kit.
5. After the sequencing of the PCR product, compare it with the histone H3 sequences available in the GenBank database using a BLAST search.

51.2.2.2.2.5 ITS Sequence PCR Oechsler et al.[220] designed primers for the end of the 18S ribosomal DNA (F18A: 5′-GCG GAG GGA TCA TTA CCG AGT T-3′) and the beginning of the 28S rRNA (F28S: 5′-CAG CGG GTA TTC CTA CCT GATC-3′) of the target *Fusarium* species, and amplified with them the ITS region comprising ITS1, 5.8S rRNA, and ITS2. Although previous ITS analyses were not very useful for distinguishing *Fusarium* species, the sequence data from this study correlated well with the morphologic classification. The authors suggested the feasibility of *Fusarium* detection and identification at the species level from ocular sources using the sequence of the ITS region.

Procedure[220]

1. Prepare the reaction mixture for a standard PCR protocol in the final volume of 25 µL containing 200 ng DNA template.
2. Perform the PCR in a thermocycler using the following reaction conditions: 95°C for 3 min; 45 cycles of 95°C for 30 s, 55°C for 30 s, and 68°C for 2 min.
3. Visualize the PCR products after electrophoresis in 1% agarose gels stained with ethidium bromide and run in 1 × TAE buffer.
4. Purify the PCR products with a commercial PCR DNA purification kit.
5. After the sequencing of the PCR product, compare it with the ITS sequences available in the GenBank database using a BLAST search.

51.2.2.2.2.6 F. oxysporum- and F. solani-Specific PCR There are a lot of species-specific primer pairs in the literature for rapid and reliable identification of *Fusarium* species.[198,214,217,221] Ghignone and Migheli[198] collected the species-specific primers for the detection of phytophatogenic fungi and created a continuously improving database from them (http://www.sppadbase.com/). This database contains primers for identification of some human pathogenic members of the *Fusarium* genus and references where the protocols are described.

Hue et al.[197] designed primer pairs for specific detection of five clinically important *Fusarium* species derived from human sources. Only the OX 31 (5′-TGA CTT GGA TGA GAC CTT GGC G-3′) and OX 32 (5′-CAG GAT TTA CCG ACA CAG CTT TTG-3′) primer pair was specific for the investigated *F. oxysporum* strains (annealing temperature = 66°C), while the SOL 31 (5′-GCT ACC GAG GCC ATC AAT TCA TG-3′) and SOL 32 (5′-TGA TGT TGT ACT TCT CCT TGC CC-3′) primer pair was specific for four of the five tested *F. solani* strains (annealing temperature = 66°C). The procedure and methodology are described in Section 51.2.2.2.1.

51.3 CONCLUSION AND FUTURE PERSPECTIVES

As human and animal diseases due to *Fusarium* spp. have become increasingly common, there is an urgent need to

develop rapid, sensitive, and specific diagnostic methods for these organisms. Correct identification of *Fusarium* organisms to species/clonal level is essential for the control of human and plant fusarial diseases. Considering the fact that the morphological features of *Fusarium* at both the genus and species level are diverse and similar to those of other fungal genera/species, it has been a challenge to identify these organisms precisely. To ensure effective solutions against problems of *Fusarium* etiology, it is critical to develop common and suitable identification systems for *Fusarium* taxonomy using a combination of conventional culture, macroscopic and microscopic characteristics, and molecular methods. For clinical strains of *Fusarium*, antimicrobial susceptibility patterns of the new isolates can be compared with the confirmed patterns for additional understanding about the species and will also help select most appropriate chemotherapy. Further analysis of *Fusarium* whole-genome sequences will facilitate comparison and reconfirmation of the species/clones in future.

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AUTHOR QUERIES

[AQ1] Plesae check the identified heading levels for correctness.

[AQ2] Can "species" be inserted after "*Fusarium*" in the sentence beginning "*Fusarium* cause…"? Please clarify.

[AQ3] All figures to be set in grayscale. Please rephrase references to color in Figure 51.1 caption accordingly.

[AQ4] Please verify the change of "Infection caused by…" to "Infections caused by…"

[AQ5] Can "species" be inserted after "*Fusarium*" in the sentence beginning "*Fusarium* have been…"? Please clarify.

[AQ6] Please check the sentence starting "Except form one…" for clarity.

[AQ7] Please clarify whether the edit to the sentence "In immunocompromised patients…" is OK.

[AQ8] In "ribosomal RNA genes (rDNAs)", please clarify whether "rDNA" should be "rRNA".

[AQ9] Please check the author name in Ref. [11].

[AQ10] Please provide more details if any for Ref. [114].

[AQ11] References [37,136], [120,153], [180,189] are exactly one and the same. So we have deleted the repeated version and renumbered the reference list and in-text citations. Please check.

[AQ12] Please provide volume number for Ref. [209].

[AQ13] Please provide in-text citation for Ref. [210].