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Phylogenetic diversity of human pathogenic *Fusarium* and emergence of uncommon virulent species

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Summary *Objectives: Fusarium* species cause a broad spectrum of infections. However, little is known about the etiological agents to the species level. We identified *Fusarium* species isolated from clinical specimens including those of high risk patients to better understand the species involved in the pathogenesis.
Methods: A set of 44 *Fusarium* isolates were identified by two-locus sequence typing using partial sequences of the second largest subunit of RNA polymerase (*RPB2*) and translation elongation factor 1 alpha (*TEF-1α*).
Results: The identified species belonged to four species complexes (SC); the most common SC was *Fusarium solani* (FSSC) (75%), followed by *Fusarium oxysporum* (FOSC) (4.5%), *Fusarium*

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fujikuroi (FFSC) (13.6%), and *Fusarium dimerum* (FDSC) (6.8%). Sites of infections were nails (n = 19, 43.2%), skin (n = 7, 15.9%), cornea (n = 6, 13.6%), blood (n = 3, 9%), wound (n = 4, 6.8%), burn (n = 2, 4.5%), tissue (n = 2, 4.5%), and urine (n = 1, 2.27%). *Fusarium acutatum* was rare and seem restricted to the Middle East. Comorbidities associated with invasive infections were hematological malignancy and autoimmune disorders.
Conclusions: Members of the FSSC predominantly caused cornea, nail and bloodstream infections. Less frequently encountered were the FOSC, FFSC and FDSC. More accurate molecular identification of *Fusarium* species is important to predict therapeutic outcome and the emergence of these species.

Introduction

Fusarium is a hyaline hyphomycete fungus that is commonly found in the environment, where it is isolated from soil, plants and water systems.[1–3] Some *Fusarium* species cause a broad spectrum of opportunistic infections in human, while invasive and disseminated infections occur predominantly in severely immunocompromised patients[3,4] and generally manifest as fever not responding to antimicrobial therapy.[5] Risk factors for the development of invasive fusariosis are neutropenia, hematologic malignancies, hematopoietic cell transplantation, and impaired cellular immunity.[4–7] More recently an invasive infection was also reported to occur in immunocompetent patients.[8] Other infections, such as keratitis or endophtalmitis and onychomycosis, are frequently manifested in immunocompetent persons and are often associated with previous trauma.[9–13] *Fusarium* species may occasionally cause infections in animals.[14]

Fusariosis is the leading mycosis affecting immunocompromised patients,[3] and represents the second most common cause of filamentous fungi infections after aspergillosis.[15,16] Nucci et al.[17] recently reported on 233 cases of invasive fusariosis from centers in 11 different countries. Another study reported 97 global cases, which were published in the medical literature between January 2000 and January 2010, and updated with an additional 26 cases reported from USA.[4] Invasive infections with *Fusarium* species are characterized with poor prognosis due to neutropenia of the infected patients[18] and the high resistance to antifungal agents and therapy.[19–22]

Often clinically important, morphologically similar *Fusarium* species are identified to species complex level only, and further genotypic characterization to species level is usually not done routinely in clinical laboratories. In the present study, we describe the molecular characterization of *Fusarium* species isolated from 43 patients with invasive and cutaneous infections, and the clinical manifestation of the disease in patients infected with fusariosis observed over 10 years at Hamad Medical Corporation (HMC), Qatar.

Materials and methods

Patients and specimens

A set of 44 *Fusarium* strains were isolated from 43 (38 immunocompetent and 5 immunocompromised) patients that were seen at Hamad Hospital, Doha, Qatar from July 2003 to June 2014. The demographic data, clinical specimens, and fungal etiology are reported in Table 1. The isolates were obtained from a variety of clinical specimens. Nineteen strains were isolated from nails, 7 from skin, 6 from cornea, 3 from blood, 4 from wounds, 2 from burn wounds, and 2 from skin tissue, and 1 from urine. One isolate was obtained from each patient except for two isolates that were taken from blood and skin respectively, from the same patient. The HMC research and ethics committee (reference number RC/104044/2015) granted approval to conduct this study.

Isolation and morphological identification

Fusarium species were isolated and identified by morphology according to standard laboratory procedures. The clinical specimens were generally cultured on either Sabouraud dextrose agar (SDA; Difco Laboratories, Detroit, MI) plus chloramphenicol (SDA), SDA without antibiotics, or brain heart infusion plus 40 U/ml streptomycin and 20 U/ml penicillin. Blood cultures were performed using the Bactec automated culturing system (BD Diagnostic Systems). For culturing from urine, cysteine lactose electrolyte-deficient agar (CLED) (Mast Diagnostics, UK) was used for the isolation of the organism.

Fusarium species isolated from nails were considered proven infections after two consecutive isolations from the same patient, direct microscopy showing compatible fungal cells, and the absence of dermatophytes in culture, according to the diagnostic criteria of Gupta et al.[23] Organisms isolated from specimens that did not meet such criteria were excluded from the study. Culture plates were incubated at 26 °C and 37 °C and were observed daily for growth up to 10 days.

The isolates were sent to CBS-KNAW Fungal Biodiversity Center, Utrecht, The Netherlands for molecular identification, where they were subcultured on oatmeal agar (OA; home-made at CBS) and incubated for 5 days at 28 °C. Strains were deposited under accession numbers in the reference collection of the CBS-KNAW Fungal Biodiversity Centre (Table 1).

DNA extraction

Hyphae were harvested in 2 ml screw capped tubes with sterile sand, 750 μl of Lysis buffer and 750 μl of phenol-chloroform (1:1; pH 8.0) were added and the mixtures were bead beaten at 2500 rpm for 3 min. The crude extract was