Cluster of *Fusarium verticillioides* bloodstream infections among immunocompetent patients in an internal medicine department after reconstruction works in Larissa, Central Greece

S.P. Georgiadou [a,†], A. Velegraki [b,†], M. Arabatzis [b], I. Neonakis [c], S. Chatzipanagiotou [d], G.N. Dalekos [a], E. Petinaki [c,*]

[a] Department of Medicine and Research Laboratory of Internal Medicine, Medical School, University of Thessaly, Thessaly, Greece
[b] Mycology Research Laboratory, Department of Microbiology, Medical School, National and Kapodistrian University of Athens, Athens, Greece
[c] Department of Microbiology, Medical School, University of Thessaly, Thessaly, Greece
[d] Department of Biopathology and Clinical Microbiology, Athens Medical School, Aeginition Hospital, Athens, Greece

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SUMMARY

Background: Fusarium spp. can cause disseminated infections, particularly in immuno-compromised patients. *Fusarium verticillioides* is a human pathogen, and sporadic cases of fusariosis have been reported.
Aim: To report a nosocomial cluster of *F. verticillioides* bloodstream infections among seven immunocompetent inpatients following reconstruction works.
Methods: Identification was performed using macroscopic and microscopic morphology, and molecular assays (sequencing the nuclear ribosomal internal transcribed spacer region and translation elongation factor-1α gene). Susceptibility testing was performed in accordance with the guidelines of the Clinical and Laboratory Standards Institute. Environmental surveillance specimens were taken and cultured on Sabouraud dextrose agar plates.
Findings: In total, 16 blood cultures obtained from the seven patients were positive for *F. verticillioides*. All surveillance cultures were negative.
Conclusions: In order to prevent fungaemia, it is important to implement effective infection control measures, before, during and after demolition and construction activities in healthcare settings.
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Introduction

Fusarium spp. are filamentous fungi, widely distributed in soil and plants, that can infect humans and animals, causing superficial locally invasive or disseminated disease.[1,2] Infection is mainly through inhalation of air-borne conidia or via breaks in the skin due to trauma and/or burns. Contamination of hospital

* Corresponding author. Address: Department of Microbiology, University of Thessaly, School of Medicine, Biopolis 41110, Larissa, Greece. Tel.: +30 241 3502508; fax: +30 241 3501570.
   E-mail address: petinaki@med.uth.gr (E. Petinaki).
† Contributed equally to this work.

water systems has been reported to result in dispersal of air-borne conidia.[3] Patients undergoing haematopoietic stem cell transplantation or solid organ transplantation, those with haematological malignancies and those undergoing immuno-suppressive therapy are at high risk of fusariosis.[4] Although *Fusarium verticillioides* (formerly *Fusarium moniliforme*) is considered to be one of the most common *Fusarium* spp., information regarding its clinical significance is limited, and is mainly found in case reports of infections in immunodeficient patients.[5,6] This article describes a cluster of *F. verticillioides* bloodstream infections among immunocompetent patients in an internal medicine department in Central Greece following reconstruction works, and the control measures implemented.

Methods

Setting

The study institution is a 650-bedded tertiary care hospital that serves a population of approximately one million people. The internal medicine department has 44 beds distributed in five four-bedded units, nine two-bedded units and six single rooms. All 20 rooms are on the same floor, and none of the rooms have high-efficiency particulate air filters or negative pressure.[7] The internal medicine department admits patients 24 h/day, four days/week, making simultaneous evacuation of the entire unit impossible.

Reconstruction works

The floors of all rooms in the internal medicine department were reconstructed over a seven-day period (1st–7th November 2012). Each day, three different rooms were evacuated for reflooring. The air-conditioning unit of each room was dismantled and disinfected. Additional measures, such as sealing rooms with plastic coverings, were not implemented. After renovation, each room was cleaned thoroughly and aerated for 24 h before the return of the patients; no environmental cultures were taken at this time. After the outbreak, all rooms in the internal medicine department were disinfected with quaternary ammonium fungicidal compounds, and surveillance cultures were taken.

Patients

Between 8th and 26th November 2012, seven cases of fever with fungaemia occurred among inpatients in the internal medicine department. These patients were distributed in four closely located rooms. All patients were elderly males (median age 77 years, range 63–86 years). According to their clinical data, none of the seven patients were malnourished. Six of the seven patients did not have any risk factors for fusarium infection, such as neutropenia, or hepatic or renal insufficiency.[8,9] One patient with idiopathic thrombocytopenic purpura was on methylprednisolone therapy. Epidemiological data of the patients and final outcomes are shown in Table I.

Surveillance specimens during the outbreak

After the third case of fungaemia, environmental swab samples were taken in all rooms of the internal medicine department, before and after disinfection, in order to identify the source of the outbreak. Swabs were taken from consumables, benches, incubators, porous materials (e.g. blankets and

Table I
Clinical characteristics and outcome of patients with fusariosis, Larissa, Greece, 8th–26th November 2012 (N = 7)

Age in years/sex	Date of admission	Cause of admission	Date of positive blood cultures	Source of positive blood cultures	Treatment	Outcome	Comments
74/M	02/11/2012	UTI/BSI[a]	08/11/2012 26/11/2012	Peripheral	Yes	Death	Date of death 01/12/2012
85/M	02/11/2012	UTI/BSI[b]	12/11/2012 29/11/2012	Central venous catheter	Yes	Survival	Discharged 12/12/2012
86/M	08/11/2012	Endocarditis–spondylodiscitis[c]	13/11/2012	Peripheral	No	Death	Date of death in ICU 20/11/2012
70/M	15/09/2012	ITP	17/11/2012 04/12/2012 09/12/2012	Peripheral	Yes	Death	Date of death 11/12/2012
63/M	13/10/2012	Pneumonia[d]	18/11/2012 02/12/2012 05/12/2012	Peripheral	Yes	Death	Date of death in ICU 12/12/2012
82/M	10/11/2012	Pneumonia[e]	20/11/2012 09/12/2012	Peripheral	Yes	Survival	Discharged 30/12/2012
80/M	18/11/2012	BSI[f]	26/11/2012 06/12/2012 08/12/2012	Central venous catheter	Yes	Survival	Discharged 07/01/2013

M, male; UTI, urinary tract infection; BSI, bloodstream infection; ICU, intensive care unit; ITP, idiopathic thrombocytopenic purpura.
[a] Due to *Klebsiella pneumoniae*.
[b] Due to *Proteus* spp.
[c] Due to *Staphylococcus aureus* (meticillin sensitive).
[d] Due to *K. pneumoniae* and *Serratia marcescens*.
[e] Due to *S. marcescens*.
[f] Due to *Proteus* spp., *Escherichia coli* and *Providencia stuartii*.

bed linen), bed surfaces, tables, sinks and medical equipment. The ventilation system within the microbiology laboratory was also included to rule out potential laboratory contamination. All samples were plated on to Sabouraud dextrose agar with chloramphenicol. The hospital water system was not suspected as a source of contamination and was not tested as no other clusters of fusarium fungaemia were concurrently identified elsewhere in the hospital. However, the water system may have been implicated, as a recent study in a French tertiary care institution revealed the presence of *Fusarium* spp. in the water distribution system.[10] In addition, screening blood cultures were performed once per week in all inpatients in the internal medicine department (with and without suspicion of infection), between 13th November 2012 and 13th January 2013. Single peripheral blood culture samples (an aerobic bottle) were taken by registered nurses and phlebotomists using an aseptic technique; a 10% povidone-iodine swab (Betadine) was applied to the venepuncture site and was allowed to dry.

Laboratory investigation

Based on macroscopic and microscopic morphology on potato dextrose agar (PDA) prepared in house and incubated at 25 °C for seven days, the clinical isolates were broadly placed within the *Gibberella fujikuroi* species complex. Briefly, colonies on PDA were floccose with white aerial mycelium, a bright salmon reverse and a brownish centre at the inoculation point. Fusiform to clavate microconidia were produced abundantly from long monophialides in long chains, and macroconidia were thin walled with foot-shaped basal cells, almost straight, long and slender. In comparison, colonies on malt extract agar were faster growing and had a deep centre. All isolates displayed maximum growth at 37 °C and minimum growth at 3 °C. Following the protocol for mould sequence-based identification, the isolates' nuclear ribosomal internal transcribed spacer region was sequenced (GenBank accession number KC709665), confirming the isolates' phenotypic placement within the *G. fujikuroi* species complex.[11] Sequencing translation elongation factor-1α gene (GenBank accession number KC709666) and comparison of the derived sequences into the Fusarium-ID database (http://isolate. fusariumdb.org/index.php) identified the isolates to species level as *Fusarium verticillioides* (teleomorph *G. moniliformis*).[12] Susceptibility testing was performed in accordance with the guidelines of the Clinical and Laboratory Standards Institute.[13]

Subtyping of the isolates was attempted by a polymerase-chain-reaction-based method originally developed for subtyping *Aspergillus fumigatus* clinical isolates.[14] Briefly, two primers, designated 'IGSL' and 'IGSR', targeting conserved intergenic spacer regions were used, as described previously.[14] Although all *F. verticillioides* isolates demonstrated the same DNA type, testing a single available *F. verticillioides* isolate of nosocomial origin but unrelated epidemiologically to those described in this study did not provide sufficient evidence on the reliability of this method for recognizing intraspecific DNA types. Clearly, it would be useful if more clinically and epidemiologically linked and unrelated strains were tested.

Results

On 2nd November 2012, a 74-year-old male patient with a history of diabetes mellitus, hypertension and ischaemic stroke was transferred to the internal medicine department from a long-term care facility because of a two-day history of fever and malaise. Urosepsis was suspected. Urine grew *Klebsiella pneumoniae* producing extended-spectrum beta lactamase, and the patient was treated with meropenem. Six days later, fever recurred and blood cultures revealed *Fusarium* spp. Minimum inhibitory concentrations of antifungals and the candins were recorded: amphotericin B (>32 mg/L), 5-fluorocytosine (>32 mg/L), anidulafungin (>16 mg/L), caspofungin (>16 mg/L), micafungin (>16 mg/L), itraconazole (>32 mg/L), posaconazole (32 mg/L) and voriconazole (1 mg/L). Although the new echinocandin drugs such as micafungin and caspofungin are very important for treating common aspergillus and candida infections, they are inactive for *Fusarium* spp. and *Mucor* spp.[15] The patient was commenced on intravenous voriconazole (6 mg/kg on day 1, then 4 mg/kg) immediately (i.e. the same day as the fusarium-positive culture).

A new chest X-ray showed bilateral pulmonary infiltrates. Microscopic examination and cultures of sputum specimens were negative for *Fusarium* spp. Bronchoalveolar lavage was not performed as bronchoscopy was not clinically indicated. The patient responded initially to the antifungal treatment with remission of fever, but his condition deteriorated on day 24 and he presented with hypotension and lethargy. Broad-spectrum antibiotics were added empirically and new blood cultures were obtained, which were positive for *Fusarium* spp. alone.

The patient died 30 days after admission. Death was attributed to fusarium infection, as the requested autopsy was denied. Following the first case, six other patients (all male, median age 80 years, range 63–86 years) were diagnosed with fusarium bloodstream infections (Table I).

All fusarium isolates had identical MICs, with the lowest (1 mg/L) recorded for voriconazole. Six of the seven patients received intravenous voriconazole; one patient, who was not treated, died in the intensive care unit before blood cultures flagged positive for *Fusarium* spp. Overall, four of the seven patients with fusarium bloodstream infection died (median time of death 24 days after diagnosis, range seven to 30 days) (Table I) but autopsy was denied in all cases.

In total, 16 blood cultures were obtained from the seven patients, all of which were positive for *Fusarium* spp. (Table I).

Following identification of the third case of *F. verticillioides* on 13 November 2012, the hospital's infection prevention and control committee decided that strict infection control measures were required urgently. Each day from 13th November to 4th December 2012, three rooms in the internal medicine department were evacuated and disinfected with quaternary ammonium. Simultaneous evacuation of all rooms was not an option, as the internal medicine department is active 24 h/day, four days/week. Equipment and surfaces, storage rooms and medication preparation rooms were also subjected to enhanced disinfection with quaternary ammonium fungicidal compounds, followed by aeration of each disinfected room. An infection control nurse made daily rounds on the internal medicine department to monitor compliance with the recommended infection control practices. Patients were moved back to their rooms after 48 h.

All surveillance specimens, blood cultures and environmental samples were negative for *Fusarium* spp. Several random samples from the suction tubes in the internal medicine department and

povidone-iodine solution bottles used for skin disinfection were negative for contaminants. No other cases of fusarium bloodstream infection were detected after implementation of the infection control measures in the internal medicine department; however, the possibility of factors leading to spontaneous termination of the outbreak cannot be excluded.

Discussion

Fusarium spp. are saprophytic moulds that can be found in soil and air. In addition to local infections, they can also cause invasive fungal infections. Common portals of entry are the skin and the airways. *Fusarium* spp. are usually resistant to antifungal agents, and invasive fusarioses are life-threatening conditions with high mortality rates. *F. verticillioides* is considered to be a human pathogen, and cases of fusariosis occur sporadically, mainly in immunocompromised patients. To the authors' knowledge, this is the first study to report a cluster of *F. verticillioides* bloodstream infections among immunocompetent inpatients.

Six of the seven patients appeared to be immunocompetent, and no characteristic signs of disseminated fusarium infection (e.g. skin lesions, organ involvement) were observed.[16] The possibility of a pseudo-outbreak could not be excluded; however, the isolation of *Fusarium* spp. from multiple blood cultures from these patients, connected in space and time, would support the hypothesis of an outbreak. Although it is not possible to propose an unambiguous mechanism for the dispersal of *Fusarium* spp. conidia, it is possible that spores were released from the old floors during building work, settled on room surfaces and materials, and acted as reservoirs for conidia dissemination. The fact that *Fusarium* spp. were not isolated from the surveillance cultures, which were obtained after the third case, does not exclude this possibility as: (1) the air in the ward was not sampled during reconstruction works, or immediately after, to assess the concentration of *Fusarium* spp. conidia at particular points in time before the occurrence of the index case; and (2) surface sampling was performed at a different time under a different ward microclimate, which may have influenced the outcome of the environmental surveillance, causing qualitative and quantitative variations in the concentrations of any fungal spores.[17]

A nosocomial pseudo-outbreak caused by *F. verticillioides* was reported in Italy in 1994–1995, but, to the authors' knowledge, this is the first report of nosocomial *F. verticillioides* bloodstream infections following reconstruction works.[18] Hospital reconstruction works and renovations have been associated with increased risk for nosocomial mould infections, especially among severely immunocompromised patients.[19,20] Potential sources of nosocomial fungal pathogens include airborne conidia, although colonization or high air-borne spore concentrations are not necessarily predictive of fungal infection as contaminated intravenous fluids, catheters and hospital water contaminating the hands of healthcare workers have also been implicated.[21] Implementation of the recommended infection control measures during reconstruction/building activities in healthcare settings is imperative.[22] However, due to budgetary constraints, most of the recommended measures were not taken prior to, or during, the reconstruction works in the study department, which is likely to have led to dispersion of fungal spores in ward areas. Successful control of the fungaemia cases underlines the importance of application of effective control measures, before, during and after construction and demolition activities in healthcare settings.

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