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CASE REPORT

Breakthrough Invasive *Candida glabrata* in Patients on Micafungin: a Novel *FKS* Gene Conversion Correlated with Sequential Elevation of MIC

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***Candida glabrata* strains sequentially isolated from blood developed resistance to micafungin (MICs from <0.015 to 4 μg/ml). A novel mutation identified in micafungin-resistant strains at bp 262 of *FKS2* (containing a deletion of F659 [F659del]) was inserted into the homologous region in *FKS1*.**

CASE REPORT

A 93-year-old man was admitted to our hospital with a diagnosis of pulmonary tuberculosis (day 1). The patient had been receiving treatment for essential hypertension with chronic renal failure for a decade. On admission, the patient's vital signs were normal; however, serum laboratory data showed a marked elevation of creatinine (Cr) (4.7 mg/dl) and blood urea nitrogen (BUN) (74.5 mg/dl). After initiation of antituberculous therapy with oral isoniazid (300 mg/day) plus rifampin (450 mg/day), renal failure progressed (Cr, 7.0 mg/dl) due to drug-induced myoglobinemia (1,000 ng/ml) with uremic symptoms. Although an urgent flexible double lumen (FDL) catheter was introduced into the internal jugular vein, readministration of isoniazid (day 18) caused severe rhabdomyolysis (myoglobinemia, 25,650 ng/ml), with a recurrence of the uremic symptoms.

On day 27, the patient suddenly went into a state of shock with high fever and was empirically treated with intravenous meropenem (0.5 g/day), vancomycin (0.5 g, every 48 h [q48h]), and fluconazole (200 mg/day) based on a tentative diagnosis of aspiration pneumonia or catheter-related bloodstream infection complicated by sepsis. On the same day, two sets of blood cultures and serum endotoxin antigen were negative except for an elevation of β-D-glucan (133 pg/ml). On day 32, the patient's serum value of β-D-glucan rose to 530 pg/ml, and he had a positive result for serum galactomannan (*Aspergillus* antigen) of 4.5, thrombocytopenia (6.4×10^3 platelets/μl), and leukocytopenia (2.0×10^3 leukocytes/μl). Therefore, the fluconazole was changed to voriconazole (6 mg/kg of body weight/day, q12h) with the intent of targeting *Aspergillus* spp. However, on day 35, a blood culture collected on day 32 (strain NO1) was identified as *Candida glabrata*; therefore, voriconazole was changed to intravenous micafungin (100 mg/day) according to the Infectious Diseases Society of America (IDSA) 2009 guidelines (1). A blood culture taken on day 34 (NO2) also was positive for *C. glabrata*; however, after initiation of treatment with micafungin, the persistent fever subsided, and a blood culture taken at day 37 was negative for the yeast. Both strain NO1 and strain NO2 were susceptible to micafungin (MIC, <0.015 μg/ml) but susceptible-dose dependent to fluconazole (MIC <8 μg/ml) by Clinical and Laboratory Standards Institute (CLSI) broth microdilution (BMD) methods (CLSI document M27-S4). With regard to voriconazole, no breakpoint was determined for *C. glabrata*. In spite of two rounds of replacement of the FDL catheter, the serum value of β-D-glucan remained high (>600 pg/ml), and blood cultures taken on day 48 (NO3) and day 51 (NO4) again yielded *C. glabrata*. Based on the suspicion of a micafungin-resistant strain, micafungin treatment was changed to intravenous liposomal amphotericin B (3 mg/

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TABLE 1 Drug susceptibilities of isolated *Candida glabrata* strains

Strain	MIC (μg/ml) of[a]:						
	MCFG (S, R)	AMPH-B (S)	5-FC (S)	FLCZ (SDD)	ITCZ (S, SDD)	VRCZ	PSCZ
NO1	<0.015	1	<0.12	8	1	0.5	2
NO2	<0.015	1	<0.12	8	1	0.5	0.5
NO3	<0.015	0.5	<0.12	4	0.5	0.25	0.5
NO4	2	1	<0.12	4	1	0.25	0.5
NO5	4	0.5	<0.12	8	1	1	2

[a] AMPH-B, amphotericin B; 5-FC, 5-flucytosine; FLCZ, fluconazole; ITCZ, itraconazole; MCFG, micafungin; VRCZ, voriconazole; PSCZ, posaconazole; R, isolates were resistant; S, isolates were susceptible; SDD, isolates were susceptible, depending on the dose.

TABLE 2 Genetic characterization of isolated *Candida glabrata* strains[b]

Strain (MIC [μg/ml] of MCFG)	Characterisitic(s) of gene:	
	FKS1	*FKS2*
NO1 (<0.015)	Wild type	Wild type
NO2 (<0.015)	Wild type	Wild type
NO3 (<0.015)	Wild type	F659Δ L1767Δ
NO4 (2)	Gene conversion[a]	F659Δ
NO5 (4)	Gene conversion[a]	F659Δ

[a] The gene with the insertion is predicted to encode an Fks1 protein with the following changes: M555T, V558I, L563V, V568I, T583S, H600Q, A620S, and Y623Δ.
[b] MCFG, micafungin. The sequence type for every strain was 22.

kg/day) on day 53. However, *C. glabrata* was still isolated from a blood culture taken on day 56 (NO5), and the patient died of septic shock on day 59. Following the patient's death, the NO3 strain was shown to be susceptible to micafungin (MIC, <0.015 μg/ml), while the NO4 and NO5 strains showed resistance to micafungin (based on CLSI document M27-S4), the MICs of which were 2 and 4 μg/ml, respectively (Table 1).

We performed morphological and genetic analyses for isolates NO1 to NO5. Interestingly, colonies observed in the isolation step for the NO1 and NO2 strains (Fig. S1A in the supplemental material) were of a size typical of *C. glabrata* ("normal"; they were light purple); in contrast, colonies for the NO3 and NO4 strains (Fig. S1B in the supplemental material) were heterogeneous, consisting of a mixture of small dark-purple and normal light-purple colonies, and the isolation step for NO5 (Fig. S1C in the supplemental material) yielded colonies of uniform size that were consistently small and darkly colored on CHROMagar *Candida* medium (Becton, Dickinson and Company). The change in colony size was thought to be due to mitochondrial deficiency (petite mutant) or *FKS* mutations, as described below. These results also suggest that the blood culture from which NO3 and NO4 were isolated contain several *C. glabrata* clones with heterogeneous growth rates. To examine whether the five clinical isolates originated from a single strain, randomly amplified polymorphic DNA (RAPD) and multilocus sequence typing (MLST) analyses were performed. Briefly, the template genomic DNA was extracted from *C. glabrata* cells, and a series of PCR and DNA sequencing reactions were performed using the primers indicated in Table S1 in the supplemental material. In RAPD assays (performed per previously reported methods [2]), all five of the tested strains yielded identical amplification patterns (data not shown). Furthermore, MLST analysis revealed that the five strains demonstrate a shared sequence type, ST22 (Table 2) (http://cglabrata.mlst.net/). These results suggest that the strains were probably derived from a single parental strain; however, more-detailed genetic analyses would be necessary for identification of the source of the strains.

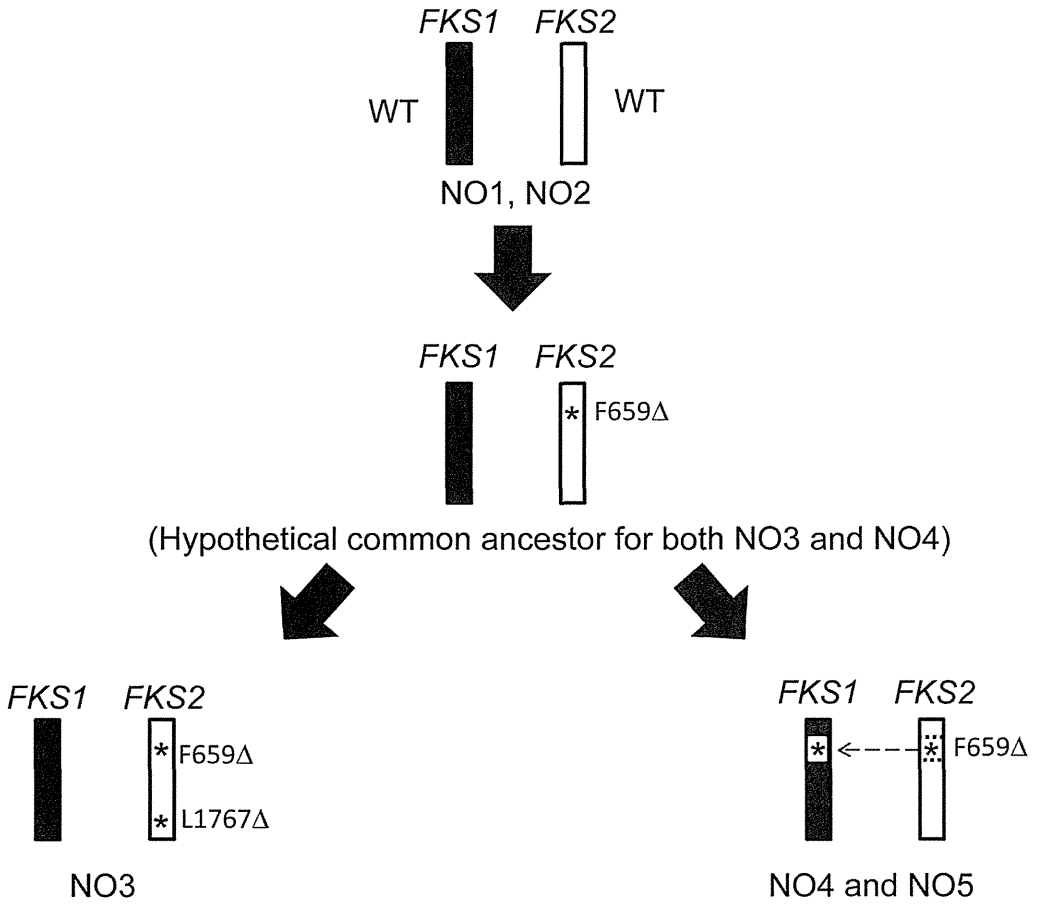
Cases harboring *Candida* spp. with reduced susceptibility to echinocandins are still uncommon, and strains of *C. glabrata* ranked as nonsusceptible to echinocandin have been reported only rarely in Japan (3, 4, 5). In previous reports, reduced susceptibility to echinocandins was related primarily to single point mutations within the *FKS* genes, which code for β-1,3-glucan-synthases. A total of 48 distinct mutations have been reported in 5 different yeast species, with 44 lesions occurring in hot spot 1 and 4 lesions occurring in hot spot 2 (6, 7, 8). The majority of the *FKS1* mutations are predicted to result in an amino acid substitution at S629P (9), Phe625 (F625S or F625I), or Asp632 (D632G, D632E, or D632Y); *FKS2* mutations are predicted to result in a substitution (F659V, F659S, or F659Y) (10) or a deletion (a deletion of F659 [F659del]) (11) in Phe659, as well as multiple mutations in residues 662 to 667, especially S663P (12, 13).

We determined the entire coding sequences of *FKS1* and *FKS2* in the five isolates (NO1 to NO5). Briefly, the entire *FKS* genes (7,332 bp and 7,941 bp for *FKS1* and *FKS2*, respectively) were amplified by PCR from genomic DNA extracted from each strain, and DNA sequences of the PCR fragments were determined as described elsewhere but with different set of primers (see the primer list in Table S1 in the supplemental material) (5). The *FKS* sequences of the *C. glabrata* strains were reconfirmed with an independent analysis. In comparison to database sequences (the GenBank [https://www.ncbi.nlm.nih.gov/genbank/] accession numbers for *FKS1* and *FKS2* are HM366440 and HM366442, respectively), both NO1 and NO2 do not have any mutations which cause amino acid substitutions in both *FKS1* and *FKS2*. The subsequently isolated strain NO3 harbored two deletion mutations (F659del and L1767del) in *FKS2* (Table 2 and Fig. 1A). It was curious that NO3 was still susceptible to micafungin (MIC <0.015 μg/ml); however, F659del in *FKS2* was thought to confer echinocandin resistance, as mentioned above (11, 12). It was also demonstrated that the laboratory-constructed *C. glabrata* mutant which had F659del in *FKS2* was susceptible to echinocandins (8). These observations all together suggest that F659del in *FKS2* alone does not necessarily confer echinocandin resistance to *C. glabrata*. It is also conceivable that L1767del in *FKS2* or an unknown genetic modification(s) other than those in *FKS* genes suppress the effect of F659del; however, we have no evidence supporting these hypotheses.

In micafungin-resistant NO4 and NO5, bp 262 of the *FKS2* sequence (containing F659del) was substituted for the homolo-

FIG 1 (A) Proposed model for the introduction of mutations into *FKS* genes. The flow chart of *FKS1* and *FKS2* modification is schematically rendered. The *FKS1*-type sequence and *FKS2*-type sequence are black and white, respectively. The representative point mutations which resulted in an amino acid substitution are designated with asterisks. *FKS2* in NO3 carries two mutations, resulting in a predicted protein harboring F659del and L1767del (bottom left). In contrast, *FKS2* in strains NO4 and NO5, both isolated from the patient subsequent to NO3's isolation, carries the F659del-encoding mutation alone (bottom right). This discrepancy suggests that NO3 and NO4 originated from a common ancestor (center middle) that was presumably susceptible to micafungin. The blood culture from which NO3 and NO4 were isolated also may have contained this hypothetical strain. (B) Alignment of flanking sequences around the region of the *FKS* gene substitution. *FKS1* in NO3 (top), *FKS1* in NO4 (middle, bold), and *FKS2* in NO4 (bottom) are aligned for comparison. The numbering represents positions within the respective open reading frame, and the conserved nucleotides are highlighted by gray shading. The *FKS1* sequence in NO4 is identical to a homologous *FKS2* sequence in NO4 from bp 1635 to 1899 (numbering for *FKS1* is above the alignment), whereas the *FKS1* sequence in NO4 is identical to parental *FKS1* in NO3 both upstream of bp 1635 and downstream of bp 1899. The F659del-encoding mutation in *FKS2* also was inserted into *FKS1* in NO4 (arrow). The resulting eight amino acid substitutions from the gene conversion are designated with single letters above and below the alignment (above for parental Fks1p amino acid residues and below for Fks2p; asterisks indicate amino acid residues conserved between Fks1p and Fks2p). The hot spot region related to echinocandin resistance is underlined. WT, wild type.

A



B

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NO3 FKS1 1525-GCAGGTGCTCAACATCTTTCCCGTAGATTTTGGTTCTTATGTTTGATTTTTGCAGTTAACTTGGGTCCAATTATT-1599
NO4 FKS1 1525-GCAGGTGCTCAACATCTTTCCCGTAGATTTTGGTTCTTATGTTTGATTTTTGCAGTTAACTTGGGTCCAATTATT-1599
NO4 FKS2 1627-GCTGGTGCTCAACATTTATCTCGTAGGTTTTGGTTTTTATGTGGTATCCTAGGTGTTAATCTTGGTCCTTTAATT-1701

                                                  1635
                                                  I
                                                  * * * * * * * * * M * * V
NO3 FKS1 1600-TTTGTCTTCGCTTATGAAAAGGACACTGTTCAATCAAAGGCAGGCCACGCGGTTGCTGCGGTCATGTTCTTTGTT-1674
NO4 FKS1 1600-TTTGTCTTCGCTTATGAAAAGGACACTGTTCAATCGAAGGCTGGTCATGCTGTAGCAGCTGTAACGTTTTTCATT-1674
NO4 FKS2 1702-TTTGTCTTTGCTTATGAAAAGGACACTGTTCAATCGAAGGCTGGTCATGCTGTAGCAGCTGTAACGTTTTTCATT-1776
                                                  * * * * * * * * * T * * I

                  * * * * L * * * * V * * * * * * * * * * * * * * T
NO3 FKS1 1675-GCAGTCGCTACATTGCTATTTTTCTCAGTCATGCCATTGGGTGGTCTGTTCACGTCCTACATGCAAAAGTCTACC-1749
NO4 FKS1 1675-GCTGTGGCCACTGTTTTATTCTTCTCGATTATGCCATTAGGTGGTCTTTTCACCTCATATATGCAAAAATCAAGT-1749
NO4 FKS2 1777-GCTGTGGCCACTGTTTTATTCTTCTCGATTATGCCATTAGGTGGTCTTTTCACCTCATATATGCAAAAATCAAGT-1851
                  * * * * V * * * * I * * * * * * * * * * * * * S

                  * * * * * * * * * * * * * * * * H * * * * * * * *
NO3 FKS1 1750-AGACGTTACGTCGCTTCTCAAACCTTCACTGCCTCCTTTGCACCTTTGCATGGTCTTGACAGATGGTTGTCTTAC-1824
NO4 FKS1 1750-AGAAGATATGTTGCTTCTCAGACTTTCACCGCATCTTTTGCCCCATTACAAGGTTTGGATAGATGGTTATCTTAT-1824
NO4 FKS2 1852-AGAAGATATGTTGCTTCTCAGACTTTCACCGCATCTTTTGCCCCATTACAAGGTTTGGATAGATGGTTATCTTAT-1926
                  * * * * * * * * * * * * * * * * * Q * * * * * * * *
                                                                  1899
                                                                  I
                  * * * * * * * * * * * A * * * Y F * * * * * * * * I
NO3 FKS1 1825-CTGGTTTGGGTTACTGTTTTTGCTGCTAAGTACGCTGAATCATACTACTTCTTGATTCTATCTCTAAGAGATCCA-1899
NO4 FKS1 1825-TTAGTTTGGGTTACTGTTTTTGCTGCCAAGTACTCTGAATCGTACTTC---TTGATTTTGTCTCTAAGAGACCCT-1896
NO4 FKS2 1927-TTAGTTTGGGTTACTGTTTTTGCTGCCAAGTACTCTGAATCGTACTTC---TTGATTTTGTCTCTAAGAGACCCT-1998
                  * * * * * * * * * * * S * * * F * * * * * * * * *
                                          F659del

NO3 FKS1 1900-ATCAGAATTTTGTCTACCACTACCATGAGATGTACTGGTGAATACTGGTGGGGTTCAAAGCTATGTAGACATCAA-1974
NO4 FKS1 1897-ATCAGAATTTTGTCTACCACTACCATGAGATGTACTGGTGAATACTGGTGGGGTTCAAAGCTATGTAGACATCAA-1971
NO4 FKS2 1999-ATCAGAATTTTATCAACTACTACCATGAGATGTACTGGTGAGTATTGGTGGGGTTCAAAGTTATGTAGACATCAA-2073
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gous region of *FKS1*, resulting in a predicted protein harboring multiple amino acid mutations (M555T, V558I, L563V, V568I, T583S, H600Q, A620S, Y623del) compared to the parental sequence (Table 2 and Fig. 1B). One possible cause for the micafungin resistance in NO4 and NO5 is the multiple mutations in *FKS1* resulting from the genetic substitution from *FKS2* to *FKS1*; however, both NO4 and NO5 still keep F659del in *FKS2*, presumably related to echinocandin resistance (Fig. 1A and B). It is ambiguous which *FKS* mutation(s) conferred micafungin resistance to NO4 or NO5; therefore, each *FKS* gene in NO4 and NO5 should be separately expressed in a *C. glabrata* laboratory strain and functionally characterized in future work.

Also of note was the fact that the mutation encoding L1767del in *FKS2* (observed in NO3) was not observed in NO4 and NO5. The loss of this mutation indicates that NO3 and NO4 developed from a common ancestor whose *FKS2* gene harbored the F659del-encoding mutation alone (Fig. 1A). Thus, the heterogeneous colony sizes observed in NO3 and NO4 may reflect the existence of a population of *C. glabrata* organisms carrying heterogeneous *FKS* gene sequences in NO3 and NO4.

To our knowledge, this is the first report suggesting that a genetic addition from *FKS2* to *FKS1* can mediate micafungin resistance in *C. glabrata*. This case also suggests that morphological colony phenotypes may be associated with changes in micafungin susceptibility in *C. glabrata* isolates.

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We have no conflicts of interest to declare.

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ORIGINAL ARTICLE

Three new basidiomycetous yeasts, *Pseudozyma alboarmeniaca* sp. nov., *Pseudozyma crassa* sp. nov. and *Pseudozyma siamensis* sp. nov. isolated from Thai patients

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ABSTRACT

We previously reported the first isolation of *Pseudozyma* species from the blood of Thai patients. In this study, three additional new *Pseudozyma* species were isolated from clinical specimens from Thai patients. The *Pseudozyma* species showed relatively low sensitivity to azole antifungal agents. The names proposed for these isolates are *Pseudozyma alboarmeniaca* (DMST 17135^T = JCM 12454^T = CBS 9961^T), *Pseudozyma crassa* (DMST 17136^T = JCM 12455^T = CBS 9959^T) and *Pseudozyma siamensis* (DMST 17137^T = JCM 12456^T CBS 9960^T), where DMST is Department of Medical Sciences Culture Collection, JCM is Japan Collection of Microorganisms and CBS is Centraalbureau voor Schimmelcultures.

Key words new species, *Pseudozyma alboarmeniaca*, *Pseudozyma crassa*, *Pseudozyma siamensis*.

The anamorphic basidiomycetous genus *Pseudozyma* includes 16 species and is phylogenetically positioned in the Ustilaginales, Ustilaginomycetes and Ustilaginomycotina (1). These species are widely distributed in soil and plant environments. Although *Pseudozyma* species generally do not cause infection, several *Pseudozyma* strains have been isolated from the blood of patients in Thailand, including two taxonomically novel species named *P. thailandica* and *P. paraantarctica* (2). We isolated three new *Pseudozyma* species from clinical specimens of Thai patients.

In this study, we propose the following three new species names for these isolates: *Pseudozyma alboarmeniaca*, *Pseudozyma crassa* and *Pseudozyma siamensis*.

MATERIALS AND METHODS

Strains used

Three strains, DMST 17135, 17136 and 17137 that were isolated from the blood of Thai patients at Mae Sot Hospital, Phramongkutklao Hospital and Phitsanulok Regional Medical Center, respectively, were transferred to the Mycology section, National Institute of Health in Thailand.

Phylogenetic analysis

The D1/D2 LSUs and ITS regions of the rRNA genes were sequenced directly from PCR products using the primer pairs NL-1 (forward, 5′-GCATATCAATAAGCGGAGGAAAAG-3′) and NL-4 (reverse, 5′-GGTCCGTGTTTCAAGACGG-3′) (3), and pITS-F (forward, 5′-GTCGTAACAAGGTTAACCTGCGG-3′) and pITS-R (reverse, 5′-TCCTCCGCTTATTGATATGC-3′) (4). The sequences were aligned using Clustal W ver. 1.8 software (5). The distances between sequences were calculated using Kimura's two-parameter model for neighbor-joining analysis (6, 7). A bootstrap analysis was conducted with 100 replications (8).

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List of Abbreviations: CBS, Centraalbureau voor Schimmelcultures; DMST, Department of Medical Sciences Culture Collection; ITS, internal transcribed spacer; JCM, Japan Collection of Microorganisms; L. adj., Latin adjective; LSU, large subunit; YM, yeast-mold.

Taxonomic characteristics

Morphological, biochemical and physiological characteristics were examined using the methods described in the fifth edition of *The Yeasts, A Taxonomic Study* (9). Ubiquinone molecules were identified according to the method of Nakase and Suzuki (10).

Drug susceptibility

Drug susceptibility to micafungin, amphotericin B, flucytosin, fluconazole, itraconazole, voriconazole and miconazole were investigated using an EIKEN kit (Eiken Chemical, Tochigi, Japan) according to the manufacturer's instructions.

RESULTS

Taxonomical and physiological characteristics

We constructed phylogenetic trees of the three strains, DMST 17135, 17136 and 17137, and related species using the ITS including the 5.8S and D1/D2 LSU sequences (Fig. 1). The level of dissimilarity between the strains and the phylogenetically closest species in the ITS sequences was >10% for the ITS region. Such divergence is sufficient to identify the isolates as individual species (4). Although current fungal taxonomy has been re-constructed by revision of fungal nomenclatural rules, the taxonomical characteristics of the three strains were those of *Pseudozyma* species. We concluded that our isolates should be treated as the three new species *P. alboarmeniaca*, *P. crassa*, and *P. siamensis*.

The physiological characteristics of the three new species are shown in Tables 1 and S1. *Pseudozyma* are characterized by the ability to assimilate *myo*-inositol and D-glucuronate, and the inability to form extracellular starch-like compounds (1). The three new species shared these physiological characteristics.

Drug susceptibility

The clinical isolates (DMST 17135, 17136 and 17137) were tested for their susceptibility to seven antifungal drugs (micafungin, amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole and miconazole). They were resistant or had relatively low susceptibility to six of these (micafungin, flucytosin, fluconazole, itraconazole, voriconazole and miconazole) (Table 2).

Pseudozyma alboarmeniaca Mekha, Takashima, et Sugita sp. nov.

(L. adj. albus, white, and L. adj. armeniacus, apricot-colored, from the color of the colony).

MycoBank no.: MB 493128.

Vegetative cells were ellipsoidal or elongate (2–6.5 × 5–18 μm), single or in pairs, and short chains or in groups after 3 days at 25°C in YM broth (Fig. 2). A creeping, complete, fragile, folded pellicle and sediment were present after 1 month at 17°C. The streak culture was orange white to grayish orange, mat, slightly wrinkled to netted near the bottom, soft to butyraceous, and had an entire margin after 1 month at 17°C on YM agar. Branched and hyaline mycelia, 1.5–5 μm in diameter, and conidia were produced on corn meal agar after 3 days at 25°C. The conidia on short sterigma-like structures were fusiform to ellipsoidal. The biochemical and physiological properties are shown in Table 1.

The type strain (DMST 17135^T = JCM 12454^T = CBS 9961^T) was isolated from a human and is maintained at DMST, JCM and CBS.

Pseudozyma crassa Mekha, Takashima, et Sugita sp. nov.

(L. adj. crassus, thick, broad, from colony morphology of this yeast).

MycoBank no.: MB 493129.

Vegetative cells were ellipsoidal or elongate (2–5 × 5–15 μm), single or in pairs, or short chains or in groups after 3 days at 25°C in YM broth (Fig. 2). An almost complete pellicle layer and sediment were present after 1 month at 17°C. The streak culture was white, mat, smooth, and slightly wrinkled and soft, and the margin was fringed by mycelia after 1 month on YM agar at 17°C. Septate, branched and hyaline mycelia (2–5 μm in diameter) were produced on corn meal agar after 3 days at 25°C. Fusiform to ellipsoidal conidia and chlamydospores were present. The biochemical and physiological properties are shown in Table 1.

The type strain (DMST 17136^T = JCM 12455^T = CBS 9959^T) was isolated from a human and is maintained at DMST, JCM and CBS.

Pseudozyma siamensis Mekha, Takashima, et Sugita sp. nov.

(N. L. adj. Siamensis, pertaining to Siam).

MycoBank no.: MB 493132

Vegetative cells were ellipsoidal or elongate (1.5–6.5 × 5–20 μm), single or in pairs, or in groups after 3 days in YM broth at 25°C (Fig. 2). An almost complete ring, fragile, folded pellicle and sediment were present after 1 month at 17°C. The streak culture was light orange to brownish orange, semi-shiny, smooth and slightly wrinkled, soft to butyraceous and had a zonate margin after 1 month on YM agar at 17°C. Pseudomycelia and septate, branched and hyaline mycelia 1.5–6.5 μm in

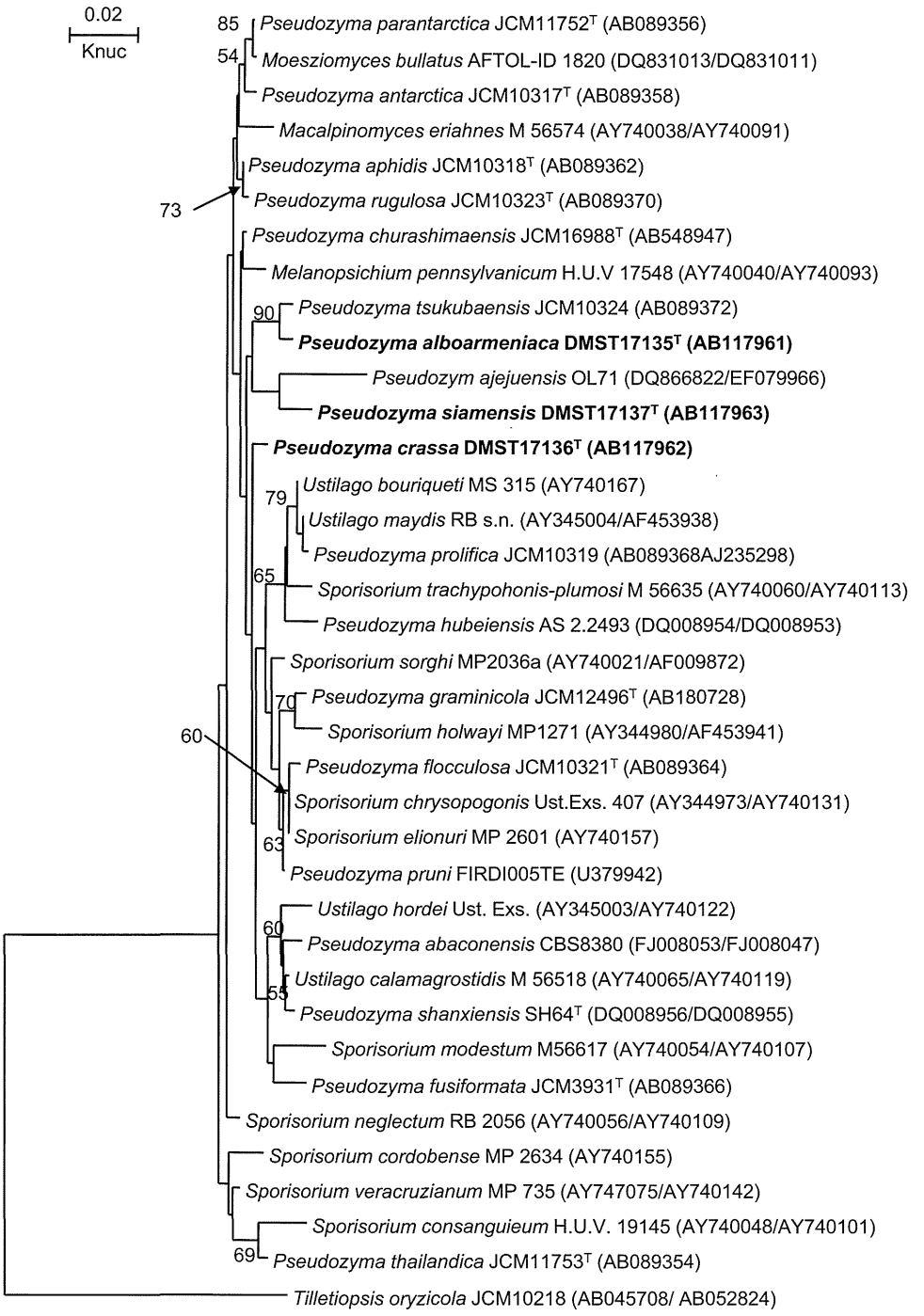

Fig. 1. Molecular phylogenetic trees constructed using the ITS including the 5.8S and D1/D2 LSU rDNA sequences of the three new *Pseudozyma* species and related ustilaginomycetous anamorphic yeasts. The DDBJ/GenBank/EMBL accession numbers are indicated in parentheses. Numbers indicate the confidence level from 100 replicate bootstrap samples (frequencies < 50% are not indicated). Knuc, Kimura's parameter (7).

Table 1. Characteristics of three *Pseudozyma* species

Compound	*Pseudozyma alboarmeniaca*	*Pseudozyma crassa*	*Pseudozyma siamensis*	Compound	*Pseudozyma alboarmeniaca*	*Pseudozyma crassa*	*Pseudozyma siamensis*
Glucose	+	+	+	Galactitol	−	−	−
Inulin	−	LW	LW	D-Mannitol	+	+	+
Sucrose	+	+	+	D-Glucitol	+	+	+
Raffinose	+	+	+	*myo*-Inositol	+	+	+
Melibiose	+	L	+	DL-Lactate	+	W	+
Galactose	+	+	LW	Succinate	+	+	+
Lactose	+	−	+	Citrate	+	+	+
Trehalose	+	−	+	D-Gluconate	+	L	+
Maltose	+	+	+	D-Glucosamine	+/L	+	+
Melezitose	+	+	+	*N*-acetyl-D-glucosamine	+	+	+
Methyl-α-D-glucoside	+	L	+	Hexadecane	+	+	+
Soluble starch	+	+	+	Nitrate	+	+	+
Cellobiose	+/L	L	L	Vitamin-free	−	+	+
Salicin	+/W	+	+	**Additional growth tests and other characteristics**			
L-Sorbose	+	LW	+				
L-Rhamnose	−	−	−	2-Keto-D-gluconate	+	−	LW
D-Xylose	+	+	+	Saccharate	+/LW	−	+
L-Arabinose	+	+	+	D-Glucuronate	+	+	+
D-Arabinose	+/L	+	L	50% Glucose	−	−	−
D-Ribose	+/L	+	L	Starch formation	−	−	−
Methanol	−	−	−	Urease	+	+	+
Ethanol	+	L	+	Nitrite	±	+	−
Glycerol	+	+	+	Major ubiquinone	Q10	Q10	Q10
Erythritol	+	+	+	Growth at 30°C	+	+	+
Ribitol	+	L	L	Growth at 37°C	+	+	+

+, positive; −, negative; L, latent; LW, latent and weakly positive; W, weakly positive.

diameter were produced after 3 days on corn meal agar at 25°C. The biochemical and physiological properties are shown in Table 1.

The type strain (DMST 17137^{T} = JCM 12456^{T} = CBS 9960^{T}) was isolated from a human and is maintained at the DMST, JCM and CBS.

DISCUSSION

We have previously identified several *Pseudozyma* strains, including two new species (*P. parantarctica* and *P. thailandica*). They are also resistant or showed low susceptibility to antifungal agents other than amphotericin B (2). Because there are few antifungal agents that are effective against *Pseudozyma* species, were unable to obtain any detailed information, including that concerning patients' life-prognoses. Since we initially isolated these new *Pseudozyma* species from clinical specimens, a few reports of cases of infection with other *Pseudozyma* species have been published from the USA (11), China (12) and South Korea, (13) in 2008, 2010 and 2011, respectively. *Pseudozyma aphidis* was isolated from patients with central venous catheter infection and mycetoma of the leg in the USA and China, respectively. Of the currently accepted 16 species in the genus *Pseudozyma*, five have been isolated from Thai patients.

Table 2. *In vitro* susceptibility of *Pseudozyma* clinical isolates to antifungal agents

Species	Strain	MIC (μg/mL)						
		MCFG	AMPH	5-FC	FCZ	ITZ	VCZ	MCZ
P. alboarmeniaca	DMST 17135	>16	0.25	>64	32	4	2	4
P. crassa	DMST 17136	>16	0.25	>64	>64	>8	2	4
P. siamensis	DMST 17137	>16	0.125	>64	32	4	2	2

5-FC, flucytosine; AMPH, amphotericin B; FCZ, fluconazole; ITZ, itraconazole; MCFG, micafungin; MCZ, miconazole; VCZ, voriconazole.

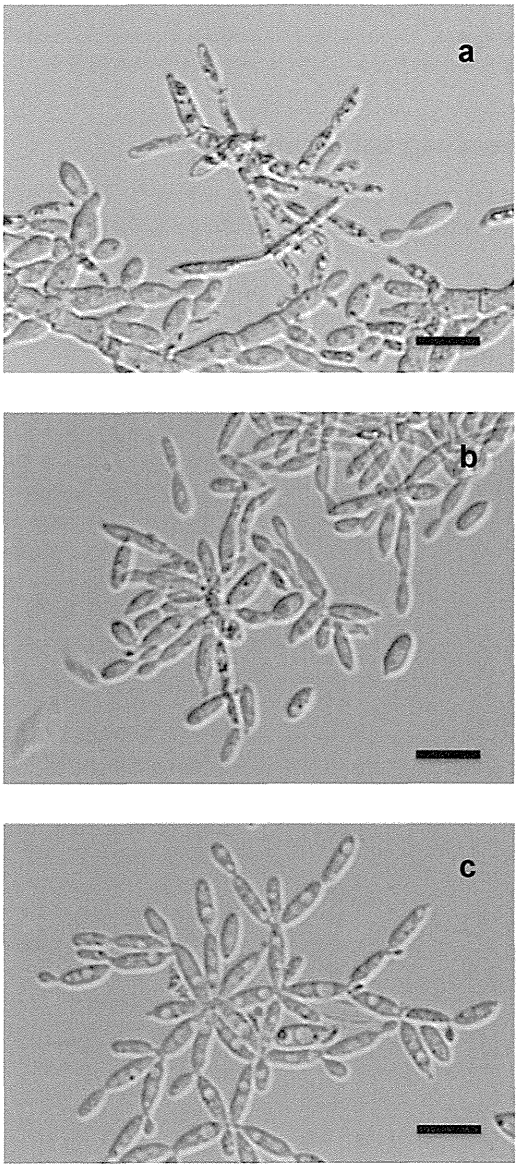

Fig. 2. Vegetative cells of *Pseudozyma alboarmeniaca* DMST 17135^T (a) *P. crassa* DMST 17136^T. (b) *P. siamensis* DMST 17137^T. (c) After growing in YM broth at 25°C for 3 days.

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DISCLOSURE

None of the authors have any conflicts of interest in any financial, commercial or other affiliations.

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Table S1. Comparison of physiologic characteristics of *P. alboarmeniaca*, *P. crassa*, *P. siamensis*, and related *Pseudozyma* species

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Management bundles for candidaemia: the impact of compliance on clinical outcomes

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Objectives: The Mycoses Forum in Japan has developed management bundles for candidaemia to incorporate into bedside practice. The aim of this study was to investigate nationwide compliance with the bundles and their impact on clinical outcomes.

Methods: Non-neutropenic patients treated with antifungals for candidaemia were surveyed. Bundles consist of nine items to complete. Data were sent to the central office between July 2011 and April 2012.

Results: Six hundred and eight patients were analysed. The compliance rate for achieving all elements was 6.9%, and it increased to 21.4% when compliance was analysed by the bundle except for oral switch. There was a significant difference in clinical success between patients with and without compliance [92.9% versus 75.8% ($P=0.011$)]. Compliance with the bundles, however, failed to be an independent factor associated with favourable outcomes. When step-down oral therapy was excluded from the elements of compliance, compliance with the bundles was revealed to be an independent predictor of clinical success (OR 4.42, 95% CI 2.05–9.52) and mortality (OR 0.27, 95% CI 0.13–0.57). Independent individual elements contributing to clinical success were removal of central venous catheters within 24 h, assessment of clinical efficacy on the third to the fifth day and at least 2 weeks of therapy after clearance of candidaemia.

Conclusions: Compliance with the bundles for candidaemia had a beneficial effect on clinical outcomes. Promotion of the bundles approach may have the potential to narrow the gap between clinical evidence and bedside practice.

Keywords: candidiasis, guidelines, intravenous catheters, invasive disease, fungal infections

Introduction

Candidaemia is the fourth most common cause of nosocomial bloodstream infections[1] and invasive candidiasis has a significant impact on patient outcomes.[2–4] In a review of randomized trials for the treatment of invasive candidiasis, overall mortality was 31.4% and the rate of treatment success was 67.4%.[5] Despite advances in the recognition of high-risk patients with invasive candidiasis and drug development, the mortality associated with invasive candidiasis has not changed substantially. In the light of the medical need to analyse the scientific evidence and make recommendations, the IDSA[6] updated the clinical practice guidelines for the management of candidiasis in 2009. The ESCMID Task Force[7] developed diagnostic and management/therapeutic guidelines for *Candida* diseases in 2012. In Japan, the Mycoses Forum Task Force published guidelines for the management of deep-seated mycoses in 2007.[8]

Although many guidelines have been published in a wide variety of areas of infectious diseases, the development of guidelines has not necessarily led to changes in clinical behaviour in a timely fashion. For integration into bedside practice, the development of bundles based on key recommendations is considered to be effective. The surviving sepsis campaign bundle is one of the most successful cases.[9–11] Levy *et al.*[10] described that the campaign was associated with sustained, continuous quality improvement and a reduction of mortality rates in participating hospitals.

To introduce the appropriate management of candidaemia into bedside practice, the Mycoses Forum in Japan developed bundles based on key guideline recommendations. Bundled care processes standardize interventions to reduce unintended variations

among clinicians by establishing a shared clinical baseline on which further appropriate management can be built. The aim of this study was to investigate nationwide compliance with the bundles and the impact of compliance on clinical outcomes in patients with candidaemia.

Methods

The ACTIONs (Appropriate Candidal Treatment Implementation of Non-neutropenic strategies) Project Committee developed bundles based on key guideline recommendations[6–8] for the diagnosis and treatment of non-neutropenic patients with invasive candidiasis in 2011. The ACTIONs Project is one of the activities of the Mycoses Forum supported by Pfizer Japan Inc. ACTIONs bundles consist of nine items to complete for candidaemia (Table 1). For the awareness of activities, briefing sessions targeting infection control doctors certified by the Japanese College of Infection Control Doctors were held in 11 geographical regions throughout Japan. Bundle checklists were available on the web site of the Mycoses Forum (http://www.mycoses.jp/actions_project/index.html#BUNDLE) and were printed and widely distributed. Data were entered into the bundle database locally or check sheets were sent to the central office of the Mycoses Forum between July 2011 and April 2012.

Entry criteria were non-neutropenic patients >17 years old treated with antifungals for candidaemia with a positive culture for *Candida* spp. in blood samples. The appropriate selection and dosing regimen of antifungals were decided according to previously published guidelines[6–8] (Table 2). If no clinical efficacy was obtained on the third to the fifth day, consideration of alternative therapy was recommended, such as a change to echinocandins or liposomal amphotericin B in patients to whom azoles were administered as initial therapy. Transition to fluconazole is recommended in clinically stable patients with infection due to *Candida albicans*.

Clinical response was judged after the end of all treatment courses, and mortality was evaluated 28 days after the start of antifungal therapy. Treatment was considered to be successful if all attributable signs and symptoms associated with candidaemia had resolved. Treatment was considered to have failed if there was unresponsive infection after at least 5 days of therapy, or if relapse occurred. In patients with treatment failure of initial antifungals or unacceptable adverse events necessitating a change of initial antifungal therapy, overall treatment was judged to be successful if a favourable clinical response was obtained with alternative therapy.

Table 1. Bundle elements in patients with candidaemia

Bundles to be accomplished at the start of therapy
1. Removal of existing CVCs within 24 h of diagnosis
2. Initial appropriate selection of antifungals
3. Initial appropriate dosing of antifungals

Bundles to be accomplished after initiation of therapy
4. Ophthalmological examinations
5. Follow-up blood cultures until clearance of candidaemia
6. Assessment of clinical efficacy on the third to fifth day to consider necessity of alternative therapy
7. Appropriate choice of alternative antifungals
8. At least 2 weeks of therapy after documented clearance of *Candida* from bloodstream and resolution of attributable symptoms (prolonged therapy for candidaemia with organ involvement)
9. Step-down oral therapy for patients with favourable clinical course

Table 2. Appropriate selection and dosing of antifungals in the bundles

Antifungals	Appropriate indication	Standard dosing
Echinocandins	patients with moderately severe to severe illness infection due to *C. glabrata* and *C. krusei* patients with candidaemia in whom CVCs cannot be removed consider poor ocular penetration in ocular candidiasis	caspofungin: loading dose of 70 mg, then 50 mg daily micafungin: 100–150 mg daily
Fluconazole	patients who are less critically ill and who have no recent azole exposure infection due to *C. parapsilosis* and *C. albicans* transition to fluconazole in clinically stable patients with infection due to *C. albicans*	loading dose of 800 mg, then 400 mg daily
Voriconazole	alternative therapy step-down oral therapy limitation of intravenous formulation in renal impairment consider therapeutic drug monitoring	6 mg/kg bid for two doses, then 3–4 mg/kg bid
Itraconazole	alternative therapy limitation of intravenous formulation in renal impairment	200 mg bid for 2 days, then 200 mg daily
Liposomal amphotericin B	patients with severe sepsis/septic shock infection due to *C. glabrata*, *C. krusei* and *C. guilliermondii* patients with candidaemia in whom CVCs cannot be removed	2.5–5.0 mg/kg daily
Amphotericin B deoxycholate	recommendation against use due to substantial renal and infusion-related toxicity	—
Flucytosine	combination use with other antifungals	25 mg/kg qid

bid, twice a day; qid, four times a day.

Table 3. Achievement of individual bundle elements in patients with candidaemia

Phase	Elements of the bundles	Population	No. of patients with achievement of the elements (%)
Bundles at the start of therapy	1. removal of existing CVCs within 24 h of diagnosis	patients with CVC placement	414/510 (81.2)
	2. initial appropriate selection of antifungals	all	534/608 (87.8)
	3. initial appropriate dosing of antifungals	all	464/608 (76.3)
Bundles after initiation of therapy	4. ophthalmological examinations	all	326/608 (53.6)
	5. follow-up blood cultures until clearance of candidaemia	all	368/608 (60.5)
	6. assessment of clinical efficacy on the third to fifth day	all	514/608 (84.5)
	7. appropriate choice of alternative antifungals	patients with alternative therapy	269/345 (78.0)
	8. at least 2 weeks of therapy after documented clearance of *Candida* from bloodstream	all	327/608 (53.8)
	9. step-down oral therapy	all	148/608 (24.3)

We defined compliance as evidence that all bundle elements except 'appropriate choice of alternative antifungals' were completely fulfilled. As this item is indicated only for patients in whom antifungals were changed, we excluded this from the analysis of compliance. The element 'removal of central venous catheters (CVCs)' was included for the evaluation of compliance in patients with CVC placement. Missing data regarding the accomplishment of bundle elements were set as 'fail'.

Clinical efficacy and mortality were evaluated according to the compliance. To identify the contribution of each element to improvement of clinical outcomes, the ORs of clinical success and mortality were adjusted for the following factors affecting clinical outcomes: surgery, chemotherapy for cancer, malnutrition, total parenteral nutrition, age >70 years, chronic renal failure/haemodialysis, severe illness, steroid/immunosuppressant use, mechanical ventilation, use of a CVC, malignancy, ICU stay, diabetes mellitus and isolation of non-*albicans Candida*. Bundle elements such as 'third to fifth day follow-up' and '2 weeks of antifungal therapy' can only be achieved in patients who survive. To exclude deaths and dropouts before completion of bundle elements to be achieved after the start of therapy, we performed sub-population analysis in patients who survived >28 days after the start of antifungal therapy.

This study was approved by the institutional review board of Hyogo College of Medicine. The institutional review board waived the need for patients' informed consent. Ethics approval was the responsibility of each participating centre. If necessary, investigators obtained formal approval of the protocol by the regional ethics committee. The crude OR in univariate analysis was estimated for each variable by the χ^2 test and potential confounders were examined by cross tabulation. The variables selected by univariate analysis ($P<0.1$) were subsequently entered into a stepwise logistic regression model to estimate the magnitude of association (adjusted OR and 95% CI). The level of significance was set at $P<0.05$. SPSS ver. 16 (SPSS Inc., Chicago, IL, USA) was used to perform these analyses.

Results

Six hundred and forty-one subjects were registered. The analysis included 608 patients for whom information on clinical efficacy was obtained. Mortality was evaluated in 479 patients. *C. albicans* was commonly identified (46.4%). The other species identified included *Candida parapsilosis* (18.4%), *Candida glabrata* (16.0%), *Candida tropicalis* (7.6%), *Candida krusei* (4.6%), *Candida guilliermondii* (3.5%) and others (3.3%). The achievement of individual elements is shown in Table 3. In 81.2% of patients, CVCs were removed within 24 h of the diagnosis of candidaemia. Components for which the achievement rate was relatively low were ophthalmological examination (53.6%), follow-up blood cultures (60.5%), at least 2 weeks of therapy after the end of candidaemia (53.8%) and step-down oral therapy (24.3%). Because of the low achievement rate of oral switch, the compliance rate for achieving all elements was as low as 6.9% (42 of 608 patients), and increased to 21.4% (130 of 608 patients) when compliance was assessed by the completion of the bundles, except for step-down oral therapy.

The clinical success rate was 77.0% (468 of 608 patients) and the mortality rate was 26.5% (127 of 479 patients). There was a significant difference in clinical outcomes between patients with and without compliance [success rate 92.9% versus 75.8% ($P=0.011$)]. The mortality rate in patients with compliance tended to be lower than that in patients without compliance [8.3% versus 27.5% ($P=0.054$)]. There was a clear correlation between the number of elements accomplished and the clinical outcomes in patients with CVC placement [clinical success: 0–2 elements, 21.6% ($n=37$); 3 elements, 43.2% ($n=44$); 4 elements, 74.3% ($n=74$); 5 elements, 76.8% ($n=95$); 6 elements, 89.2% ($n=102$); 7 elements, 95.8% ($n=120$); and 8 elements, 92.1% ($n=38$)/respective mortality rates: 58.0% ($n=31$); 52.9% ($n=34$); 21.4% ($n=56$); 32.9% ($n=76$); 27.5% ($n=91$); 6.9% ($n=101$); and 9.1% ($n=22$)].

After adjusting for host and fungal factors, compliance with the bundles was not an independent factor associated with clinical success (adjusted OR 3.93, 95% CI 0.90–17.17) or improved survival (adjusted OR 0.15, 95% CI 0.07–1.50). The small number of compliant patients might have caused the negative result. If the element of oral switch was excluded from the assessment of bundle compliance, improved clinical success (adjusted OR 4.42, 95% CI 2.05–9.52) and mortality (adjusted OR 0.27, 95% CI 0.13–0.57) were confirmed in compliant patients (Table 4). Independent bundle elements that contributed to clinical success were the removal of CVCs within 24 h after confirmation of candidaemia, assessment of clinical efficacy on the third to the fifth day to consider the necessity of alternative antifungals, and at least 2 weeks of therapy after clearance of *Candida* from the bloodstream (Table 5). As regards mortality, the removal of CVCs within 24 h, at least 2 weeks of therapy and step-down oral therapy were

Table 4. *Impact of compliance with the bundles on clinical outcomes in patients with candidaemia*

Definition of compliance	Clinical success				Mortality			
	patients with compliance no. of patients (%)	patients without compliance no. of patients (%)	crude OR (95% CI)	adjusted OR (95% CI)	patients with compliance no. of patients (%)	patients without compliance no. of patients (%)	crude OR (95% CI)	adjusted OR (95% CI)
Achievement of all evaluable bundle elements	39/42 (92.9)	429/566 (75.8)	4.15 (1.26–13.7)	3.93 (0.90–17.17)	2/24 (8.3)	125/455 (27.5)	0.24 (0.06–1.04)	0.15 (0.07–1.50)
Achievement of all evaluable bundle elements except oral switch	121/130 (93.1)	347/478 (72.6)	5.08 (2.50–10.29)	4.42 (2.05–9.52)	10/97 (10.3)	117/383 (30.5)	0.26 (0.13–0.52)	0.27 (0.13–0.57)

independent elements that were statistically associated with improved survival (Table 5). To correct for selection bias by patients who died before the latter two bundle elements were reached, we separately analysed the effect of bundle compliance in patients who survived >28 days.

Even in patients surviving >28 days, compliance of the bundles except oral switch improved clinical efficacy [compliance 97.7% versus non-compliance 86.6% ($P=0.004$)] and was an independent predictor of clinical success (adjusted OR 5.245, 95% CI 1.194–23.043). In addition, the beneficial effect was confirmed in individual elements to be achieved after initiation of therapy. Assessment on the third to the fifth day [94.3% versus 50.0% ($P<0.001$)], at least 2 weeks of therapy [96.0% versus 78.1% ($P<0.001$)], ophthalmological examination [93.9% versus 82.9% ($P=0.001$)] and follow-up blood culture [94.0% versus 80.8% ($P<0.001$)] significantly increased the clinical success rate in patients surviving >28 days. Independent bundle elements associated with clinical success were assessment on the third to the fifth day (adjusted OR 9.347, 95% CI 3.158–27.668) and at least 2 weeks of therapy after clearance of *Candida* from the bloodstream (adjusted OR 2.754, 95% CI 1.027–7.387).

Discussion

To the best of our knowledge, this is the first study to confirm the beneficial impact of 'bundles' on clinical outcomes in patients with candidaemia. In a similar study, Antworth *et al.*[12] developed a candidaemia care bundle incorporating key elements from the guidelines for the management of candidemia.[6] However, no significant differences in clinical outcomes were identified in their study. In ACTIONs bundles, independent individual elements that contributed to clinical success in patients with candidaemia were the removal of CVCs within 24 h of diagnosis, assessment of clinical efficacy on the third to the fifth day and at least 2 weeks of therapy after the clearance of candidaemia.

In candidaemia without documented organ involvement, treatment aims were to clear the infection and at the same time to avoid deep-organ involvement caused by metastatic infectious foci. This can be achieved by treatment for 2 weeks after the end of candidemia.[7] Although Oude Lashof *et al.*[13] failed to demonstrate a correlation between the duration of antifungal treatment and the development of late complications in patients with candidaemia, this recommendation is based on the results of several prospective, randomized trials in which this rule has been successfully applied, and it is generally associated with few complications and relapses.[14–16] The higher success rate and improved survival rate achieved by the removal of CVCs are consistent with other published studies[17,18] and a recent meta-analysis.[5] The ACTIONs Project Committee recommended that alternative antifungal therapy should be considered in patients with no clinical improvement or based on identified *Candida* species on the third to the fifth day after starting initial therapy. Hsu *et al.*[19] reported that a higher overall response rate was obtained in patients with early initiation (within 72 h of positive culture) of an echinocandin; they recommended that the clinical response be assessed on the third day in patients with initial fluconazole therapy, and an echinocandin is preferred as an alternative therapy in patients with a poor response.

Table 5. Impact of individual bundle elements on clinical outcomes in patients with candidaemia: univariate and multivariate analyses

	Clinical success				Mortality			
Key bundle elements	patients who achieved the element no. of patients (%)	patients who did not achieve the element no. of patients (%)	crude OR (95% CI)	adjusted OR (95% CI)	patients who achieved the element no. of patients (%)	patients who did not achieve the element no. of patients (%)	crude OR (95% CI)	adjusted OR (95% CI)
Removal of CVCs within 24 h	336/414 (81.2)	60/96 (62.5)	2.59 (1.60–4.18)	2.97 (1.51–5.85)	72/329 (21.9)	35/82 (42.7)	0.38 (0.23–0.63)	0.41 (0.23–0.74)
Appropriate initial selection of antifungals	425/534 (79.6)	43/74 (58.1)	2.81 (1.69–4.67)	—	112/424 (26.4)	15/55 (27.3)	0.96 (0.51–1.80)	—
Appropriate dosing	375/464 (80.8)	93/144 (64.6)	2.31 (1.53–3.49)	—	98/372 (26.3)	29/107 (27.1)	0.96 (0.59–1.56)	—
Assessment of clinical efficacy on the third to fifth day	431/514 (83.9)	37/94 (39.4)	8.00 (4.97–12.87)	5.53 (2.54–12.04)	92/406 (22.7)	35/73 (47.9)	0.32 (0.19–0.53)	—
At least 2 weeks of therapy after clearance of *Candida* from bloodstream	302/327 (92.4)	166/281 (59.1)	8.37 (5.22–13.42)	4.65 (2.35–9.19)	32/256 (12.5)	95/223 (42.6)	0.19 (0.12–0.30)	0.23 (0.13–0.40)
Ophthalmological examinations	281/326 (86.2)	187/282 (66.3)	3.17 (2.13–4.73)	—	47/259 (18.1)	80/220 (36.4)	0.39 (0.26–0.59)	—
Follow-up blood cultures	318/368 (86.4)	150/240 (62.5)	3.87 (2.57–5.67)	—	60/292 (20.5)	67/187 (35.8)	0.46 (0.31–0.70)	—
Step-down oral therapy	133/148 (89.9)	335/460 (72.8)	3.31 (1.87–5.86)	—	13/108 (12.0)	114/371 (30.7)	0.31 (0.17–0.57)	0.34 (0.15–0.76)

In patients with a poor clinical response to initial antifungals, overall treatment was judged to be successful if a favourable clinical response was obtained with alternative therapy. With early alternative therapy, initial inappropriate selection of antifungals might not have affected clinical outcomes in our study. Poor prognosis as a result of initial inappropriate therapy was demonstrated in patients with septic shock[20] and was closely related to the severity of infection. However, patient severity ranged from mild to fatal in our study. Kollef *et al.*[18] demonstrated that concurrent performance of early appropriate therapy and adequate source control were required to improve survival among patients with septic shock caused by candidaemia. The presence of both delayed antifungal administration and inadequate source control had a risk of mortality similar to the presence of either one of these variables alone.

ESCMID guidelines[7] suggest simplification of treatment by stepping down to an oral azole if the patient is stable and tolerates the oral route, and if the species is susceptible. Step-down oral therapy could have benefits, such as reduced use of intravenous catheters, earlier patient discharge and cost savings.[21,22] We demonstrated a significantly higher clinical success rate and lower mortality rate in patients with adherence to step-down oral therapy. As step-down therapy was indicated in patients with a favourable course, the results should be interpreted with caution. Owing to the small number of compliant patients, compliance with the bundles failed to be an independent factor associated with favourable clinical outcomes. Upon exclusion of the element of oral switch, compliance was revealed to have a beneficial effect on clinical outcomes.

The bundle approach is also a useful way to assess the present status of guideline adherence.[10] However, even when compliance was defined as achievement of all bundle elements except step-down oral therapy, the rate remained 21.4%. We regarded this value as the baseline, and intend to compare it with the compliance rate in a future follow-up study to ensure the effect of the project. In the case of severe sepsis bundles, Levy *et al.*[10] reported that the compliance rate of the resuscitation bundle was 10.9% in the first quarter and increased over time. The other components for which the achievement rate was relatively low were ophthalmological examination, follow-up blood cultures and at least 2 weeks of therapy after clearance of *Candida* from the bloodstream. Considering the high ocular involvement in patients with candidaemia,[23] a more vigorous promotion of ophthalmological examination should be considered. The recommendation of follow-up cultures is difficult to accept by general physicians in patients whose symptoms have already resolved when candidaemia is diagnosed. In such patients, 2 weeks of therapy is performed without confirmation of the end of candidaemia.

Certain limitations must be considered in interpreting these findings. Firstly, as the main participants were certified infection control doctors and participation was entirely voluntary, the compliance rates for bundles are not necessarily representative of those by general clinicians, and the universality of our findings is therefore speculative. Secondly, as participants judged the appropriateness of antifungal selection according to the recommendations described in the checklist, the committee did not have detailed information on how they judged this item. Thirdly, the small number of patients who achieved step-down oral therapy might have caused a negative result in compliant patients. To confirm the usefulness of compliance with the bundles, an increase in the achievement of this item is required. Finally, a cluster randomized trial including hospitals with and without the bundles would provide better scientific evidence. In conclusion, the introduction of a multifaceted performance improvement initiative with bundles was useful in the treatment of candidaemia. Although the efficacy of bundles should be evaluated in more rigorous studies, promotion of the bundle approach may have the potential to narrow the gap between clinical evidence and bedside practice.

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