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Medical Mycology, 2023, **61**, myad057
https://doi.org/10.1093/mmy/myad057
Original Article
Advance access publication date: 19 June 2023

Epidemiology and susceptibility of *Nakaseomyces* (formerly *Candida*) *glabrata* bloodstream isolates from hospitalised adults in South Africa

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Abstract

During 2016–2017, *Nakaseomyces glabrata* (formerly *Candida glabrata*) caused 14% of cases of candidaemia in South Africa. We aimed to describe the clinical characteristics of adults with *N. glabrata* candidaemia at 20 sentinel hospitals (accounting for 20% (172/917) of cases) and the antifungal susceptibility of the corresponding isolates. A higher proportion of patients with *N. glabrata* candidaemia were older (median age: 55 years [interquartile range (IQR): 41–65 years] vs. 49 years [IQR: 35-63 years]; $p = 0.04$), female (87/164, 53% vs. 283/671, 42%; $p = 0.01$), admitted to a public-sector hospital (152/172, 88% vs. 470/745, 63%; $p < 0.001$), treated with fluconazole only (most with suboptimal doses) (51/95, 54% vs. 139/361, 39%; $p < 0.001$), and had surgery (47/172, 27% vs. 123/745, 17%; $p = 0.001$) and a shorter hospital stay (median 7 days [IQR: 2-20 days] vs. 13 days [IQR: 4-27 days]; $p < 0.001$) compared to patients with other causes of candidaemia. Eight *N. glabrata* isolates (6%, 8/131) had minimum inhibitory concentrations in the intermediate or resistant range for ≥ 1 echinocandin and a R1377K amino acid substitution encoded by the hotspot 2 region of the *FKS2* gene. Only 11 isolates (8%, 11/131) were resistant to fluconazole. Patients with confirmed *N. glabrata* candidaemia are recommended to be treated with an echinocandin (or polyene), thus further guideline training is required.

Lay summary

Nakaseomyces (formerly *Candida*) *glabrata* is a yeast-like fungus that forms part of the commensal gut flora and among people with certain risk factors, can invade into the bloodstream. *Nakaseomyces glabrata* is a relatively more common cause of candidaemia in high-income vs. low- and middle-income countries. There are no *N. glabrata* clinical isolates that are considered susceptible to fluconazole, and thus echinocandins are recommended for treatment. However, echinocandin resistance is emerging. We described the characteristics of South African patients with *N. glabrata* bloodstream infections and the antifungal susceptibility of corresponding isolates. We found that patients infected with *N. glabrata* were more likely to be older, female, admitted to public hospitals and to be post-surgery and these patients were also more likely to be treated with fluconazole monotherapy and to have stayed a shorter time in hospital compared to patients infected with other *Candida* species. Only 6% of *N. glabrata* isolates were echinocandin-resistant with mutations in specific resistance genes that we have found in South African *N. glabrata* isolates previously. Eight percent of *N. glabrata* isolates were resistant to fluconazole and the remainder were in the susceptible dose dependent category, requiring higher fluconazole treatment doses. Patients with confirmed *N. glabrata* bloodstream infection should ideally be treated with an echinocandin or polyene rather than fluconazole and training is required for doctors treating these patients.

Key words: *Nakaseomyces glabrata*, epidemiology, susceptibility, candidaemia.

Introduction

Candidaemia is the most common presentation of invasive candidiasis and contributes substantially to morbidity and mortality among critically ill patients.[1,2] *Candida* is among the top four pathogens causing healthcare-associated bloodstream infections and causes the most common fungal infection in patients admitted to intensive care units (ICUs).[3] The crude mortality associated with candidaemia was 29% in a large United States (US) study,[4] 31% (220/720) in Spain,[5] 54% (383/712) in Brazil,[6] and 60% (122/203) in South Africa.[7] *Nakaseomyces* (formerly *Candida*) *glabrata*, a commensal of the human gastrointestinal tract[8,9] is classified as high priority on the first World Health Organization fungal priority pathogens list.[10] The genus also includes the pathogenic cryptic species: *Nakaseomyces bracarensis* and *Nakaseomyces nivariensis*.[11] *Candida albicans* followed by *N. glabrata* and *Candida parapsilosis* are the most common causes of candidaemia in the US,[12] Australia,[13] and Europe.[14] However, across Latin America and in South Africa, *C. albicans* and *C. parapsilosis* are the dominant species.[2,15–17] In

Received: March 8, 2023. **Revised:** June 7, 2023. **Accepted:** June 14, 2023
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South African national laboratory surveillance from 2016 to 2017, *N. glabrata* was the fourth most common cause of candidaemia after *C. parapsilosis*, *C. albicans*, and *Candida auris*.[17]

Nakaseomyces glabrata infection mostly affects immunocompromised patients such as those living with HIV,[18] diabetes mellitus, patients admitted to ICU,[19] and those with a prolonged hospital stay.[18] It is more commonly reported in adults than in children and neonates.[8] Furthermore, no *N. glabrata* clinical isolates are susceptible to fluconazole, and echinocandin resistance is emerging.[20–24] In two large surveillance programs (the SENTRY Global Surveillance Program and the US Centers for Disease Control and Prevention population-based surveillance), approximately 10% of *N. glabrata* isolates were resistant to fluconazole[20] and echinocandin resistance ranged from 0% to 4% in the US.[25,26] The mechanism of echinocandin resistance involves mutations in the highly-conserved hotspot (HS) regions of the 1,3-β-D-glucan synthase-encoding *FKS* genes.[27,28] Triazole resistance involves mutation of the transcription factor *PDR1* that leads to the overexpression of drug efflux pumps.[27] Mutations in the *ERG6* gene have been associated with polyene resistance.[29] Antifungal susceptibility testing can detect phenotypic echinocandin resistance and screening *N. glabrata* isolates for mutations in the *FKS* genes has been a useful method to detect resistance.[30]

In a South African national surveillance study conducted between 2009 and 2010, only 10 of 1252 (0.8%) bloodstream *Candida* isolates had micafungin or anidulafungin minimum inhibitory concentrations (MICs) in the resistant/intermediate categories.[16] *FKS* gene sequencing of the ten isolates, including three *N. glabrata* isolates, yielded no mutations in the HS regions. We aimed to describe the overall contribution of *N. glabrata* to candidaemia among South African hospitalised adults during 2016–2017, including patient characteristics and the antifungal susceptibility and mechanism of echinocandin resistance of bloodstream *N. glabrata* isolates.

Materials and methods

Study design and sample selection

We conducted a cross-sectional study nested within laboratory-based surveillance for candidaemia. During 2016–2017, national surveillance included 269 private- and public-sector hospitals.[17] A case was defined as a person with any *Candida* species isolated from blood culture (an episode of infection was defined by an arbitrary 30-day period). For this study, we only included adults aged $\geq$ 18 years at 20 enhanced surveillance sites (ESS). Three private- and 17 public-sector hospitals, most in large urban centres, were classified as ESS in 2016–2017.[31] Surveillance officers either interviewed these case patients or reviewed their medical charts to abstract clinical data, including risk factors, treatment, and in-hospital outcome. Unique cases with viable isolates were included. If more than one blood culture was submitted from the same patient within a 30-day period, then the patient's first blood culture contributed to a unique case and the other blood cultures were considered as duplicates and excluded. Patients with contaminated/missing isolates were also excluded. We also excluded cases where multiple species of *Candida* were isolated from a single episode (i.e. mixed infections). Repeat episodes of candidaemia (blood cultures from the same patient more than 30 days apart) were included. Isolates accompanied by a laboratory report with patient metadata were submitted by diagnostic laboratories to a reference laboratory at the National Institute for Communicable Diseases (NICD) in Johannesburg for confirmation of identification by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker, Bremen, Germany).

Antifungal susceptibility testing

Antifungal susceptibility testing was performed for nine antifungal agents (anidulafungin, micafungin, caspofungin, flucytosine, posaconazole, itraconazole, voriconazole, fluconazole, and amphotericin B) using Sensititre YeastOne YO10 broth microdilution plates (Thermo Fisher Scientific, Cleveland, OH, USA). Additionally, amphotericin B MICs were determined by Etest (bioMérieux, Marcy l'Etoile, France) on RPMI 1640 agar plates containing 2% glucose (Diagnostic Media Products [DMP], NHLS, Johannesburg, South Africa). Results were read visually after 24 hours and for Sensititre plates, the MIC was recorded as the lowest concentration of drug that resulted in a significant decrease in growth compared to the control well. The broth microdilution MIC endpoints for echinocandins, azoles, and flucytosine were 50% inhibition. The broth microdilution and Etest MIC endpoint for amphotericin B was 100% inhibition. Minimum inhibitory concentrations were interpreted using CLSI M27M44S breakpoints.[32] Isolates with an intermediate or resistant MIC value to one or more of the echinocandins had anidulafungin MIC values re-checked using the Etest method on RPMI 1640 agar plates containing 2% glucose (DMP) read by two independent readers.

FKS screening

Amplification of the HS regions of the *FKS* genes was performed as previously described to screen for *FKS* mutations.[33] *Nakaseomyces glabrata* isolates with intermediate or resistant MICs to $\geq$ 1 echinocandin were screened for mutations at all four HS regions.[22]

Statistical analyses

The Chi-square test on Stata software (Version 14; StataCorp LP, TX) was used to perform statistical data analysis. We included all candidaemia cases (using the case definition described above) from 2016 to 2017 from enhanced laboratory-based surveillance. We compared cases at ESS and non-ESS in terms of their age, sex, *Candida* species distribution, and health sector to check for selection bias (Supplementary Table 1). We then compared adults with *N. glabrata* candidaemia to those with non-*N. glabrata* candidaemia at ESS. We used the NICD species identification for the two groups and when this was missing, the sending laboratory's identification was used. We also included both in-hospital outcome and 30-day mortality in our descriptive analysis since there seemed to be no difference between the data for both variables. A quick Pitt score was calculated based on the sum of the following variables with a score of $\geq$ 2 predicting death: body temperature $<$ 35°C (one point), systolic blood pressure $<$ 90 mm Hg (one point), cardiac arrest (one point), mechanical ventilation (one point), and altered mental status (one point).[34]

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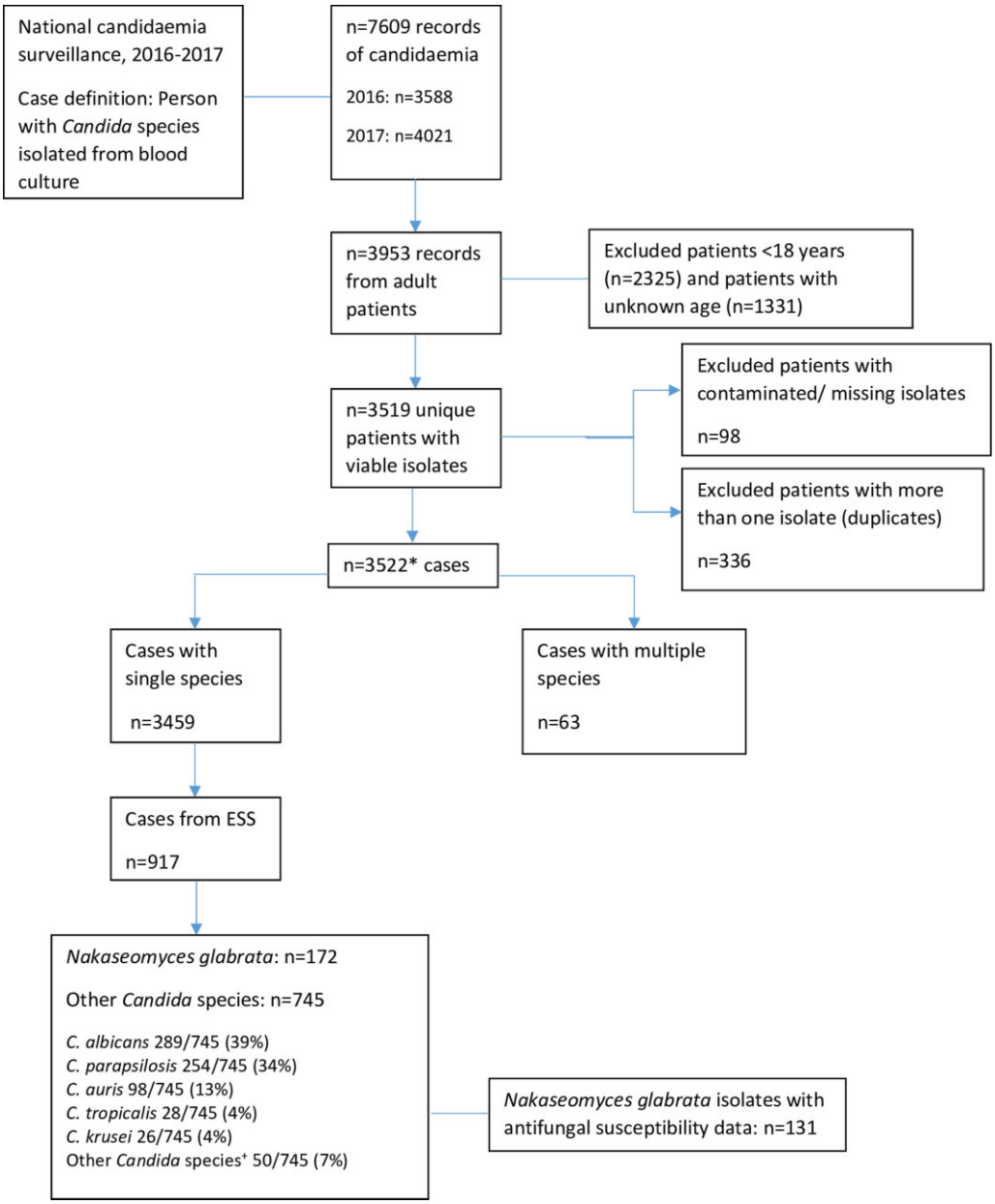


Figure 1. Flow diagram illustrating the inclusion and exclusion criteria for adult patients with candidaemia in this study from national laboratory-based surveillance in South Africa, 2016–2017 Note: ESS—Enhanced surveillance sites, *included three patients with recurrent episodes of candidaemia, +other *Candida* species included *C. dubliniensis*, *C. fabianii*, *C. famata*, *C. guilliermondii*, *C. haemulonii*, *C. kefyr*, *C. lipolytica*, *C. lusitaniae*, *C. metapsilosis*, *C. nivariensis*, *C. orthopsilosis*, *C. pelliculosa*, *C. sphaerica,* and *Candida* species not specified.

Ethics approval

Ethics clearance for GERMS surveillance was obtained from the Human Research Ethics Committee (Medical), University of the Witwatersrand with clearance certificate number: M140159 and from relevant University and Provincial Ethics Committees for other ESS.[31]

Results

Descriptive epidemiology of *N. glabrata* candidaemia

There were 7609 episodes of candidaemia reported during 2016–2017. Fifty-two percent (3953/7609) of records were from adult patients. Overall, there were 3459 cases of candidaemia with a single species isolated and 484 (14%) of these were caused by *N. glabrata*. There were 917 cases of candidaemia at ESS in total (Fig. 1). There were important differences in age, *Candida* species distribution, and health sector among cases at ESS vs. non-ESS (Supplementary Table 1).

At ESS, 172 (19%) candidaemia cases were caused by *N. glabrata* vs. 745 cases (81%) of non-*N. glabrata* candidaemia (Fig. 1). Compared to patients infected by other *Candida* species, those with *N. glabrata* candidaemia (Table 1) were older (median age: 55 years [interquartile range (IQR): 41–65 years] vs. 49 years [IQR: 35-63 years]; $p = 0.04$), mostly female (87/164, 53% vs. 283/671, 42%; $p = 0.01$), and admitted to public sector hospitals (152/172, 88% vs. 470/745, 63%; $p < 0.001$) in other provinces rather than in the Gauteng Province (92/172, 53% vs. 518/745, 70%; $p = 0.002$).

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Table 1. Characteristics of adult South African patients with *Nakaseomyces glabrata* candidaemia compared to those with non-*N. glabrata* candidaemia at enhanced surveillance sites, 2016–2017

Characteristics	All cases $n = 917$	*N. glabrata* candidaemia $n = 172$	non-*N. glabrata* candidaemia[+] $n = 745$	*P* value
Age (median[IQR]) (years)	51 (36–64)	55 (41–65)	49 (35–63)	0.04
18–44	370 (40)	55 (32)	315 (42)	
45–64	319 (35)	66 (38)	253 (34)	
≥ 65	228 (25)	51 (30)	177 (24)	
Gender*				0.01
Male	465/835 (56)	77/164 (47)	388/671 (58)	
Female	370/835 (44)	87/164 (53)	283/671 (42)	
Health sector				<0.001
Public	622 (68)	152 (88)	470 (63)	
Private	295 (32)	20 (12)	275 (37)	
Province				0.002
Gauteng	610 (66.5)	92 (53)	518 (70)	
KwaZulu-Natal	78 (8.5)	18 (10)	60 (8)	
Free State	44 (5)	14 (8)	30 (4)	
Eastern Cape	65 (7)	17 (10)	48 (6)	
Western Cape	68 (7)	20 (12)	48 (6)	
Limpopo/Mpumalanga/Northern Cape/ North West	52 (6)	11 (6)	41 (6)	
Year of diagnosis				0.44
2016	445 (49)	88 (51)	357 (48)	
2017	472 (51)	84 (49)	388 (52)	
Any reported co-morbid condition				0.20
Yes	252 (27)	54 (31)	198 (27)	
No	665 (73)	118 (69)	547 (73)	
HIV infection*				0.53
Yes	168/382 (44)	31/76 (41)	137/306 (45)	
No	214/382 (56)	45/76 (59)	169/306 (55)	
Mental status*				0.59
Alert	216/487 (44)	40/98 (41)	176/389 (45)	
Disoriented/Stuporous	164/487 (34)	33/98 (34)	131/389 (34)	
Comatose/Sedated	107/487 (22)	25/98 (26)	82/389 (21)	
ICU stay during admission*				0.16
Yes	413/680 (61)	79/142 (56)	334/538 (62)	
No	267/680 (39)	63/142 (44)	204/538 (38)	
Treatment*				<0.001
Fluconazole only	190/456 (42)	51/95 (54)	139/361 (39)	
Amphotericin B alone or in combination with fluconazole	123/456 (27)	32/95 (34)	91/361 (25)	
Echinocandin alone or in combination[1]	143/456 (31)	12/95 (13)	131/361 (36)	
Prior antifungal use*				0.003
Yes	94/674 (14)	9/143 (6)	85/531 (16)	
No	580/674 (86)	134/143 (94)	446/531 (84)	
Surgery (abdominal/non-abdominal)				0.001
Yes	170 (19)	47 (27)	123 (17)	
No	747 (81)	125 (73)	622 (83)	
In-hospital outcome*				0.30
Died	414/714 (58)	92/149 (62)	322/565 (57)	
Discharged	300/714 (42)	57/149 (38)	243/565 (43)	
30 day mortality*				0.14
Died	370/670 (55)	88/145 (61)	282/525 (54)	
Recovered	300/670 (45)	57/145 (39)	243/525 (46)	
Length of hospital stay in days[2](median [IQR])	11 (3–26)	7 (2–20)	13 (4–27)	<0.001
Time to death in days[2](median [IQR])	6 (2–17)	4 (1–10)	6 (2–18)	0.005
Quick Pitt score				0.82
0	161 (36)	31 (33)	130 (37)	
1	113 (26)	27 (29)	86 (25)	
2	145 (33)	31 (33)	114 (33)	
3	20 (4)	5 (5)	15 (4)	
4	2 (1)	0 (0)	2 (1)	
5	0 (0)	0 (0)	0 (0)	

[+] *C. albicans* 289/745; *C. parapsilosis* 254/745; *C. auris* 98/745; *C. tropicalis* 28/745; *C. krusei* 26/745; other *Candida* species 50/745
*missing data, denominator shown for these variables
[1]echinocandin with fluconazole, amphotericin B, and/or voriconazole
[2]from specimen collection

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Additionally, more patients with *N. glabrata* candidaemia were treated with fluconazole only (51/95, 54% vs. 139/361, 39%; $p < 0.001$), had surgery (47/172, 27% vs. 123/745, 17%; $p = 0.001$) and a shorter hospital stay (median: 7 days [IQR: 2–20 days] vs. 13 days [IQR: 4-27 days]; $p < 0.001$). Ninety percent (46/51) of patients with *N. glabrata* candidaemia who were treated with fluconazole only were given a daily dose of < 800 mg, while the remainder received ≥ 800 mg daily (5/51, 10%) (Supplementary Table 2). Fewer patients with *N. glabrata* candidaemia vs. other causes had a record of prior antifungal use (9/143, 6% vs. 85/431, 16%; $p = 0.003$). During the 2016–2017 surveillance period, there was one case of *N. nivariensis* candidaemia identified (included in the comparison group in this study) and no cases of *N. bracarensis* infection.

MIC distribution and FKS screening results of *N. glabrata* isolates

Of 172 *N. glabrata* cases, 131 had isolates with antifungal susceptibility data available for analysis (Fig. 1). Applying the CLSI breakpoints, there were eight isolates (6%) with intermediate or resistant MICs to ≥ 1 echinocandin. Two *N. glabrata* isolates (2%) were resistant to anidulafungin; and three isolates each (2%) had intermediate or resistant MICs to micafungin and/or caspofungin. All eight *N. glabrata* isolates with intermediate or resistant echinocandin MICs had the R1377K substitution encoded by *FKS2* hotspot 2 (FKS2HS2) and no mutations in *FKS1* hotspots 1 (FKS1HS1) and 2 (FKS1HS2) or *FKS2* hotspot 1 (FKS2HS1). These eight isolates had susceptible anidulafungin MICs when tested in duplicate by the commercial Etest method (Table 3). The fluconazole MICs of these eight *N. glabrata* isolates were all in the susceptible dose-dependent category (range: 4–32 mg/L). Five of the eight patients were admitted to a public-sector hospital and three to a private-sector hospital. Outcome data was only available for five of eight patients; three died and two survived. Eleven isolates (8%) were resistant to fluconazole (Table 2). Ten of these 11 fluconazole-resistant isolates also had MICs in the non-wild type range for posaconazole and voriconazole and four had an MIC greater than the itraconazole epidemiological cut-off value (ECV).[35] Overall, 80 (61%), 26 (20%), and 5 (4%) of 131 isolates had MICs above the voriconazole, posaconazole, and itraconazole ECV, respectively. There were no isolates with non-wild-type amphotericin B MICs.

Discussion

Nakaseomyces glabrata accounted for 14% of cases in South Africa during national laboratory-based surveillance for candidaemia in 2016–2017. *Nakaseomyces glabrata* was also the fourth most common cause of candidaemia in Brazil[36] and India.[37] In South African public-sector hospitals during 2016–2017, the incidence risk for candidaemia caused by *C. albicans* was highest compared to other *Candida* species. However, in private-sector hospitals during the same time period, *C. parapsilosis* candidaemia had the highest incidence risk. Additionally, the incidence risk for *N. glabrata* candidaemia was twice as high in public- vs. private-sector hospitals.[17] The reasons why *N. glabrata* is more common in patients admitted to public-sector hospitals is unknown but this could be related to the characteristics of the inpatient population such as older age, female predominance, and underlying co-morbid

Table 2. Antifungal susceptibility distribution of *Nakaseomyces glabrata* isolates from adult South African patients collected through national candidaemia surveillance during 2016–2017, $n = 131$

Antifungal agent	Test method	Number of isolates with MIC (mg/L) of:													MIC_{50}	MIC_{90}
		0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256		
Anidulafungin	BMD	35	83	11			1	1									0.03	0.03
Micafungin	BMD	126	1	1	1		1	1									0.015	0.015
Caspofungin	BMD	2	18	86	22	2	1										0.06	0.125
Fluconazole	BMD					1		1	10	12	22	55	19	4	6	1	16	32
Posaconazole	BMD	1			5	7	23	69	21		2	3					1	2
Voriconazole	BMD	2	5	6	14	24	53	22	2		3						0.5	1
Itraconazole	BMD	1		2	10	10	61	36	6		1	4					0.5	1
Flucytosine	BMD	3		107	17	1	1	1						1			0.06	0.12
Amphotericin B*	BMD				2	2	32	82	12								1	1
Amphotericin B*	Etest	6	4	3	11	33	40	25	6								0.38	1

Note: BMD—Broth microdilution, MIC—Minimum inhibitory concentration (mg/L)
*Missing values for amphotericin B, resistant isolates in bold for fluconazole, intermediate/resistant isolates in bold for anidulafungin, micafungin, and caspofungin

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Table 3. *FKS* gene mutation screening of eight *Nakaseomyces glabrata* isolates, from adult South African patients identified through national candidaemia surveillance during 2016–2017, with intermediate or resistant MIC values to one or more of the echinocandins

Isolate	Anidulafungin BMD MIC	Anidulafungin interpretation	Micafungin BMD MIC	Micafungin interpretation	Caspofungin BMD MIC	Caspofungin interpretation	Anidulafungin Etest MIC Reader 1	Anidulafungin Etest MIC Reader 2	*FKS2* hotspot 2 mutation*
3264	0.03	S	0.008	S	0.25	I	0.016	0.016	R1377K
3659	1	R	1	R	0.5	R	0.012	0.012	R1377K
3970	0.5	R	0.5	R	0.25	I	0.012	0.012	R1377K
3973	0.06	S	0.12	I	0.06	S	0.012	0.012	R1377K
4193	0.03	S	0.015	S	0.25	I	0.016	0.012	R1377K
4219	0.015	S	0.015	S	0.25	I	0.016	0.012	R1377K
4280	0.03	S	0.008	S	0.25	I	0.016	0.012	R1377K
5954	0.03	S	0.008	S	0.25	I	0.016	0.012	R1377K

Note: S—Susceptible, R—Resistant, I—Intermediate, BMD—broth microdilution, and MIC—Minimum inhibitory concentration (mg/L)
*No mutations in *FKS1* hotspots 1 and 2; and in *FKS2* hotspot 1

conditions. *Nakaseomyces glabrata* could also appear to be more common in the public sector since *C. auris* and *C. parapsilosis* are relatively less common pathogens.[17] Other pathogenic species in the *Nakaseomyces* genus are found very rarely in South Africa; we only found one case of *N. nivariensis* infection. One review paper reported that both *N. nivariensis* and *N. bracarensis* are found at a lower prevalence compared to *N. glabrata*.[38]

Only 6% of *N. glabrata* isolates in our study were resistant to the echinocandins and 8% resistant to fluconazole. We found no multidrug-resistant isolates in our collection. The eight *FKS* mutants from eight patients that we found in our study all had the same R1377K substitution encoded by *FKS2HS2*. Most of these patients were admitted to a public-sector hospital. We found the same mutation previously in two echinocandin-resistant *N. glabrata* isolates cultured from urine specimens of two patients treated in the private health sector.[39] In a recent German study, the researchers found other substitutions encoded by *FKS2HS2* (R1378H and R1378S); these mutants were isolated from abdominal fluid and blood culture respectively.[40] *FKS1* and *FKS2* gene mutations usually lead to reduced echinocandin susceptibility.[21] However, we found that *FKS* mutations were associated with intermediate/resistant caspofungin BMD MICs (for seven out of eight isolates). Three isolates were either resistant or intermediate to micafungin and/or anidulafungin by BMD. All eight isolates had susceptible anidulafungin Etest MICs. This was surprising since MICs obtained by BMD and Etest usually correlate well.[41] We also screened for *FKS* mutations in 12 micafungin-/anidulafungin-susceptible isolates and all 12 isolates did not have a *FKS* mutation (data not shown). It is possible that heteroresistance occurs in some *N. glabrata* populations but this has only been described for fluconazole to date.[42]

In one study, the Ser-663 substitution encoded by *FKS2HS1* resulted in a worse outcome. Additionally, these isolates also had the following MICs (in mg/L) for anidulafungin: 2 or 4; caspofungin: > 8 and fluconazole: > 64.[40] We had limited data, therefore, we could not determine whether patients infected with a *FKS* mutant had an adverse outcome. Treatment failure has been found to be more common in patients infected with *N. glabrata FKS* mutants [80%, 8/10[21] and 60%, 6/10[43]]. Furthermore in previous studies, 9%[26] and 14%[40] of *N. glabrata* fluconazole-resistant isolates were also resistant to the echinocandins. However, we did not find a relationship between the echinocandin and fluconazole MIC for the *FKS* mutants. The pre-disposing factors through which *N. glabrata* can acquire resistance to fluconazole and the echinocandins are by prior antifungal exposure,[44] loss of function mutations in the DNA mismatch repair (MMR) gene *MSH2*,[29] exposure to sub-therapeutic drug concentrations, and biofilm formation.[44] One would expect to find resistant *N. glabrata* from urine specimens due to the low echinocandin concentrations achieved in the urinary tract[39] and healthcare-associated urinary tract infection surveillance would be useful to screen for resistant *N. glabrata*.

A higher percentage of older female patients admitted to a South African public-sector hospital had *N. glabrata* candidaemia. *Nakaseomyces glabrata* has been found to mainly cause infection in older individuals.[18,19,45] Since *N. glabrata* is a commensal of the digestive system, it is possible that conditions such as diverticulitis, which is more common in the elderly population[46] predispose them to *N. glabrata* infection. In our study, patients infected with *N. glabrata* candidaemia had a shorter hospital stay and a shorter time to death compared to the non-*N. glabrata* candidaemia group. We found no difference in the quick Pitt score nor the crude mortality for patients infected with *N. glabrata*. When comparing *N. glabrata* and *C. albicans* infection cases, the crude in-hospital case fatality ratio for *N. glabrata* was 50.8% compared to 50.2% for *C. albicans*.[17] Even though *N. glabrata* is intrinsically less virulent than *C. albicans*, the crude mortality is similar. We can only speculate that *N. glabrata*'s virulence factors together with varying degrees of decreased susceptibility lead to similarly high mortality. *Nakaseomyces glabrata* may elicit a lesser inflammatory immune response and can also survive and replicate within macrophages similar to *Cryptococcus neoformans*[18,47] although it is also possible that host factors play a role. We also found that a higher percentage of patients infected with *N. glabrata* candidaemia were on fluconazole monotherapy. A fluconazole dose of less than 800 mg daily would generally be inadequate for treatment of *N. glabrata* infections.[48] These patients were also less likely to be treated with an echinocandin. According to the Infectious Diseases Society of America and Southern African guidelines for management of candidiasis, an echinocandin is recommended as initial therapy for candidaemia.[48,49] It is possible that with the lack of availability of echinocandins in the South African public sector during 2016–2017, clinicians would have been more likely to prescribe fluconazole for empiric and targeted treatment of candidaemia. However, we do not have access to antifungal consumption data in the public sector to corroborate these findings. Echinocandins have now been added to the South African standard treatment guidelines for treatment of invasive candidiasis, but are still restricted to tertiary

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centres.[50] Antifungal stewardship programs are needed to make sure South African patients infected with *N. glabrata* are appropriately treated with an echinocandin or polyene.

A limitation of our study was selection bias observed for detailed clinical data since we focused on ESS cases in our analysis. However, most of the non-ESS cases were from the private sector with limited clinical data available and therefore could not be analysed further. Another limitation was that antifungal susceptibility testing and screening of *N. glabrata* isolates for mutations in the *FKS* genes are unable to detect low-level resistance mechanisms such as heteroresistance[42] or tolerance.[28]

Our study provides insight into a common pathogen causing candidaemia in South African patients. We were able to describe clinical characteristics of patients infected with *N. glabrata* and its antifungal susceptibility pattern. Low levels of resistance to echinocandins and azoles were observed. Most patients with *N. glabrata* candidaemia were treated with suboptimal doses of fluconazole but should ideally be treated with an echinocandin or polyene.

Supplementary material

Supplementary material are available at *Medical Mycology* online.

Acknowledgements

Members of GERMS-SA (2016–2017)

John Black, Vanessa Pearce (Eastern Cape); Anwar Hoosen, Vicky Kleinhans (Free State); Alan Karstaedt, Caroline Maluleka, Charl Verwey, Charles Feldman, David Moore, Gary Reubenson, Khine Swe Swe Han, Jeannette Wadula, Jeremy Nel, Kathy Lindeque, Maphoshane Nchabeleng, Nazlee Samodien, Nicolette du Plessis, Norma Bosman, Ranmini Kularatne, Sharona Seetharam, Teena Thomas, Theunis Avenant, Trusha Nana, Vindana Chibabhai (Gauteng); Adhil Maharj, Asmeeta Burra, Fathima Naby, Halima Dawood, Jade Mogamberry, Koleka Mlisana, Lisha Sookan, Praksha Ramjathan, Prasha Mahabeer, Romola Naidoo, Sumayya Haffejee, Yacoob Coovadia (KwaZulu-Natal); Ken Hamese, Ngoaka Sibiya, Ruth Lekalakala (Limpopo); Greta Hoyland, Jacob Lebudi (Mpumalanga); Pieter Jooste (Northern Cape); Ebrahim Variava, Erna du Plessis (NW); Andrew Whitelaw, Kessendri Reddy, Mark Nicol, Preneshni Naicker (Western Cape); Adrian Brink, Elizabeth Prentice, Inge Zietsman, Maria Botha, Peter Smith, Xoliswa Poswa (AMPATH); Chetna Govind, Keshree Pillay, Suzy Budavari (LANCET); Carel Haumann, Catherine Samuel, Marthinus Senekal (PathCare); Andries Dreyer, Khatija Ahmed, Louis Marcus, Warren Lowman (Vermaak and Vennote); Angeliki Messina, Dena van den Bergh, Karin Swart (Netcare); Cynthia Whitney (CDC); Keith Klugman (Emory); Ananta Nanoo, Andries Dreyer, Anne von Gottberg, Anthony Smith, Arvinda Sooka, Cecilia Miller, Charlotte Sriruttan, Cheryl Cohen, Chikwe Ihekweazu, Claire von Mollendorf, Desiree du Plessis, Erika Britz, Frans Radebe, Genevie Ntshoe, Gillian Hunt, Hlengani Mathema, Jacqueline Weyer, Jenny Rossouw, John Frean, Karen Keddy, Kerrigan McCarthy, Linda de Gouveia, Linda Erasmus, Lucille Blumberg, Marshagne Smith, Martha Makgoba, Motshabi Modise, Nazir Ismail, Nelesh Govender, Neo Legare, Nicola Page, Ntsieni Ramalwa, Nuraan Paulse, Phumeza Vazi, Olga Perovic, Penny Crowther-Gibson, Portia Mutevedzi, Riyadh Manesen, Ruth Mpembe, Sarona Lengana, Shabir Madhi, Sibongile Walaza, Sonwabo Lindani, Sunnieboy Njikho, Susan Meiring, Thejane Motladiile, Tiisetso Lebaka, Vanessa Quan, Verushka Chetty (NICD).

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Conflict of interest

None

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Received: March 8, 2023. **Revised:** June 7, 2023. **Accepted:** June 14, 2023
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