AAC Accepted Manuscript Posted Online 1 April 2019
Antimicrob. Agents Chemother. doi:10.1128/AAC.02503-18
Copyright © 2019 Arastehfar et al.
This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Accepted Manuscript Posted Online
Antimicrobial Agents and Chemotherapy
AAC
Antimicrobial Agents and Chemotherapy

1 **Low level of antifungal resistance in Iranian isolates of *Candida glabrata* recovered from blood samples**
2 **from multicenter (2015-2018): Potential prognostic values of genotyping and sequencing of *PDR1***

3 **Running title: First evaluation of Iranian isolates of *C. glabrata***

4 Amir Arastehfar[1], Farnaz Daneshnia[1], Kamiar Zomorodian[2], Mohammad-Javad Najafzadeh[3], Sadegh
5 Khodavaisy[4,5], Hossein Zarrinfar[6], Ferry Hagen[1], Zahra Zare Shahrabadi[2], Michaela Lackner[7], Hossein
6 Mirhendi[8], Mohammadreza Salehi[9], Maryam Roudbary[10*], Weihua Pan[11*], Markus Kostrzewa[12], and Teun
7 Boekhout[1,13]

8 [1] Westerdijk Fungal Biodiversity Institute, Utrecht, Netherlands

9 [2] Basic Sciences in Infectious Diseases Research Center, and Department of Medical Mycology and
10 Parasitology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran

11 [3] Department of Medical Mycology and Parasitology, School of Medicine, Mashhad University of
12 Medical Sciences, Mashhad, Iran

13 [4] Department of Medical Parasitology and Mycology, Tehran University of Medical Sciences, Tehran,
14 Iran

15 [5] Zoonoses Research Center, Kurdistan University of Medical Sciences, Sanandaj, Iran

16 [6]Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

17 [7] Division of Hygiene and Medical Microbiology, Medical University of Innsbruck, Innsbruck, Austria

18 [8] Department of Medical Parasitology and Mycology, School of Medicine, Isfahan University of Medical
19 Sciences, Isfahan, Iran

20 [9]Department of infectious diseases and Tropical Medicine, Faculty of Medicine, Tehran University of Medical
21 Sciences, Tehran, Iran

22 [10] Department of Medical Mycology and Parasitology, School of Medicine, Iran University of Medical Sciences,
23 Tehran, Iran

24 [11] Department of Dermatology, Shanghai Key Laboratory of Molecular Medical Mycology, Shanghai Institute of
25 Medical Mycology, Shanghai Changzheng Hospital, Second Military Medical University, Shanghai 200003,
26 China

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

Accepted Manuscript Posted Online

27 [12]Bruker Daltonik GmbH, Bremen, Germany

28 [13]Institute of Biodiversity and Ecosystem Dynamics, University of Amsterdam, Amsterdam1012 WX, the
29 Netherlands

30 #

31 ***Maryam Roudbary**, Department of Medical Mycology and Parasitology, School of Medicine, Iran University
32 of Medical Sciences, Tehran, Iran Tel: 009886703296, Fax: 009888602217, email address,
33 m_roudbary@yahoo.com, Roudbari.mr@iums.ac.ir

34 ***Weihua Pan**, department of Dermatology, Shanghai Key Laboratory of Molecular Medical Mycology,
35 Shanghai Institute of Medical Mycology, Shanghai Changzheng Hospital, Second Military Medical University,
36 Shanghai 200003, China' Tel:+8602181885494, Fax: +8602181885493, panweihua@smmu.edu.cn

37

38 **Key words:** *C. glabrata*, Candidemia, Iran, Antifungal susceptibility testing, Genotyping,
39 HS1 of *FKS1* and *FKS2*, *CgPDR1*, and *ERG11*

40

41

42

43

44

45

46

47

48

AAC Antimicrobial Agents and Chemotherapy

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

Accepted Manuscript Posted Online
Antimicrobial Agents and Chemotherapy
AAC
Antimicrobial Agents and Chemotherapy
Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

49 **Abstract**

50 Establishing an effective empirical antifungal therapy requires conducting national
51 surveillance studies. Herein, we report the clinical outcome and microbiological features of
52 Iranian isolates of *C. glabrata* derived from patients suffering from candidemia. *C. glabrata*
53 isolates were retrospectively collected from four major cities of Iran, identified by a 21-plex
54 PCR, MALDI-TOF MS, and LSU rDNA sequencing, and genotyped by Amplified fragment
55 length polymorphism (AFLP). Mutations in *PDR1*, *ERG11*, and hotspot1 of *FKS1* and *FKS2,*
56 were investigated, and antifungal susceptibility testing (AFST) was performed (CLSI M27-
57 A3/S4). Seventy isolates of *C. glabrata* were collected from 65 patients with median age of
58 58. Fluconazole (29.23%) was the most widely used and least effective antifungal agent. The
59 overall crude mortality rate was 35.4%. Only one strain was resistant to fluconazole and
60 57.7% and 37.5% of isolates were non-wild type (non-WT) against caspofungin and
61 voriconazole, respectively. All of isolates showed WT phenotype for AMB, posaconazole,
62 and itraconazole. HS1 of *FKS1* and *FKS2* did not harbor any mutations, while numerous
63 missense mutations were observed in *PDR1* and *ERG11*. AFLP clustered our isolates into
64 nine genotypes, among them genotypes 1 and 2 were significantly associated with a higher
65 mortality rate (P=0.034 and P=0.022, α<0.05). Moreover, 83.3% of patients infected with
66 strains harboring a single new mutation of T745A in *PDR1* died despite of treatment with
67 fluconazole or caspofungin. Overall, Iranian isolates of *C. glabrata* were susceptible to major
68 antifungal drugs. Application of genotyping techniques and sequencing of specific genes,
69 *PDR1*, might have prognostic implications.

70

71 **Introduction**

Accepted Manuscript Posted Online

Antimicrobial Agents and Chemotherapy

AAC

Antimicrobial Agents and Chemotherapy

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

72 *Candida glabrata* is considered as the second most common cause of candidemia in the USA
73 and some European countries (1–4) and the third in Spain (5). Patients infected with *C.*
74 *glabrata,* compared to those infected with *C. albicans,* require higher expenses of health care
75 and a longer stay in the hospitals (6). Emergence of strains resistant to fluconazole (7),
76 echinocandins and/or other antifungals (multidrug resistant) (8, 9) along with a limited
77 number of antifungal drugs created a therapeutic challenge.

78 Although gain of function mutations in transactivating transcription factor of Cg*PDR1* have
79 been considered as the main cause of azole resistance in *C. glabrata* (10), some mutations in
80 *ERG11* are linked to MDR strains highly resistant to FLC, VRC, and AMB (11). Resistance
81 to echniocandins is mainly mediated by mutations in the hotspot1 of the *FKS1* and *FKS2* (12),
82 which are considered to be independent prediction factors for therapeutic failures of
83 echinocandins (13).

84 Although, *C. glabrata* is recognized as an asexual *Candida* species, genomic studies showed a
85 high genetic variability for clinical isolates of *C. glabrata* obtained from various countries
86 (14). Moreover, it has been known that some genotypes are attributable to a higher mortality
87 rate (15) and even it might be hypothesized that some genotypes are more virulent and
88 resistant (15). Hence, utilization of genotyping techniques such as multi locus sequence
89 typing (MLST) (15), microsatellite typing (9), pulsed field gel electrophoresis (16), amplified
90 fragment length polymorphism (AFLP) analysis (17), and polymorphic locus sequence typing
91 (18) are relevant for infection control measures. Although MLST has been extensively used
92 for genotyping of clinical isolates of *C. glabrata*, AFLP showed a higher resolution (19) and it
93 is also a preferred typing method for *C. auris* (20) and *Aspergillus terreus* (21).

94 Determination of antifungal susceptibility pattern on a national level is a prerequisite to
95 understand the evolving susceptibility profile of *C. glabrata*. Lack of systematic and
96 nationwide information on microbiological and clinical data of Iranian isolates of *C. glabrata*

Accepted Manuscript Posted Online

97 recovered from blood samples prompted us to conduct the present study. Isolates of *C.*
98 *glabrata* were retrospectively collected from four major clinical cities of Iran from 2015-
99 2018. Antifungal susceptibility testing was performed according to CLSI M27-A3/S4,
100 characterization of genotypes was carried out by AFLP and presence of mutations in genes
101 conferring resistance to azoles (*PDR1* and *ERG11*) and echinocandins (HS1 of *FKS1*and
102 *FKS2*) were explored. Moreover, important clinical data were mined from the history of
103 infected patients and presented.

104

105 **Results**

106 **Clinical outcomes**

107 Clinical data used in this study are listed in the Supplementary table 2 (Excel file). In total, 70
108 isolates of *C. glabrata* were recovered from 65 patients with the median age of 58 years,
109 among them 47.7 (n=31) were female and 52.3% (n=34) were male. The majority of the
110 isolates (86.1%; n=56) were recovered from blood, followed by central venous catheter and
111 abdominal fluids each 3.08% (n=2), and abdominal wound, dialysis fluid, cerebrospinal fluid
112 CSF, and DL and TL each 1.54% (n=1) (Supplementary table 2). ICU, CCU, NICU, and
113 PICU accommodated the majority of the patients (47.69%), followed by other hospital units,
114 including surgery (18.46%), emergency (15.38%), internal medicine (12.31%,), children
115 (3.08%), infectious diseases (1.54%) and general men (1.54%). Regarding underlying
116 conditions, other infections and tumors were observed in 47.7% of patients, followed by
117 trauma and surgery (20.00%), metabolic disorder (9.23%), blood-associated disease (7.69%),
118 autoimmune disease and liver and kidney dysfunctions each 4.62%, gastrointestinal bleeding
119 (GIB) (3.08%), and poisoning (1.54%). The majority of patients were treated with fluconazole
120 (29.23%), followed by caspofungin (18.46%), AMB (10.77%), voriconazole (3.08%), and

Antimicrobial Agents and Chemotherapy
AAC
Antimicrobial Agents and Chemotherapy
Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

Accepted Manuscript Posted Online

Antimicrobial Agents and Chemotherapy

AAC

Antimicrobial Agents and Chemotherapy

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

121 ointment clotrimazole (1.54%). Patients treated with caspofungin showed the highest rate of
122 survival (83.3%), followed by those treated with AMB (71.43%) and fluconazole (52.63%).
123 Twenty four (36.92%) patients did not receive any treatment and nine of them (37.50%) died
124 and 62.50% (n=15) survived. The overall crude mortality rate of patients infected with *C.*
125 *glabrata* was 35.4% (n= 23).

126 **Screening of mutations in *PDR1*, *ERG11*, and HS1 of *FKS1* and *FKS2***

127 Sequencing of *PDR1* showed that 54.92% (*n*=39) isolates contained non-synonymous
128 mutations (Table 1, Supplementary table 3 and 4, and Supplementary Figure 3), 45.08%
129 (*n*=39) isolates were wild-type, and 64.78% (*n*=39) harbored silent mutations (Supplementary
130 table 4). Twenty eight percent of mutations were located in the intervening region between the
131 binding and middle homology domains and found in isolates that showed the highest MIC
132 values for fluconazole (≥32 and 64). As for association of occurrence of mutation in *PDR1*
133 and voriconazole MIC values, 45.1% of the *PDR1* wild types and 30.7% of non-WT *PDR1*
134 isolates (carrying various non-synonymous mutations) had the MIC values higher than
135 epidemiological cut-off value (MIC≥0.5) (Table 5). Among strains with non-synonymous
136 mutations in *PDR1*, K67N (MIC=2 μg/ml), G128E, G493A (MIC=0.5 μg/ml), K430M,
137 T745A (MIC=0.5 μg/ml), E555K (MIC=4 μg/ml), and T745+C930R (MIC=0.5 μg/ml) were
138 exclusively occurred in strains with voriconazole MIC>ECV (Table 5). Regarding *ERG11*,
139 36.6% (*n*=26) of isolates showed non-synonymous mutations, 63.38% (*n*=26) were wild-type,
140 and 81.69% (*n*=58) harbored silent mutations (Table 2, Supplementary table 3 and 4, and
141 Supplementary Figure 3). Almost 22.53% (*n*=16) of isolates simultaneously contained
142 mutations in both genes of *PDR1* and *ERG11* (Supplementary Table 4). Hotspot1 of both
143 *FKS1* and *FKS2* were devoid of any mutations. Isolates harboring simultaneous mutations in
144 both *PDR1* and *ERG11* and those with mutation in either genes did not show a significantly
145 higher MIC values compared to those of wild-types. Surprisingly, five out of six patients

Accepted Manuscript Posted Online

146 infected with strains containing a single mutation of T745A in *PDR1* died, despite of
147 treatment with fluconazole or caspofungin or combination of both. These strains were found
148 in two cities of Mashhad (*n*=5) and Shiraz (*n*=1) and using AFLP they were clustered into five
149 distinguished genotypes (two strains from Mashhad shared the same genotype).

150 **Genotyping of isolates using AFLP**

151 AFLP divided our isolates into 9 distinct clusters (G1-G9) and genotype 2 was comprised of
152 three sub-genotypes of G2A, G2B, and G2C (Figure 1). Two isolates, collected from Tehran
153 and Isfahan, showed a bizarre banding pattern compared to the rest of *C. glabrata* isolates and
154 they clustered with *C. nivariensis* and *C. uthaithanina*. Subsequently, subjecting respective
155 DNA samples to the 21-plex PCR revealed two bands representing *C. glabrata* and *C.*
156 *parapsilosis* indicating that DNA samples were mixed of both aforementioned species. As a
157 result, the DNA samples obtained from these two isolates were excluded from downstream
158 genotyping analysis. There was no significant difference between resistance profile and
159 genotype clusters (Table 4). Associations of various genotypes with resistance profile to
160 fluconazole are summarized in Table 4. Although, through Chi-squared test (two-tailed)
161 clinical outcome was only significantly associated with G3 (*P*=0.025), logistic regression and
162 path analysis showed that G1 (*P*=0.034) and G2 (*P*=0.022) were significantly associated with
163 a higher rate of mortality (α<0.05), while G3 was significantly associated with survival
164 (*P*=0.001, α<0.05) (See supplementary files, statistical analysis section). Moreover, through
165 Chi-squared test (two-tailed) there was no significant association between clinical outcome
166 and VRZ resistance profile (*P*=0.555) and clinical outcome and clinical failure (*P*=0.504).
167 Additionally, multivariate logistic regression analysis did not show significant association
168 between clinical outcome and hospitalization duration (*P*=0.291) (See supplementary files,
169 statistical analysis section).

170 **Antifungal susceptibility pattern**

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

AAC Antimicrobial Agents and Chemotherapy

Accepted Manuscript Posted Online
AAC
Antimicrobial Agents and Chemotherapy
Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

171 All of the MIC values obtained in this study are summarized in Table 3 and Supplementary
172 table 3. Resistance to fluconazole (MIC≥64) was only noted in one isolate (1.4%) and the rest
173 were susceptible dose-dependent (SDD), while 36.43% (*n*=28) of the isolates showed the
174 MIC values higher than ECV for voriconazole (MIC≥0.5 µg/ml), and all of isolates showed
175 the WT phenotype for posaconazole (MIC≥2 µg/ml), and itraconazole (MIC≥4 µg/ml). No
176 cross-resistance between azole drugs was observed. As for caspofungin, 57.74% of isolates
177 (*n*=41) showed the MICs above the ECV (≥0.5 µg/ml), while for AMB none of isolates
178 showed MIC>ECV (AMB ECV>2 µg/ml) (31). Although, resistance to echinocandins is
179 noted when resistance is observed for at least two antifungal agents in this class (12, 32),
180 caspofungin was the only echinocandin agent that was available in our study. Moreover, due
181 to the interlaboratory variation observed for caspofungin (33) and as a surrogate the MIC
182 values of caspofungin were combined with the sequence data of HS1 of *FKS1* and *FKS2*.
183 Almost 24% (*n*=17) of isolates simultaneously had the MIC values higher than ECV for both
184 caspofungin and voriconazole (MIC≥0.5 µg/ml), among which 35.29% (*n*=6) had the MIC
185 values of ≥ 1µg/ml and ≥0.5 µg/ml for voriconazole and caspofungin, respectively.
186 Fluconazole showed the highest geometric mean value (10.31), followed by amphotericin B
187 (0.57), itraconazole (0.51), caspofungin (0.41), posaconazole (0.41), and voriconazole (0.32).

188 **Discussion**

189 The steady increase in the incidence of candidemia due to *C. glabrata* along with a
190 concerning development of resistance to azoles, echinocandins, and even emergence of strains
191 with MDR traits have highlighted the importance of studying antifungal susceptibility, the
192 involved subcellular mechanisms of resistance, and genotyping of clinical isolates of *C.*
193 *glabrata* (9, 12). Previously, studies conducted in China (9, 34), South Korea (15), India (35),
194 and USA (12) had investigated the aforementioned aspects of clinical isolates of *C. glabrata*,
195 and showed a variability in rate of resistance to azoles and echinocandins in those countries.

Accepted Manuscript Posted Online
Antimicrobial Agents and Chemotherapy
AAC
Antimicrobial Agents and Chemotherapy
Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

196 As, these information in a nationwide scale is lacking for Iranian isolates of *C. glabrata*, we
197 conducted a multicenter study to investigate the clinical and microbiological features of this
198 species.

199 In our study, no difference was observed in occurrence of candidemia due to *C. glabrata*
200 between males and females. Consistent with the other studies infections due to *C. glabrata*
201 were mainly observed in elderlies (6, 36), with the median age of 58 years. Moreover,
202 underlying conditions observed for our patients, namely extensive use of broad-spectrum
203 antibiotics, cancer, other infections, and surgery are recognized risk factors for development
204 of candidemia (6, 36). Although clinical guidelines consider echinocandins as the frontline
205 therapy for *C. glabrata* (37), in our study caspofungin ranked as the second treatment option
206 and showed the highest rate of survival compared to those treated with fluconazole. Lower
207 utilization of echniocandins compared to azoles in developing countries might reflect the
208 higher expenses associated with these drugs (35). Unlike other studies with a reported
209 mortality rate of 58%-61% (38), in our study approximately 35% of our patients died, similar
210 to what is reported from USA (6).

211 As no mutations were observed in HS1 of *FKS1* and *FKS2,* none of our isolates were
212 categorized as echinocandin resistant. Due to unreliability of the MIC values of caspofungin
213 (33) and superiority of presence of mutations in HS1 of FKS1 and FKS2 (39) resistance to
214 echniocandins were inferred only based on the presence of mutation in HS1 of the
215 aforementioned genes. This is in line with our findings, where the vast majority of isolates
216 (57.74%) had the MIC>ECV (0.5µg/ml), while there were no mutations in the HS1 of *FKS1*
217 and *FKS2*. Contrary to USA with a rate of echinocandin resistance up to 13% (12), the lack of
218 echinocandin resistance in our study is similar toother Asian countries, including South Korea
219 (0%), India (0%), China (1.9%), Turkey (2%) (15, 34, 35, 40), European and South American
220 countries (38, 41-44). Likely, this variation in rate of resistance to echinocandins reflects the

Accepted Manuscript Posted Online

Antimicrobial Agents and Chemotherapy

AAC

Antimicrobial Agents and Chemotherapy

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

221 variation in therapeutic regimen implemented in a specific region/country (35), and the
222 genetic difference between isolates of *C. glabrata* (15).

223 A low level of resistance was observed for fluconazole (one isolate, 1.4%), and the rest of
224 isolates was categorized as the SDD phenotype. This rate of resistance to fluconazole is
225 similar to what is observed in the other Asian and South American countries where the
226 incidence of fluconazole resistance varies from 0%-8.9% (15, 34, 35, 40, 41). As strains
227 harboring mutations in *PDR1* or *ERG11* compared to those of wild-types did not exhibit
228 higher MIC values (Tables 2 and 3), it could be inferred that those mutations were not
229 engaged in resistance. The fluconazole resistant isolate carried a previously described
230 mutation (P76S, P145T, D243N) (34) that was also found in isolates with the SDD phenotype
231 (Table 2). Although, in some other *Candida* species, such as *C. albicans* (45) the fluconazole
232 and vroiconazole resistance are governed by the same mechanism, none of our strains showed
233 concurrent cross-resistance/non-WT phenotype for FLZ and VRZ. Moreover, the majority of
234 non-synonymous mutations occurring in *PDR1* (*n*=26; 66.6%) had the VRZ MIC <ECV and
235 among those with the MIC >ECV, only one third were exclusively found in VRZ non-WT
236 strains (K67N, G128E+G493A, K430M+T745A, E555K, and T745+C930R). Besides, *PDR*1
237 WT strains compared to those of non-WTs had a higher proportion of non-WT phenotype for
238 VRZ (45.1% WT versus 30.7% non-WT) (Table 5). Collectively, these observations point to
239 the fact that in *C. glabrata*, resistance to fluconazole and voriconazole might not be controlled
240 by the same mechanism. As for *ERG11*, all non-synonymous mutations occurred in
241 fluconazole SDD strains. X-ray crystallography studies on *ERG11* of *S. cerevisiae* (46) and
242 homology modelling in *C. glabrata* (47) showed that missense mutations in the residues of
243 132, 140, 143, 464 and 146, 243, and 246, respectively, are linked to azole resistance. On the
244 contrary, in our study none of the isolates with substitution in the neighborhood of those
245 residues (196, 425, 430, 456-458) showed resistance to fluconazole. Moreover, unlike *S.*

Accepted Manuscript Posted Online

246 *cerevisiae* (46), occurrence of mutation in the residue of 315 (G315D) of a clinical strain of *C.*
247 *glabrata* caused multidrug resistance to fluconazole (> 256μg/ml), voriconazole (>
248 256μg/ml), and AMB (> 32μg/ml) (11). None of isolates showed the MIC values higher than
249 ECV (MIC> 2μg/ml) for AMB. Low level and lack of resistance to azoles and
250 AMB/echniocandins in this study might be explained by the fact that none of our patients
251 experienced previous and prolonged exposure with these antifungals (48, 49).

252 Although, mutations in MSH2 gene (DNA mismatch repair pathway) correspond to
253 hypermutable phenotypes of *C. glabrata* that can facilitate development of azole R and MDR
254 strains (8), studies from India (35), France (50) and China (34) found that mutations in this
255 gene are more associated with rare and specific genotypes. Therefore, we did not include this
256 gene in our study.

257 Observation of hyper-variation in virulence patterns for each strains of *C. glabrata* (51) along
258 with the association of certain genotypes with a higher rate of mortality (15), revealed the
259 importance of genotyping techniques in clinical settings. In line with these findings, in our
260 study two genotypes, G1 and G2, showed a significant association with a higher rate of
261 mortality ($\alpha<0.05$, $P=0.034$ and $P=0.022$), while G3 was significantly associated with
262 survival ($\alpha<0.05$, and $P=0.001$). Additionally, it has been shown that mutations in *PDR1* have
263 implications in virulence and strains carrying certain mutations showed reduced adherence to
264 macrophages and increased adhesion to epithelial cells (10). Interestingly, we noticed that five
265 out of six patients infected with strains carrying a single mutation of T745A in *PDR1* (not in
266 combination with the other mutations in *PDR1*) died despite of treatment with either
267 fluconazole or caspofungin or a combination of both. Five of those isolates belonging to four
268 genotypes (two strains shared the same genotype) were found in the same city (Mashhad) and
269 the same hospital for which 80% of infected patients died (n=4). As for the other isolate
270 belonging to a different genotype was found in Shiraz and the infected patient died. Although,

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

Antimicrobial Agents and Chemotherapy
AAC
Antimicrobial Agents and Chemotherapy

Accepted Manuscript Posted Online

Antimicrobial Agents and Chemotherapy

AAC

Antimicrobial Agents and Chemotherapy

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

271 drawing this conclusion based on a small number of strains is not conclusive, due to the
272 pleiotropic functions of *PDR1*, this specific mutation (T745A) might deserves further in-vivo
273 studies. Surprisingly, in our study each genotype of *C. glabrata* contained isolates recovered
274 from patients hospitalized in different cities. Admitting the fact that AFLP might not have the
275 genotyping resolution of whole genome sequencing platforms, this observation might be an
276 indicative of nosocomial transmission of *C. glabrata* isolates. Although rarely reported, some
277 studies have shown the nosocomial transmission of *C. glabrata* isolates in clinical settings
278 (18, 52).

279 **Materials and methods**

280 **Collection of isolates and ethical approval**

281 Isolates of *C. glabrata* were retrospectively collected from Tehran, Isfahan, Shiraz, and
282 Mashhad from 2015-2018 (Supplementary Figure 1). The procedure of study in each center
283 was evaluated by regional ethical committee members and accordingly they were provided
284 with ethical codes (IR.SUMS.REC.1397.365, IR MUMS fm REC.1397.268, IR. TUMS.
285 .SPH.REC.1396.4195). Prior to studying the isolates and analyzing the clinical data, each
286 patient and the isolates derived from them were designated with specific codes to prevent
287 exposing their personal data.

288 **Identification**

289 Preliminary isolates were identified by a 21-plex PCR (22). Isolates were serially coded from
290 1-70. They were re-identified by MALDI-TOF MS (MALDI Biotyper; Bruker Daltonik
291 GmbH, Bremen, Germany) (23) and sequencing of the D1/D2 domains of the large subunit of
292 rDNA (LSU rDNA) sequencing) (24).

293 **DNA extraction**

Accepted Manuscript Posted Online

294 DNA samples were extracted with the CTAB method (100 mM Tris-HCl pH 8,4; 1,4 M NaCl;
295 25 mM EDTA pH 8.0; 2% CTAB) (25). The quality of DNA samples were assessed by
296 NanoDrop (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and running 5μl of
297 DNA sampels on 0.7% agarose gel, the quality and their quantity was evaluated by QuBit
298 dsDNA BR Assay Kit (Thermo Fisher Scientific corporation, Waltham, Massachusetts,
299 USA).

300 **Primer design, PCR, and sequencing for FKS1, FKS2, PDR1, and ERG11**

301 DNA sequences of HS1 of *FKS1* and *FKS2*, and *PDR1*, and *ERG11* were determined and
302 screened for presence of mutations. Fourteen primers were used to sequence *PDR1*
303 comprising two external primers and 12 internal primers and eight primers for *ERG11*,
304 including two external and 6 internal primers (Supplementary Table 1 and Figure 2). Primers
305 were synthesized by the IDT Company (Integrated DNA Technology, Leuven, Belgium).

306 PCR reactions for *FKS1*, *FKS2*, *PDR1*, and *ERG11* were prepared in a volume of 50μl as the
307 following, 5μl 10X buffer (10X NH4, No $MgCl_2$), 2mM $MgCl_2$, 0.2mM dNTP mix (dNTP
308 mix, 100Mm, Biolab), 5 picomol of primers (FKS1-F, FKS1R, FKS2F, FKS2R, PDR1Fex,
309 PDR1Rex, ERG11Fex, and ERG11Rex), 2.5 units of *Taq* polymerase enzyme (Bio Taq DNA
310 Polymerase, Biolab), and using MiliQ water to adjust the volume to 50μl.

311 All PCR reactions were set at the same annealing temperature but with variable incubation
312 time of the extension phase. PCR programs contained the following steps, 95 °C for 5 min,
313 followed by 95 °C for 30 sec, 58 °C for 30 sec, 72 °C for 30 seconds (*FKS1*), 1 min (*FKS2*), 2
314 min for *ERG11*, and 3 min for *PDR1*, followed by 72 °C for 8 min. PCR products were run on
315 2% agarose gel.

316 **Sequencing and analysis of sequences**

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

Antimicrobial Agents and Chemotherapy
AAC
Antimicrobial Agents and Chemotherapy

Accepted Manuscript Posted Online

Antimicrobial Agents and Chemotherapy

AAC

Antimicrobial Agents and Chemotherapy

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

317 Primers presented in table 1 were used in bidirectional dideoxy chain terminated Sanger
318 sequencing. Contigs were assembled and edited by SeqMan software (DNASTAR, Madison,
319 USA) and obtained sequences were aligned by MEGA software V.7.0 (Temple University,
320 Philadelphia). Following sequences of FJ550269.1 (10) and XM_445876 (26) were used as
321 the WT references for *PDR1* and *ERG11* sequences, respectively.

322 **Genotyping using amplified fragment length polymorphism (AFLP)**

323 AFLP as suggested by Alessia *er al.* (27) was employed to evaluate the genotypic patterns of
324 our isolates of *C. glabrata*. AFLP data were analyzed by Bionumerics software V7.6 (Applied
325 Math Inc, Austin, Texas, USA). The reference and type strains of *C. glabrata* (CBS 138 and
326 CBS 2175) and the other closely-related species, including, *C. nivariensis* (CBS 9983-85 and
327 CBS 10161)*, C. bracarensis* (CBS 10154)*, C. uthaithanina* (CBS 10932)*, C. kungkrabaensis*
328 (CBS 10927)*, N. delphensis* (CBS 2170)*, N. bacillisporous* (CBS 7720) and a clinical isolate
329 of *C. bracarensis* (generously provided by professor W. Liao, Shanghai) were included in the
330 AFLP experiment.

331 **Antifungal susceptibility testing**

332 Minimum inhibitory concentration values of antifungal drugs were determined by broth
333 microdilution procedure according to CLSI-M27/A3 (28). The following antifungal drugs
334 were included, fluconazole (Pfizer, New York, USA), voriconazole (Pfizer, New York, USA),
335 itraconazole (Santa Cruz Biotech, Dallas, USA), posaconazole (MSD, Kenilworth, USA),
336 caspofungin (Merck & Co., Inc.), and amphotericin B (Sigma Chemical Corporation, St.
337 Louis, MO). For quality control purposes, *C. parapsilosis* (CBS 604) and *C. krusei* (CBS
338 5147) were used. Species-specific breakpoints were adopted from CLSI-M27/S4 (29).
339 Minimum inhibitory concentration was read visually after 24 hours and noted as the lowest
340 concentration of fluconazole (FLZ) and caspofungin (CAS) resulting in at least 50% reduction

Accepted Manuscript Posted Online

341 of growth compared to the control. Resistance to FLZ and CAS was noted when the MIC
342 values were ≥ 64 μg/ml and ≥ 0.5 μg/ml, respectively. The MIC values of other azole drugs
343 including vorconazole (VRC) (≥ 1 μg/ml), posaconazole (PSC) (≥ 4 μg/ml), and itraconazole
344 (ITC) (≥ 4 μg/ml) were interpreted according to epidemiological cut-off values (29, 30). MIC
345 values of AMB were noted at the lowest concentration of the drug that showed 100%
346 reduction compared to an AMB-free control strain, and MIC values > 2.0 were considered as
347 potential resistant isolates (29, 30, and 31).

348 **Deposition of strains in the culture collection of Westerdijk Institute and accessibility of**
349 **sequences**

350 All the isolates of *C. glabrata* studied in this project were deposited in the culture collection
351 Westerdijk Fungal Biodiversity Institute and they were designated with the following CBS
352 numbers: CBS 15665-15720, CBS 15722-15733, and CBS 15744. Sequences obtained for
353 *PDR1, ERG11*, and HS1 of *FKS1* and *FKS2* are attached in the supplementary text files of 6-
354 9.

355 **Statistical analysis**

356 Logistic regression and path analysis was performed to evaluate the statistical significance
357 and association between genotypes and death or survival. As multivariate logistic regression
358 analysis does not consider the indirect influence of independent variables on dependent ones,
359 therefore, path analysis was used to overcome this problem. Using path analysis the
360 association of mortality and survival were individually assessed with genotypes 1-3.
361 Moreover, Chi-squared test (two-tailed) was used to find the association between the clinical
362 outcome and genotypes, voriconazole susceptibility profile (susceptible or resistance),
363 hospitalization duration, and clinical failure for all patients. Values <0.05 were considered as

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

AAC Antimicrobial Agents and Chemotherapy

Accepted Manuscript Posted Online

Antimicrobial Agents and Chemotherapy

AAC

Antimicrobial Agents and Chemotherapy

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

364 statistically significant. All statistical analysis were calculated by SPSS software v.24
365 (Windows, Chicago, IL, USA) (See supplementary files, statistical analysis section).
366

367 **Acknowledgement**

368 NA

369 **Conflict of interest**

370 Markus Kostrzewa is affiliated with the Bruker Company, Bremen, Germany. The rest of
371 authors do not have any commercial affiliations and consultancies roles, elsewhere.

372 **Funding**

373 This project has received funding from the European Union's Horizon 2020 research and
374 innovation program under the Marie Sklodowska-Curie grant agreement No *642095*, National
375 Health Department of China [2018ZX10101003], National Natural Science Foundation of
376 China [31770161], Second Military Medical University [2017JZ47] and Shanghai Science
377 and Technology Committee [14DZ2272900 and 14495800500].

378 **Appendix**

379 NA

380 **References**

381 1. Trouvé C, Blot S, Hayette M, Jonckheere S, Patteet S, Rodriguez-Villalobos H,
382 Symoens F, Van Wijngaerden E, Lagrou K. 2017. Epidemiology and reporting of
383 candidaemia in Belgium : a multi-centre study. Eur J Clin Microbiol Infect Dis 36:649-
384 655.

385 2. Lockhart SR, Iqbal N, Cleveland AA, Farley MM, Harrison LH, Bolden CB,

Accepted Manuscript Posted Online

386 Baughman W, Stein B, Hollick R, Park BJ, Chiller T. 2012. Species Identification and
387 Antifungal Susceptibility Testing of *Candida* Bloodstream Isolates from Population-
388 Based Surveillance Studies in Two U . S . Cities from 2008 to 2011. J Clin Microbiol
389 50:3435–3442.

390 3. Chapman B, Slavin M, Marriott D, Halliday C, Kidd S, Arthur I, Bak N, Heath CH,
391 Kennedy K, Morrissey CO, Sorrell TC, Van Hal S, Keighley C, Goeman E,
392 Underwood N, Hajkowicz K, Hofmeyr A, Leung M, Macesic N, Botes J, Blyth C,
393 Cooley L, George CR, Kalukottege P, Kesson A, McMullan B, Baird R, Robson J,
394 Korman TM, Pendle S, Weeks K, Weeks K, Liu E, Cheong E, Chen S, Australian and
395 New Zealand Mycoses Interest Group. 2017. Changing epidemiology of candidaemia
396 in Australia. J Antimicrob Chemother 72:1103–1108.

397 4. Arendrup MC, Bruun B, Christensen JJ, Fuursted K, Johansen HK, Kjaeldgaard P,
398 Knudsen JD, Kristensen L, Møller J, Nielsen L, Rosenvinge FS, Røder B, Schønheyder
399 HC, Thomsen MK, Truberg K. 2011. National Surveillance of Fungemia in Denmark (
400 2004 to 2009). J Clin Microbiol 49:325–334.

401 5. Puig-Asensio M, Padilla B, Garnacho-Montero J, Zaragoza O, Aguado JM, Zaragoza
402 R, Montejo M, Muñoz P, Ruiz-Camps I, Cuenca-Estrella M, Almirante B; CANDIPOP
403 Project; GEIH-GEMICOMED (SEIMC); REIPI. 2014. Epidemiology and predictive
404 factors for early and late mortality in *Candida* bloodstream infections : a population-
405 based surveillance in Spain. Clin Microbiol Infect 20:245–254.

406 6. Moran C, Grussemeyer CA, Spalding JR, Benjamin DK Jr, Reed SD. 2010.
407 Comparison of costs , length of stay , and mortality associated with *Candida glabrata*
408 and *Candida albicans* bloodstream infections. Am J Infect Control 38:78–80.

409 7. Lamoth F, Lockhart SR, Berkow EL, Calandra T. 2018. Changes in the

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

AAC Antimicrobial Agents and Chemotherapy

Accepted Manuscript Posted Online

410 epidemiological landscape of invasive candidiasis. J Antimicrob Chemother 73: 4–13.

411 8. Healey KR, Zhao Y, Perez WB, Healey KR, Zhao Y, Perez WB, Lockhart SR, Sobel
412 JD, Farmakiotis D, Kontoyiannis DP, Sanglard D, Taj-Aldeen SJ, Alexander BD,
413 Jimenez-Ortigosa C, Shor E, Perlin DS. 2016. Prevalent mutator genotype identified in
414 fungal pathogen *Candida glabrata* promotes multi-drug resistance. Nat Commun
415 7:11128.

416 9. Hou X, Xiao M, Chen SC, Kong F, Wang H, Chu YZ, Kang M, Sun ZY, Hu ZD, Li
417 RY, Lu J, Liao K, Hu TS, Ni YX, Zou GL, Zhang G, Fan X, Zhao YP, Xu YC. 2017.
418 Molecular Epidemiology and Antifungal Susceptibility of *Candida glabrata* in China (
419 August 2009 to July 2014): A Multi-Center Study. Front Microbiol 8:1–9.

420 10. Ferrari S, Ischer F, Calabrese D, Posteraro B, Sanguinetti M, Fadda G, Rohde B,
421 Bauser C, Bader O, Sanglard D. 2009. Gain of Function Mutations in *Cg*PDR1 of
422 *Candida glabrata* Not Only Mediate Antifungal Resistance but Also Enhance
423 virulence. PLoS Pathogen 5:1000268.

424 11. Hull CM, Parker JE, Bader O, Weig M, Gross U, Warrilow AG, Kelly DE, Kelly SL.
425 2012. Facultative Sterol Uptake in an Ergosterol-Deficient Clinical Isolate of *Candida*
426 *glabrata* Harboring a Missense Mutation in ERG11 and Exhibiting Cross-Resistance to
427 Azoles and Amphotericin B. Antimicrob Agents Chemother 56:4223–4232.

428 12. Alexander BD, Johnson MD, Pfeiffer CD, Jiménez-Ortigosa C, Catania J, Booker R,
429 Castanheira M, Messer SA, Perlin DS, Pfaller MA. 2013. Increasing Echinocandin
430 Resistance in *Candida glabrata*: Clinical Failure Correlates With Presence of FKS
431 Mutations and Elevated Minimum Inhibitory Concentrations. Clin Infect Dis 56:1724–
432 1732.

433 13. Shields RK, Nguyen MH, Press EG, Kwa AL, Cheng S, Du C, Clancy CJ. 2012. The

Antimicrobial Agents and Chemotherapy
AAC
Antimicrobial Agents and Chemotherapy
Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

Accepted Manuscript Posted Online

434 Presence of an FKS Mutation Rather than MIC is an Independent Risk Factor for
435 Failure of Echinocandin Therapy among Patients with Invasive Candidiasis Due to
436 *Candida glabrata*. Antimicrob Agents Chemother 56:4862–4869.

437 14. Carreté L, Ksiezopolska E, Pegueroles C, Gómez-Molero E, Saus E, Iraola-Guzmán S,
438 Loska D, Bader O, Fairhead C, Gabaldón T. 2018. Patterns of Genomic Variation in
439 the Opportunistic Pathogen Candida glabrata Suggest the Existence of Mating and a
440 Secondary Association with Humans Article Patterns of Genomic Variation in the
441 Opportunistic Pathogen *Candida glabrata* Suggest the Existence of Mating and a
442 Secondary Association with Humans. Curr Biol 28:15–27.

443 15. Byun SA, Won EJ, Kim M, Lee WG, Lee K, Lee HS, Uh Y, Healey KR, Perlin DS,
444 Choi MJ, Kim SH, Shin JH. 2018. Multilocus Sequence Typing (MLST) Genotypes
445 of *Candida glabrata* Bloodstream Isolates in Korea: Association With Antifungal
446 Resistance, Mutations in Mismatch Repair Gene (Msh2), and Clinical Outcomes. Front
447 Microbiol 9:1–10.

448 16. Lin CY, Chen YC, Lo HJ, Chen KW, Li SY. 2007. Assessment of *Candida glabrata*
449 Strain Relatedness by Pulsed-Field Gel Electrophoresis and Multilocus Sequence
450 Typing. J Clin Microbiol 45:2452–2459.

451 17. Wahyuningsih R, Sahbandar IN, Theelen B, Hagen F, Poot G, Meis JF, Rozalyani A,
452 Sjam R, Widodo D, Djauzi S, Boekhout T. 2008. *Candida nivariensis* Isolated from an
453 Indonesian Human Immunodeficiency Virus-Infected Patient Suffering from
454 Oropharyngeal Candidiasis. J Clin Microbiol 46:388–391.

455 18. Katiyar S, Shiffrin E, Shelton C, Healey K, Vermitsky J-P, Edlind T. 2016. Evaluation
456 of Polymorphic Locus Sequence Typing for *Candida glabrata* Epidemiology. J Clin
457 Microbiol 54:1042–1050.

AAC
Antimicrobial Agents and Chemotherapy
Antimicrobial Agents and Chemotherapy
Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

Accepted Manuscript Posted Online

Antimicrobial Agents and Chemotherapy

AAC

Antimicrobial Agents and Chemotherapy

458 19. Asadzadeh M, Ahmad S, Al-sweih N, Khan Z. 2017. Molecular Fingerprinting Studies Do
459 Not Support Intrahospital Transmission of *Candida albicans* among Candidemia
460 Patients in Kuwait. Front Microbiol 8:1–12.

461 20. Prakash A, Sharma C, Singh A, Kumar Singh P, Kumar A, Hagen F, Govender NP,
462 Colombo AL, Meis JF, Chowdhary A. 2015. Evidence of genotypic diversity among
463 *Candida auris* isolates by multilocus sequence typing, matrix-assisted laser desorption
464 ionization time-of-flight mass spectrometry and amplified fragment length
465 polymorphism. Clin Microbiol Infect 22:1–9.

466 21. Kathuria S, Sharma C, Singh PK, Agarwal P, Agarwal K, Hagen F, Meis JF,
467 Chowdhary A. 2015. Molecular Epidemiology and In-Vitro Antifungal Susceptibility
468 of *Aspergillus terreus* Species Complex Isolates in Delhi, India : Evidence of Genetic
469 Diversity by Amplified Fragment Length Polymorphism and Microsatellite Typing.
470 PLoS ONE 10:1–17.

471 22. Arastehfar A, Fang W, Pan W, , Lackner M, Liao W, Badiee P, Zomorodian K, Badali
472 H, Hagen F, Lass-Flörl C, Boekhout T. 2018. YEAST PANEL Multiplex PCR for
473 Identification of Clinically Important Yeast Species: Stepwise Diagnostic Strategy,
474 Useful for Developing Countries. Diagn Microbiol Infect Dis 18: SO732–8893.

475 23. Cassagne C, Cella AL, Suchon P, Normand AC, Ranque S, Piarroux R. 2013.
476 Evaluation of four pretreatment procedures for MALDI-TOF MS yeast identification in
477 the routine clinical laboratory. Med Mycol 51:371–377.

478 24. Stielow JB, Lévesque CA, Seifert KA, Meyer W, Iriny L, Smits D, Renfurm R, Verkley
479 GJ, Groenewald M, Chaduli D, Lomascolo A, Welti S, Lesage-Meessen L, Favel A,
480 Al-Hatmi AM, Damm U, Yilmaz N, Houbraken J, Lombard L, Quaedvlieg W, Binder
481 M, Vaas LA, Vu D, Yurkov A, Begerow D, Roehl O, Guerreiro M, Fonseca A,

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

Accepted Manuscript Posted Online

482 Samerpitak K, van Diepeningen AD, Dolatabadi S, Moreno LF, Casaregola S, Mallet
483 S, Jacques N, Roscini L, Egidi E, Bizet C, Garcia-Hermoso D, Martín MP, Deng S,
484 Groenewald JZ, Boekhout T, de Beer ZW, Barnes I, Duong TA, Wingfield MJ, de
485 Hoog GS, Crous PW, Lewis CT, Hambleton S, Moussa TA, Al-Zahrani HS,
486 Almaghrabi OA, Louis-Seize G, Assabgui R, McCormick W, Omer G, Dukik K,
487 Cardinali G, Eberhardt U, de Vries M, Robert V. 2015. One fungus, which genes?
488 Development and assessment of universal primers for potential secondary fungal DNA
489 barcodes. Persoonia 35:242–263.

490 25. Theelen B, Silvestri M, Gueho E, Van Belkum A, Boekhout T. 2001. Identification and
491 typing of *Malassezia* yeasts using amplified fragment length polymorphism (AFLP),
492 random amplified polymorphic DNA (RAPD) and denaturing gradient gel
493 electrophoresis (DGGE). FEMS Yeast Res 1:79–86.

494 26. Szweda P, Gucwa K, Romanowska E, Dzierzanowska-Fangrat K, Naumiuk L,
495 Brillowska-Dabrowska A, Wojciechowska-Koszko I, Milewski S. 2015. Mechanisms
496 of azole resistance among clinical isolates of Candida glabrata in Poland. J Med
497 Microbiol 64:610–619.

498 27. Marchetta A, Gerrits van den Ende B, Al-hatmi AMS, Hagen F, Zalar P, Sudhadham M,
499 Gunde-Cimerman N, Urzì C, de Hoog S, De Leo F. 2018. Global Molecular Diversity
500 of the Halotolerant Fungus *Hortaea werneckii*. Life (Basel) 8:1–12.

501 28. Clinical and Laboratory Standards Institute. Reference method for broth dilution
502 antifungal susceptibility testing of yeasts; Approved Standard—Third Edition: CLSI
503 Document M27 -S3. CLSI, Wayne, PA, USA, 2008.

504 29. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts:
505 Fourth Informational Supplement M27 -S4. CLSI, Wayne, PA, USA, 2012.

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

Antimicrobial Agents and Chemotherapy
AAC
Antimicrobial Agents and Chemotherapy

Accepted Manuscript Posted Online

506 30. Pfaller MA. 2012. Antifungal Drug Resistance : Mechanisms, Epidemiology, and
507 Consequences for Treatment. AM J Med 125:S3–S13.

508 31. Pfaller MA, Diekema DJ. 2012. Progress in antifungal susceptibility testing of *Candida*
509 spp. by use of Clinical and Laboratory Standards Institute broth microdilution
510 methods, 2010 to 2012. J Clin Microbiol 50:2846–2856.

511 32. Farmakiotis D, Tarrand JJ, Kontoyiannis DP. 2014. Drug-resistant Candida glabrata
512 infection in cancer patients. Emerg Infect Dis 20:1833–1840.

513 33. Espinel-Ingroff A, Arendrup MC, Pfaller MA, Bonfietti LX, Bustamante B, Canton E,
514 Chryssanthou E, Cuenca-Estrella M, Dannaoui E, Fothergill A, Fuller J, Gaustad P,
515 Gonzalez GM, Guarro J, Lass-Florl C, Lockhart SR, Meis JF, Moore CB, Ostrosky-
516 Zeichner L, Pelaez T, Pukinskas SRBS, St-Germain G, Szeszs MW, Turnidge J. 2013.
517 Interlaboratory variability of Caspofungin MICs for Candida spp. Using CLSI and
518 EUCAST methods: should the clinical laboratory be testing this agent? Antimicrobial
519 agents and chemotherapy 57:5836–5842.

520 34. Hou X, Xiao M, Wang H, Yu SY, Zhang G, Zhao Y, Xu YC. 2018. Profiling of PDR1
521 and MSH2 in *Candida glabrata* Bloodstream Isolates from a Multicenter Study in
522 China. Antimicrob Agents Chemother 25:e00153–18.

523 35. Singh A. Healey KR, Yadav P, Upadhyaya G, Sachdeva N, Sarma S, Kumar A, Tarai
524 B, Perlin DS, Chowdhary A. 2018. Absence of azole or echinocandin resistance in
525 *Candida glabrata* isolates in India despite background prevalence of strains with
526 defects in DNA mismatch repair pathway. Antimicrob Agents Chemother 62:e00195–
527 18.

528 36. McCarty TP, Pappas PG. 2015. Invasive Candidiasis. Infect Dis Clin North Am
529 30:103–24.

Antimicrobial Agents and Chemotherapy

AAC

Antimicrobial Agents and Chemotherapy

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

Accepted Manuscript Posted Online

530 37. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L,
531 Reboli AC, Schuster MG, Vazquez JA, Walsh TJ, Zaoutis TE, Sobel JD. 2016. Clinical
532 Practice Guideline for the Management of Candidiasis : 2016 Update by the Infectious
533 Diseases Society of America. Clin Infect Dis 62:1–50.

534 38. Klotz U, Schmidt D, Willinger B, Steinmann E, Buer J, Rath PM, Steinmann J. 2016.
535 Echinocandin resistance and population structure of invasive *Candida glabrata* isolates
536 from two university hospitals in Germany and Austria. Mycoses 59:312–318.

537 39. Beyda ND, John J, Kilic A, Alam MJ, Lasco TM, Garey KW. 2014. FKS mutant
538 *Candida glabrata*: risk factors and outcomes in patients with candidemia. Clin Infect
539 Dis 59:819–825.

540 40. Kiraz N, Dag I, Oz Y, Yamac M, Kiremitci A, Kasifoglu N. 2010. Correlation between
541 broth microdilution and disk diffusion methods for antifungal susceptibility testing of
542 caspofungin, voriconazole, amphotericin B, itraconazole and fluconazole against
543 *Candida glabrata*. J Microbiol Methods 82:136–140.

544 41. Rodriguez L, Bustamante B, Huaroto L, Agurto C, Illescas R, Ramirez R, Diaz A,
545 Hidalgo J. 2017. A multi-centric Study of *Candida* bloodstream infection in Lima-
546 Callao, Peru: Species distribution, antifungal resistance and clinical outcomes. PLoS
547 ONE 12:1–12.

548 42. Bassetti M, Merelli M, Righi E, Diaz-Martin A, Rosello EM, Luzzati R, Parra A,
549 Trecarichi EM, Sanguinetti M, Posteraro B, Garnacho-Montero J, Sartor A, Rello J,
550 Tumbarello M. 2013. Epidemiology, species distribution, antifungal susceptibility, and
551 Outcome of Candidemia across Five Sites in Italy and Spain. J Clin Microbiol
552 51:4167–4172.

553 43. Bourgeois N, Laurens C, Bertout S, Balard Y, Krasteva D, Rispail P, Lachaud L. 2014.

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

Antimicrobial Agents and Chemotherapy
AAC
Antimicrobial Agents and Chemotherapy

Accepted Manuscript Posted Online

554 Assessment of caspofungin susceptibility of *Candida glabrata* by the Etest ®, CLSI,
555 and EUCAST methods, and detection of FKS1 and FKS2 mutations. Eur J Clin
556 Microbiol Infect Dis 33:1247-52.

557 44. Tortorano AM, Prigitano A, Lazzarini C, Passera M, Deiana ML, Cavinato S, De Luca
558 C, Grancini A, Lo Cascio G, Ossi C, Sala E, Montagna MT. 2013. A 1-year
559 prospective survey of candidemia in Italy and changing epidemiology over one decade.
560 Infection 41:655–662.

561 45. Xiang M-J, Liu J-Y, Ni P-H, Wang S, Shi C, Wei B, Ni Y-X, Ge H-L. 2013. Erg11
562 mutations associated with azole resistance in clinical isolates of *Candida albicans*.
563 FEMS Yeast Res 13:386–393.

564 46. Sagatova AA, Keniya MV, Wilson RK, Monk BC, Tyndall JD. 2015. Structural Insights
565 into Binding of the Antifungal Drug Fluconazole to *Saccharomyces cerevisiae*.
566 Antimicrob Agents Chemother 59:4982–4989.

567 47. Nabili M, Abdollahi Gohar A, Badali H, Mohammadi R, Moazeni M. 2016. Amino
568 acid substitutions in Erg11p of azole-resistant *Candida glabrata*: Possible effective
569 substitutions and homology modelling. J Glob Antimicrob Resist 5:42–46.

570 48. Perlin DS. 2015. Echinocandin Resistance in *Candida*. Clin Infect Dis 61:612–617.

571 49. Lewis JS 2nd, Wiederhold NP, Wickes BL, Patterson TF, Jorgensen JH. 2013. Rapid
572 Emergence of Echinocandin Resistance in *Candida glabrata* Resulting in Clinical and
573 Microbiologic Failure. Antimicrob Agents Chemother 57:4559–4561.

574 50. Dellière S, Healey K, Gits-muselli M, Carrara B, Barbaro A, Guigue N, Lecefel C,
575 Touratier S, Desnos-Ollivier M, Perlin DS, Bretagne S, Alanio A. 2016. Fluconazole
576 and Echinocandin Resistance of *Candida glabrata* Correlates Better with Antifungal

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

AAC Antimicrobial Agents and Chemotherapy

Accepted Manuscript Posted Online

577 Drug Exposure Rather than with MSH2 Mutator Genotype in a French Cohort of
578 Patients Harboring Low Rates of Resistance. Front Microbiol 7:1–9.

579 51. Lopez-Fuentes E, Gutiérrez-Escobedo G, Timmermans B, Van Dijck P, De Las Peñas
580 A, Castaño I. 2018. *Candida glabrata* 's Genome Plasticity Confers a Unique Pattern
581 of Expressed Cell Wall Proteins. J Fungi 4:E67.

582 52. Farmakiotis D, Kontoyiannis DP. 2017. Epidemiology of antifungal resistance in
583 human pathogenic yeasts: current viewpoint and practical recommendations for
584 management. Int J Antimicrob Agents 50:318–324.

585

586 **Table legends**

587 Table 1. Frequency of resistance to fluconazole in wild type and mutated strains for *PDR1*

588 Table 2. Frequency of resistance to fluconazole in wild type and mutated strains for *ERG11*.

589 Table 3. Antifungal susceptibility data derived from *C. glabrata* isolates in this study

590 Table 4. MIC distribution of fluconazole among genotypes of *C. glabrata*.

591 Table 5. Frequency of isolates with wild type and mutated *PDR1* profile along with their MIC values for
592 voriconazole

593

594 **Figure legends**

595 Figure 1.AFLP genotyping for studied strains of *C. glabrata*. Our isolates using AFLP clustered into nine
596 genotypes and each genotypes was distinctively color-coded.

597

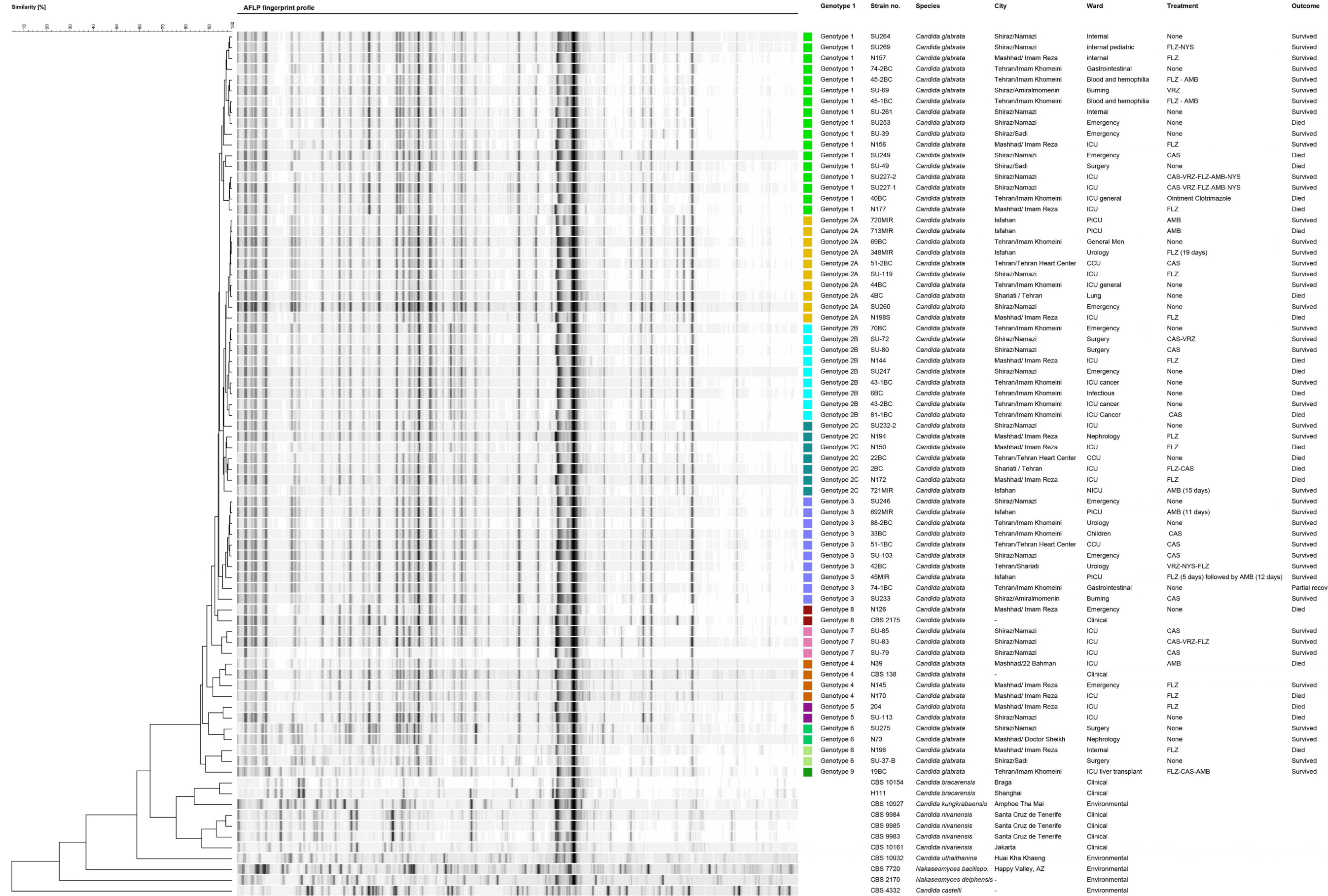
Antimicrobial Agents and Chemotherapy
AAC
Antimicrobial Agents and Chemotherapy
Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

Accepted Manuscript Posted Online

Antimicrobial Agents and Chemotherapy

AAC

Antimicrobial Agents and Chemotherapy



Genotype 1	Strain no.	Species	City	Ward	Treatment	Outcome
Genotype 1	SU264	*Candida glabrata*	Shiraz/Namazi	Internal	None	Survived
Genotype 1	SU269	*Candida glabrata*	Shiraz/Namazi	internal pediatric	FLZ-NYS	Survived
Genotype 1	N157	*Candida glabrata*	Mashhad/ Imam Reza	internal	FLZ	Survived
Genotype 1	74-2BC	*Candida glabrata*	Tehran/Imam Khomeini	Gastrointestinal	None	Survived
Genotype 1	45-2BC	*Candida glabrata*	Tehran/Imam Khomeini	Blood and hemophilia	FLZ - AMB	Survived
Genotype 1	SU-69	*Candida glabrata*	Shiraz/Amiralmomenin	Burning	VRZ	Survived
Genotype 1	45-1BC	*Candida glabrata*	Tehran/Imam Khomeini	Blood and hemophilia	FLZ - AMB	Survived
Genotype 1	SU-261	*Candida glabrata*	Shiraz/Namazi	Internal	None	Survived
Genotype 1	SU253	*Candida glabrata*	Shiraz/Namazi	Emergency	None	Died
Genotype 1	SU-39	*Candida glabrata*	Shiraz/Sadi	Emergency	None	Survived
Genotype 1	N156	*Candida glabrata*	Mashhad/ Imam Reza	ICU	FLZ	Survived
Genotype 1	SU249	*Candida glabrata*	Shiraz/Namazi	Emergency	CAS	Died
Genotype 1	SU-49	*Candida glabrata*	Shiraz/Sadi	Surgery	None	Died
Genotype 1	SU227-2	*Candida glabrata*	Shiraz/Namazi	ICU	CAS-VRZ-FLZ-AMB-NYS	Survived
Genotype 1	SU227-1	*Candida glabrata*	Shiraz/Namazi	ICU	CAS-VRZ-FLZ-AMB-NYS	Survived
Genotype 1	40BC	*Candida glabrata*	Tehran/Imam Khomeini	ICU general	Ointment Clotrimazole	Died
Genotype 1	N177	*Candida glabrata*	Mashhad/ Imam Reza	ICU	FLZ	Died
Genotype 2A	720MIR	*Candida glabrata*	Isfahan	PICU	AMB	Survived
Genotype 2A	713MIR	*Candida glabrata*	Isfahan	PICU	AMB	Died
Genotype 2A	69BC	*Candida glabrata*	Tehran/Imam Khomeini	General Men	None	Survived
Genotype 2A	348MIR	*Candida glabrata*	Isfahan	Urology	FLZ (19 days)	Survived
Genotype 2A	51-2BC	*Candida glabrata*	Tehran/Tehran Heart Center	CCU	CAS	Survived
Genotype 2A	SU-119	*Candida glabrata*	Shiraz/Namazi	ICU	FLZ	Survived
Genotype 2A	44BC	*Candida glabrata*	Tehran/Imam Khomeini	ICU general	None	Survived
Genotype 2A	4BC	*Candida glabrata*	Shariati / Tehran	Lung	None	Died
Genotype 2A	SU260	*Candida glabrata*	Shiraz/Namazi	Emergency	None	Survived
Genotype 2A	N198S	*Candida glabrata*	Mashhad/ Imam Reza	ICU	FLZ	Died
Genotype 2B	70BC	*Candida glabrata*	Tehran/Imam Khomeini	Emergency	None	Survived
Genotype 2B	SU-72	*Candida glabrata*	Shiraz/Namazi	Surgery	CAS-VRZ	Survived
Genotype 2B	SU-80	*Candida glabrata*	Shiraz/Namazi	Surgery	CAS	Survived
Genotype 2B	N144	*Candida glabrata*	Mashhad/ Imam Reza	ICU	FLZ	Died
Genotype 2B	SU247	*Candida glabrata*	Shiraz/Namazi	Emergency	None	Died
Genotype 2B	43-1BC	*Candida glabrata*	Tehran/Imam Khomeini	ICU cancer	None	Survived
Genotype 2B	6BC	*Candida glabrata*	Tehran/Imam Khomeini	Infectious	None	Died
Genotype 2B	43-2BC	*Candida glabrata*	Tehran/Imam Khomeini	ICU cancer	None	Survived
Genotype 2B	81-1BC	*Candida glabrata*	Tehran/Imam Khomeini	ICU Cancer	CAS	Died
Genotype 2C	SU232-2	*Candida glabrata*	Shiraz/Namazi	ICU	None	Survived
Genotype 2C	N194	*Candida glabrata*	Mashhad/ Imam Reza	Nephrology	FLZ	Survived
Genotype 2C	N150	*Candida glabrata*	Mashhad/ Imam Reza	ICU	FLZ	Died
Genotype 2C	22BC	*Candida glabrata*	Tehran/Tehran Heart Center	CCU	None	Died
Genotype 2C	2BC	*Candida glabrata*	Shariati / Tehran	ICU	FLZ-CAS	Died
Genotype 2C	N172	*Candida glabrata*	Mashhad/ Imam Reza	ICU	FLZ	Died
Genotype 2C	721MIR	*Candida glabrata*	Isfahan	NICU	AMB (15 days)	Survived
Genotype 3	SU246	*Candida glabrata*	Shiraz/Namazi	Emergency	None	Survived
Genotype 3	692MIR	*Candida glabrata*	Isfahan	PICU	AMB (11 days)	Survived
Genotype 3	88-2BC	*Candida glabrata*	Tehran/Imam Khomeini	Urology	None	Survived
Genotype 3	33BC	*Candida glabrata*	Tehran/Imam Khomeini	Children	CAS	Survived
Genotype 3	51-1BC	*Candida glabrata*	Tehran/Tehran Heart Center	CCU	CAS	Survived
Genotype 3	SU-103	*Candida glabrata*	Shiraz/Namazi	Emergency	CAS	Survived
Genotype 3	42BC	*Candida glabrata*	Tehran/Shariati	Urology	VRZ-NYS-FLZ	Survived
Genotype 3	45MIR	*Candida glabrata*	Isfahan	PICU	FLZ (5 days) followed by AMB (12 days)	Survived
Genotype 3	74-1BC	*Candida glabrata*	Tehran/Imam Khomeini	Gastrointestinal	None	Partial recovery
Genotype 3	SU233	*Candida glabrata*	Shiraz/Amiralmomenin	Burning	CAS	Survived
Genotype 8	N126	*Candida glabrata*	Mashhad/ Imam Reza	Emergency	None	Died
Genotype 8	CBS 2175	*Candida glabrata*	-	Clinical		
Genotype 7	SU-85	*Candida glabrata*	Shiraz/Namazi	ICU	CAS	Survived
Genotype 7	SU-83	*Candida glabrata*	Shiraz/Namazi	ICU	CAS-VRZ-FLZ	Survived
Genotype 7	SU-79	*Candida glabrata*	Shiraz/Namazi	ICU	CAS	Survived
Genotype 4	N39	*Candida glabrata*	Mashhad/22 Bahman	ICU	AMB	Died
Genotype 4	CBS 138	*Candida glabrata*	-	Clinical		
Genotype 4	N145	*Candida glabrata*	Mashhad/ Imam Reza	Emergency	FLZ	Survived
Genotype 4	N170	*Candida glabrata*	Mashhad/ Imam Reza	ICU	FLZ	Died
Genotype 5	204	*Candida glabrata*	Mashhad/ Imam Reza	ICU	FLZ	Died
Genotype 5	SU-113	*Candida glabrata*	Shiraz/Namazi	ICU	None	Died
Genotype 6	SU275	*Candida glabrata*	Shiraz/Namazi	Surgery	None	Survived
Genotype 6	N73	*Candida glabrata*	Mashhad/ Doctor Sheikh	Nephrology	None	Survived
Genotype 6	N196	*Candida glabrata*	Mashhad/ Imam Reza	Internal	FLZ	Died
Genotype 6	SU-37-B	*Candida glabrata*	Shiraz/Sadi	Surgery	None	Survived
Genotype 9	19BC	*Candida glabrata*	Tehran/Imam Khomeini	ICU liver transplant	FLZ-CAS-AMB	Survived
	CBS 10154	*Candida bracarensis*	Braga	Clinical		
	H111	*Candida bracarensis*	Shanghai	Clinical		
	CBS 10927	*Candida kungkrabaensis*	Amphoe Tha Mai	Environmental		
	CBS 9984	*Candida nivariensis*	Santa Cruz de Tenerife	Clinical		
	CBS 9985	*Candida nivariensis*	Santa Cruz de Tenerife	Clinical		
	CBS 9983	*Candida nivariensis*	Santa Cruz de Tenerife	Clinical		
	CBS 10161	*Candida nivariensis*	Jakarta	Clinical		
	CBS 10932	*Candida uthaithanina*	Huai Kha Khaeng	Environmental		
	CBS 7720	*Nakaseomyces bacilispo.*	Happy Valley, AZ	Environmental		
	CBS 2170	*Nakaseomyces delphensis*	-	Environmental		
	CBS 4332	*Candida castelli*	-	Environmental		

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

Accepted Manuscript Posted Online

Antimicrobial Agents and Chemotherapy

AAC

Antimicrobial Agents and Chemotherapy

Table 1. Frequency of resistance to fluconazole in wild type and mutated strains for *PDR1*.

Polymorphism in PDR1	**# of isolates along with their MIC values (µg/ml)**										**Total**
	≤0.5	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**≥256**	
WT				5	15	10	1				32
K67N					1						1
P68S, P135T, D235N						1	1				2
P76S, P145T, D243N*					3	1	1	1*			6
P117S				1							1
G128E						1					1
G128E, G493A					1						1
N162S						1					1
N162S, F944S					1						1
G189V					1						1
Y285N, T286A, K430M, T745A					1						1
K430M				2							2
K430M, E441K						1					1
K430M, L454P						1					1
K430M, T745A						1					1
K430M, G493A, T745A						1					1
E555K						1					1
G574S					1						1
T745A			1		3	2					6
T745A, C930R						1					1
A828T							1				
C930R				2		3	1				6
A1004C					1						1

* Only one of the isolates with this mutation (P76S, P145T, D243N) was resistant to fluconazole and the rest of isolates were 100% SDD to this drug.

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

Accepted Manuscript Posted Online

Antimicrobial Agents and Chemotherapy

AAC

Antimicrobial Agents and Chemotherapy

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

Table 2. Frequency of resistance to fluconazole in wild type and mutated strains for *ERG11*.

Polymorphism in ERG11	**# of isolates along with their MIC values (µg/ml)**										**Total**
	≤0.5	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**≥256**	
WT			1	7	18	13	5	1*			45
D196N					1						
N368T				2	3	7					12
N368T, H430P					1	1					2
N368T, K456R, G457C, V458F						1					1
N425I				1							1
H430P				1	4	2					7
K456R, G457C, V458F					1						1

* Only one of the *ERG11* wild-type isolates was fluconazole resistance and the rest of wild-type and *ERG11* mutated isolates were 100% SDD to this drug.

Table 3. Antifungal susceptibility data derived from *C. glabrata* isolates in this study

Antifungal drugs	MIC Values													Range	GM mean
	≤0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	≥64		
FLC								1	11	28	24	5	1	2-64	10.11
VRC			2	20	21	16	6	4	1		1			0.064-16	0.32
PSC		1	1	1	15	27	26							0.032-1	0.41
ITC			2	3	21	34	10	1						0.064-2	0.51
CASP				8	22	22	19							0.125-1	0.41
AMB					3	52	15	1						0.25-2	0.57

Accepted Manuscript Posted Online

AAC

Antimicrobial Agents and Chemotherapy

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

Accepted Manuscript Posted Online

Table 4. MIC distribution of fluconazole among genotypes of *C. glabrata*.

Genotypes	# of isolates along with their MIC values (µg/ml)										Total
	≤0.5	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**≥256**	
G1*				2	8	4	2	1*			17
G2 (A, B, and C)				4	9	12	1				26
G3				2	4	3	1				10
G4				1		2					3
G5					1	1					2
G6			1		3						4
G7				1	1	1					3
G8					1						1
G9					1						1

* Only one of the isolates within genotype 1 (G1) was resistant to fluconazole and the rest of isolates were SDD to this drug.

Antimicrobial Agents and Chemotherapy
AAC
Antimicrobial Agents and Chemotherapy
Downloaded from http://aac.asm.org/ on April 3, 2019 by guest

Accepted Manuscript Posted Online
Antimicrobial Agents and Chemotherapy
AAC
Antimicrobial Agents and Chemotherapy

Table 5. Frequency of isolates with wild type and mutated *PDR1* profile along with their MIC values for voriconazole

Polymorphism in PDR1	**<ECV%**	**>ECV%**	**# of isolates along with their MIC values (µg/ml)**										**Total**
			≤0.0625	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**≥32**	
WT	54.9%	45.1%	1	7	9	6	4	3			1		31
K67N	0.00%	100%						1					1
P68S, P135T, D235N	100%	0.00%		2									2
P76S, P145T, D243N	67.67%	33.33%		2	2	2							6
P117S	100%	0.00%		1									1
G128E	100%	0.00%			1								1
G128E, G493A	0.00%	100%				1							1
N162S	100%	0.00%			1								1
N162S, F944S	100%	0.00%		1									1
G189V	100%	0.00%			1								1
Y285N, T286A, K430M, T745A	100%	0.00%			1								1
K430M	100%	0.00%		1	1								2
K430M, E441K	100%	0.00%			1								1
K430M, L454P	100%	0.00%		1									1
K430M, T745A	0.00%	100%				1							1
K430M, G493A, T745A	100%	0.00%		1									1
E555K	0.00%	100%							1				1
G574S	100%	0.00%			1								1
T745A	50%	50%	1	1	1	2	1						6
T745A, C930R	0.00%	100%				1							1
A828T	0.00%	100%				1							1
C930R	67.67%	33.33%		2	2	1	1						6
A1004C	100%	0.00%			1								1

Downloaded from http://aac.asm.org/ on April 3, 2019 by guest