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Evaluation of Polymorphic Locus Sequence Typing for *Candida glabrata* Epidemiology

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The opportunistic yeast *Candida glabrata* is increasingly refractory to antifungal treatment or prophylaxis and relatedly is increasingly implicated in health care-associated infections. To elucidate the epidemiology of these infections, strain typing is required. Sequence-based typing provides multiple advantages over length-based methods, such as pulsed-field gel electrophoresis (PFGE); however, conventional multilocus sequence typing (targeting 6 conserved loci) and whole-genome sequencing are impractical for routine use. A commercial sequence-based typing service for *C. glabrata* that targets polymorphic tandem repeat-containing loci has recently been developed. These CgMT-J and CgMT-M services were evaluated with 56 epidemiologically unrelated isolates, 4 to 7 fluconazole-susceptible or fluconazole-resistant isolates from each of 5 center A patients, 5 matched pairs of fluconazole-susceptible/resistant isolates from center B patients, and 7 isolates from a center C patient who responded to then failed caspofungin therapy. CgMT-J and CgMT-M generated congruent results, resolving isolates into 24 and 20 alleles, respectively. Isolates from all but one of the center A patients shared the same otherwise rare alleles, suggesting nosocomial transmission. Unexpectedly, Pdr1 sequencing showed that resistance arose independently in each patient. Similarly, most isolates from center B also clustered together; however, this may reflect a dominant clone since their alleles were shared by multiple unrelated isolates. Although distinguishable by their echinocandin susceptibilities, all isolates from the center C patient shared alleles, in agreement with the previously reported relatedness of these isolates based on PFGE. Finally, we show how phylogenetic clusters can be used to provide surrogate parents to analyze the mutational basis for antifungal resistance.

Candida species are among the most common agents of health care-associated infections and, in particular, nosocomial bloodstream infections (1–8). At highest risk are those aged 65 and older, which is consistent with their increased likelihood of being diabetic, in the ICU, or on immunosuppressive or prolonged antibiotic therapies, all major risk factors for invasive yeast infection (4, 5). The species responsible for roughly half of these infections is *Candida albicans*, for which the source is predominantly endogenous, as evidenced by comparing genotypes of invasive isolates with those from mucosal isolates obtained before invasive infection (9–11). Endogenous transmission is consistent with the fact that *C. albicans* colonizes the mucosal epithelium in up to 70% of humans, which is mediated by multiple adherence mechanisms, yeast-hyphae dimorphism, secretion of hydrolytic enzymes, and biofilm formation (12).

These colonization factors are relatively deficient or absent in *Candida glabrata*, the second most likely cause of invasive yeast infection (4, 5, 13). However, *C. glabrata* exhibits intrinsically low susceptibility to azole antifungals and readily develops full resistance to these agents due to mutations in transcription factor Pdr1 (14–17). Since azoles such as fluconazole are widely used for prophylaxis or empirical therapy in high-risk patients (18), this could drive nosocomial transmission. Indeed, nosocomial transmission of *C. glabrata* has been repeatedly documented, although with various degrees of confidence (4, 11, 19–27). To make this connection, a strain-typing system is required that combines high resolution (diversity index of >0.9, where 1.0 represents 100% resolution) with reproducibility (facilitating comparisons over periods of months or longer) and, ideally, portability (facilitating comparisons to other labs and to strain databases).

Multiple approaches have been developed to type *C. glabrata* isolates (28). The most widely applied include (i) multilocus sequence typing (MLST), which analyzes 6 relatively conserved housekeeping loci for single nucleotide polymorphisms (SNPs) (29, 30), (ii) pulsed-field gel electrophoresis (PFGE), which compares total DNA banding patterns with or without restriction enzyme digestion (14, 23, 31), (iii) multilocus variable-number tandem-repeat analysis (MLVA, also known as microsatellite analysis), which examines length variation in 6 to 9 PCR-amplified loci that contain polymorphic tandem repeats (32–35), and (iv) random amplification of polymorphic DNA (RAPD), which compares banding patterns following PCR with a nonspecific primer (26, 36). In general, these methods are comparable in their strain resolution, achieving diversity indexes of ca. 0.9. However, these four approaches are also characterized by shared or unique limitations. First, most require facilities and expertise not generally available to clinical laboratories. Second, with the exception of MLST, which is sequence-based, the typing data are length-based,

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which has inherently lower reproducibility and portability. Third, these methods can be expensive, particularly MLST, due to the requirement for 6 sets of sequencing reactions. Finally, turnaround time can be slow, precluding real-time use for outbreak detection and investigation.

Here, we evaluate an approach to *C. glabrata* strain typing that directly addresses these limitations. This approach employs sequence-based typing and hence shares the high reproducibility and portability of MLST but targets tandem-repeat-containing loci rather than housekeeping loci. The inherently higher polymorphism of tandem repeats (due to DNA slippage during replication) is also exploited by MLVA, but sequencing can be considerably more informative than length analysis alone due to the complex patterns of insertions or deletions (indels) as well as SNPs. Thus, epidemiologically useful strain resolution can be achieved with a single sequencing reaction, reducing cost, complexity, and turnaround time. Furthermore, this approach can be outsourced to a commercial typing service, which eliminates the need for in-house facilities and expertise. Polymorphic locus sequence typing (PLST) is well established for bacterial pathogens, including *Staphylococcus aureus* (*spa* typing) and *Streptococcus pyogenes* (emm typing), and was recently introduced for *Listeria monocytogenes* (37–39). With respect to fungi, the polymorphic tandem repeat CSP has similarly been exploited to type isolates of *Aspergillus fumigatus*, a haploid mold (40, 41). Although PLST is largely precluded in *C. albicans* and related yeast due to their diploid, frequently heterozygous genomes (our unpublished data), we show here that it is well suited for typing haploid *C. glabrata*.

TABLE 1 *C. glabrata* strains used in this study

Strain(s)	Source
66032	American Type Culture Collection (Manassas, VA)
BG2	B. Cormack (Johns Hopkins University, Baltimore, MD)
20409	D. Diekema and M. Pfaller (U. Iowa, Iowa City, IA)
0016, 0037, 1437	S. Lockhart (CDC, Atlanta, GA)
34-031-010, 34-016-042, 34-028-512	J. Rex (University of Texas Health Science Center, Houston, TX)
CE-03, CE-06, CE-08, CE-14, CE-16, CE-18	D. Soll (University of Iowa, Iowa City, IA)
DPL23, DPL34, DPL36, DPL38, DPL39, DPL41	D. Perlin (University of Medicine and Dentistry of New Jersey, Newark, NJ)
107-798, 131-11625, 004-184, 102-11677,4771, 4719, 4743	M. Castanheira (JMI Laboratories, North Liberty, IA)
BWJ–BXM series (geographically diverse vaginal isolates)	S. Gygax (Medical Diagnostic Laboratories, Hamilton, NJ)
KM1–KM5 series (patients 1–5, center A)	K. Marr (Fred Hutchinson Cancer Research Center, Seattle, WA)
DSY patient-matched pairs and SFY92 (center B)	D. Sanglard (University Hospital Center, Lausanne, Switzerland)
LC-A–LC-G series (single patient, center C)	L. Cowen (University of Toronto, Ontario, Canada)

MATERIALS AND METHODS

Strains and culture. The strains used in this study and their sources are listed in Table 1. From frozen glycerol stocks, strains were streaked for isolation on yeast extract-peptone-dextrose (YPD) (1% yeast extract, 2% peptone, 2% dextrose) agar plates, with incubation at 35°C for 48 h. Fluconazole susceptibility or resistance (MIC of ≤32 μg/ml or ≥64 μg/ml, respectively) and the CRS-MIS phenotype (≥4-fold caspofungin reduced susceptibility and micafungin increased susceptibility) were determined by broth microdilution as previously described (15, 42).

Template preparation. To prepare lysates, single isolated colonies were suspended in 0.3 ml Tris-EDTA (TE) (10 mM Tris, 1 mM EDTA, pH 7.5) buffer in 1.5-ml tubes containing an equal volume of 0.5-mm glass beads. Tubes were vigorously shaken for 10 min, heated to 100°C for 10 min, and centrifuged 5 min to pellet beads and debris. To purify DNA, lysates were extracted with an equal volume of phenol-chloroform (24:1), and the aqueous layer was subjected to ethanol precipitation twice with final resuspension in 50 μl TE.

Amplification and sequencing. For typing, purified DNAs or lysates were transported to MicrobiType (Plymouth Meeting, PA) at ambient temperature. Tubes were briefly centrifuged, and 0.5-μl aliquots were used as the template in 15-μl PCR mixtures with *Taq* polymerase as recommended by the manufacturer (New England BioLabs, Ipswich, MA), for 30 (purified DNA) or 32 (lysate) cycles. Products (4 μl) were analyzed by 1% agarose gel electrophoresis and were visualized with blue light illumination after staining with SYBR Safe (Invitrogen, Grand Island, NY). For sequencing, products (1.5 μl) were treated with ExoSAP-IT as recommended by the manufacturer (Affymetrix, Santa Clara, CA), primer was added to 2 μM, and samples were submitted to Genewiz (South Plainfield, NJ). For CgMT-J and CgMT-M, amplification and sequencing primers (proprietary sequences; synthesized by IDT, Coralville, IA) were subjected to extensive laboratory testing to maximize sensitivity and specificity. For *C. glabrata* MLST, the primers described by Dodgson et al. (29) were used with minor modifications.

To identify Pdr1 substitutions in fluconazole-resistant KM isolates, lysates (1 μl) were used as the templates as described above but in 50-μl PCR mixtures. Primers were CgPDR1uF (5′-CTTCCATTACTTCGTACCCCA) and CgPDRc555R (5′-CGTCGAGAGCAAGCTGTTCT) for amplification of the N-terminal coding region and CgPDR1c432F (5′-AGAGAGAATACCGCAACCGTT) and CgPDR1dR (5′-ATACAGGCTATGCACACTGTC) for the C-terminal coding region. Following agarose gel confirmation and ExoSAP-IT treatment, aliquots were sequenced with the same and additional internal primers to span the full length of the 3,324-bp coding region. Substitutions relative to strain CBS138 were identified by BLAST analyses on the CGD website (www.candidagenome.org) and were confirmed by visual inspection of the sequence chromatograms. To characterize the 2,055-bp Ptp2 gene, the procedure above was modified by the use of primers CgPTP2uF (5′-TGGGATGCTGGATGTAGTGA) and CgPTP2dR (5′-CATGCTAGCTTTTCGAGCGA) for amplification and these and additional internal primers for sequencing. Amplification and sequencing of the Ifa38 gene was as previously described (42).

Bioinformatics. To identify candidate typing loci within the *C. glabrata* CBS138 genome, PatMatch on the CGD website (www.candidagenome.org) was employed with various simple tandem-repeat queries. BLASTN searches were conducted on the NCBI website (www.ncbi.nlm.nih.gov) against the nucleotide (strain CBS138) and whole-genome shotgun (strain M202019) databases. For phylogenetic analysis, sequences (edited as needed by visual inspection of chromatograms and trimmed to common termini) were aligned with Clustal Omega (www.ebi.ac.uk/Tools/msa/clustalo). Alignments were saved in PHYLIP format, analyzed using dnapars (PHYLIP version 3.69) with default parameters, and visualized as dendrograms using drawgram (http://evolution.genetics.washington.edu/phylip.html). Diversity index (Simpson dominance index) was calculated (www.alyoung.com/labs/biodiversity_calculator.html) using the formula $D = 1 - [\sum n(n-1)/N(N-1)]$, where n is the number of strains with a given allele and N is the total number of

FIG 1 Alignment of partial CgMT-M (A) and CgMT-J (B) sequences from representative *C. glabrata* strains.

(epidemiologically unrelated) strains. For MLST, sequences were trimmed to specified termini, and allele numbers and sequence types were determined with reference to available databases (http://cglabrata.mlst.net).

Nucleotide sequence accession numbers. CgMT-J and CgMT-M sequences representing each allele identified in this study have been submitted to GenBank with accession numbers KU172441 to KU172461 and KU172462 to KU172481, respectively.

RESULTS AND DISCUSSION

Identification of candidate loci for *C. glabrata* PLST. This project was initiated when the genome sequence for only one strain, CBS138, was available (43). PatMatch searches of this sequence on the CGD website (www.candidagenome.org) identified numerous simple tandem repeats (e.g., poly AC), which were screened for (i) proximity to other tandem repeats, (ii) location within an intergenic region, (iii) flanking regions likely to be conserved, and (iv) a total length of 0.5 to 1 kbp. Candidate loci were then evaluated by amplification and sequencing from small panels of unrelated strains, and CgMT-M (on chromosome M, upstream of CAGL0M00902g) emerged as the most promising. This locus was independently identified by Grenouillet et al. (35) as one of the four most informative targets within their MLVA scheme, which assesses length variation alone. A partial alignment of CgMT-M sequences from representative strains is shown in Fig. 1A, illustrating polymorphism in the form of indels and SNPs.

More recently, the partial genome sequence for a second *C. glabrata* strain, M202019, became available (GenBank accession number AYJS01000000). This sequence is highly similar to that for strain CBS138 (e.g., their CgMT-M loci are identical). This similarity was exploited by searching for rare regions of tandem-repeat-containing polymorphisms between the two genomes, which led to the identification of several candidate loci. Again, these loci were evaluated by amplification and sequencing from small panels of diverse strains. The most promising, CgMT-J (on chromosome J), represents the coding region for an uncharacterized protein (CAGL0J02530g) with central and C-terminal repeat regions; the latter represents the region of polymorphism between CBS138 and M202019. A partial alignment of CgMT-J sequences from representative strains is shown in Fig. 1B, illustrating polymorphism primarily in the form of indels but also several SNPs.

Phylogenetic analyses of CgMT-J and CgMT-M sequences. To evaluate CgMT-J and CgMT-M for epidemiological studies, 104 total strains (Table 1) were assembled that include (i) 59 epidemiologically unrelated mucosal (primarily vaginal) or bloodstream isolates, (ii) 27 fluconazole-susceptible or fluconazole-resistant isolates from 5 patients at medical center A, (iii) 11 fluconazole-susceptible or fluconazole-resistant isolates from 6 patients at medical center B, and (iv) 7 sequential isolates exhibiting stepwise decreases in caspofungin susceptibility from one patient at medical center C. All strains yielded sequenceable prod-

ucts for the two loci, with the exception of the DSY2253-DSY2254 pair from one center B patient, which did not yield a CgMT-J product despite repeated attempts with different primer combinations. Although most amplifications employed purified DNA templates, crude lysates (generated by vigorously shaking a colony suspension with glass beads following by heating to 100°C) also yielded high-quality sequence chromatograms (not shown).

All CgMT-J and CgMT-M sequences, including those from strains CBS138 and M202019 downloaded from NCBI databases, were aligned and phylogenetically analyzed using dnapars (PHYLIP), which weighs indels and SNPs. From the resulting dendrograms (Fig. 2 and 3), it is apparent that the two loci yield largely congruent results. Specifically, the two loci yielded 10 clusters (i.e., sharing identical or nearly identical sequences) of 2 to 21 strains, with equivalent or nearly equivalent sets of strains for CgMT-J and CgMT-M. For three examples, cluster N (defined by strain BWN) includes the equivalent set of 21 strains in the two dendrograms, cluster V (BWV) includes the equivalent set of 11 strains, and cluster J (BWJ) includes the equivalent set of 5 strains. Note that cluster C, defined by strain CE-14, is unambiguous by CgMT-M but less so by CgMT-J. With respect to singletons, two strains (CE-06 and BXG) and one matched pair (DSY738/DSY739) were identified as such by CgMT-J and CgMT-M (Fig. 2 and 3). In total, CgMT-J and CgMT-M yielded 24 (including the DSY2253-DSY2254 null) and 20 alleles, respectively. Correspondingly, the diversity indexes (excluding epidemiological replicates; see below) were 0.94 and 0.91. These values are comparable to those obtained with other typing methods and strain sets (14, 24, 32, 34, 35).

As expected, resolution was enhanced by combining CgMT-J and CgMT-M loci, i.e., concatenating their sequences and repeating the alignment and phylogenetic analysis. Specifically, CgMT-J/M typing yielded 32 total alleles and a diversity index of 0.95. The CgMT-J/M dendrogram (not shown) demonstrates equivalent strain clusters as CgMT-J and CgMT-M (Fig. 2 and 3), which is consistent with the congruency of the individual loci as noted above.

Comparison of PLST to MLST and other typing methods. The 6 CE isolates typed here by PLST were previously typed by Dodgson et al. (29) using MLST, RAPD, and Southern blot analysis with repetitive sequence-containing probes Cg6/Cg12. By PLST, CE-03, CE-16, and CE-18 are indistinguishable (Fig. 2 and 3). Consistent with this, all three were typed to group I by MLST, RAPD, and Cg6/Cg12, although CE-03 was assigned a distinct sequence type due to differences in one of the 6 loci (29). Strains CE-08 and CE-14 are closely related or identical by CgMT-J and CgMT-M, respectively (Fig. 2 and 3). Similarly, the two strains typed to group V by MLST (with identical sequence type), RAPD, and Cg6/Cg12 (29). These three methods also typed CE-06 to group V; in contrast, it is a singleton by PLST (Fig. 2 and 3).

Since cluster K includes one or both of the *C. glabrata* strains (CBS138 and M202019) with sequenced genomes, it was selected for further comparison of CgMT-J and CgMT-M typing to MLST. For the remaining 8 strains in this cluster, the 6 MLST loci (29) were amplified, sequenced, and trimmed to the appropriate termini. Allele numbers and sequence type were determined with reference to the *C. glabrata* MLST database (http://cglabrata.mlst.net). This analysis revealed that all but one of the 10 cluster K strains are sequence type 15 (ST15); the exception is strain 34-016-042, which is ST26. In comparison, cluster K is resolved into 3 types by CgMT-J and CgMT-M (Fig. 2 and 3) and into 4 types by CgMT-J/M (not shown).

Epidemiological analyses of clinical isolates from medical centers A, B, and C. As a national referral center for hematopoietic stem cell transplantation, center A receives patients from geographically diverse locations. These patients are at high risk for *Candida* infection and were routinely prophylaxed or treated with fluconazole during the period when the isolates tested here were collected (14). Four of the 5 center A patients—KM1, KM2, KM4, and KM5—yielded multiple isolates that shared the same CgMT-J and CgMT-M alleles (Fig. 2 and 3). This was unexpected since these alleles are otherwise rare, shared by only one noncenter A strain (BWP, a vaginal isolate from a Michigan patient), which defines cluster P. Patients KM4 and KM5 were additionally infected with unrelated strains typing to clusters V and S, respectively. Together, these results strongly suggest nosocomial transmission to, or between, these 4 patients. Additionally, their cluster P isolates were all (KM4 and KM5) or in part (KM1 and KM2) fluconazole resistant. Thus, it was hypothesized that this transmission was driven by selection for a fluconazole-resistant clone. However, analysis of the resistance mechanisms in these strains demonstrated that this was not the case. Resistance is generally mediated by mutations in Pdr1, the transcription factor regulating expression of multidrug transporter genes, particularly *CDR1* (15–17). By comparing Pdr1 sequences from fluconazole-susceptible and fluconazole-resistant cluster P isolates (see also below), the mutation responsible for resistance was deduced to be Asn283Ile in the KM1 isolates, Gly348Asp in the KM2 isolate, Trp780Gly in the KM4 isolates, and Lys274Glu in the KM5 isolates (or, in isolate KM5-67, a Lys274Glu Ile373Asn double mutation). The fluconazole-resistant KM4 isolates typing to cluster V had a distinct Pdr1 mutation, Gly558Cys. Thus, transmission preceded fluconazole resistance, which was selected independently in each patient. This is consistent, in retrospect, with isolation of susceptible strains from two different patients (KM1 and KM2).

With respect to the DSY strains from center B, each pair of fluconazole-susceptible and fluconazole-resistant isolates from 5 different patients shared the same CgMT-J and CgMT-M alleles, which is consistent with their identification as matched pairs (17). It is also apparent that 3 of these DSY pairs, along with strain DSY562 (parent of strain SFY92 analyzed here), are identical to one another as well, falling within cluster N (Fig. 2 and 3). This again suggests the possibility of nosocomial transmission followed by selection for fluconazole resistance. In contrast to the situation with center A, however, cluster N does not represent a rare allele, since 8 additional epidemiologically unrelated strains share this allele. Thus, the relatedness of the center B isolates may reflect a dominant regional clone rather than nosocomial transmission. More definitive typing data, perhaps from whole-genome sequencing, are required to resolve this.

Although they were collected over a period of 10 months and are distinguishable by their echinocandin susceptibilities (31), all 7 sequential isolates (LC-A through LC-G) from the center C patient shared CgMT-J and CgMT-M alleles (Fig. 2 and 3). This agrees with their previously reported relatedness based on PFGE and, for the first and final isolates, whole-genome sequence-based SNP analysis (31). (As is often the case with "whole-genome sequences" generated by next-generation sequencing technologies, many of their tandem repeat regions, including the CgMT-J and CgMT-M loci, are incomplete.)

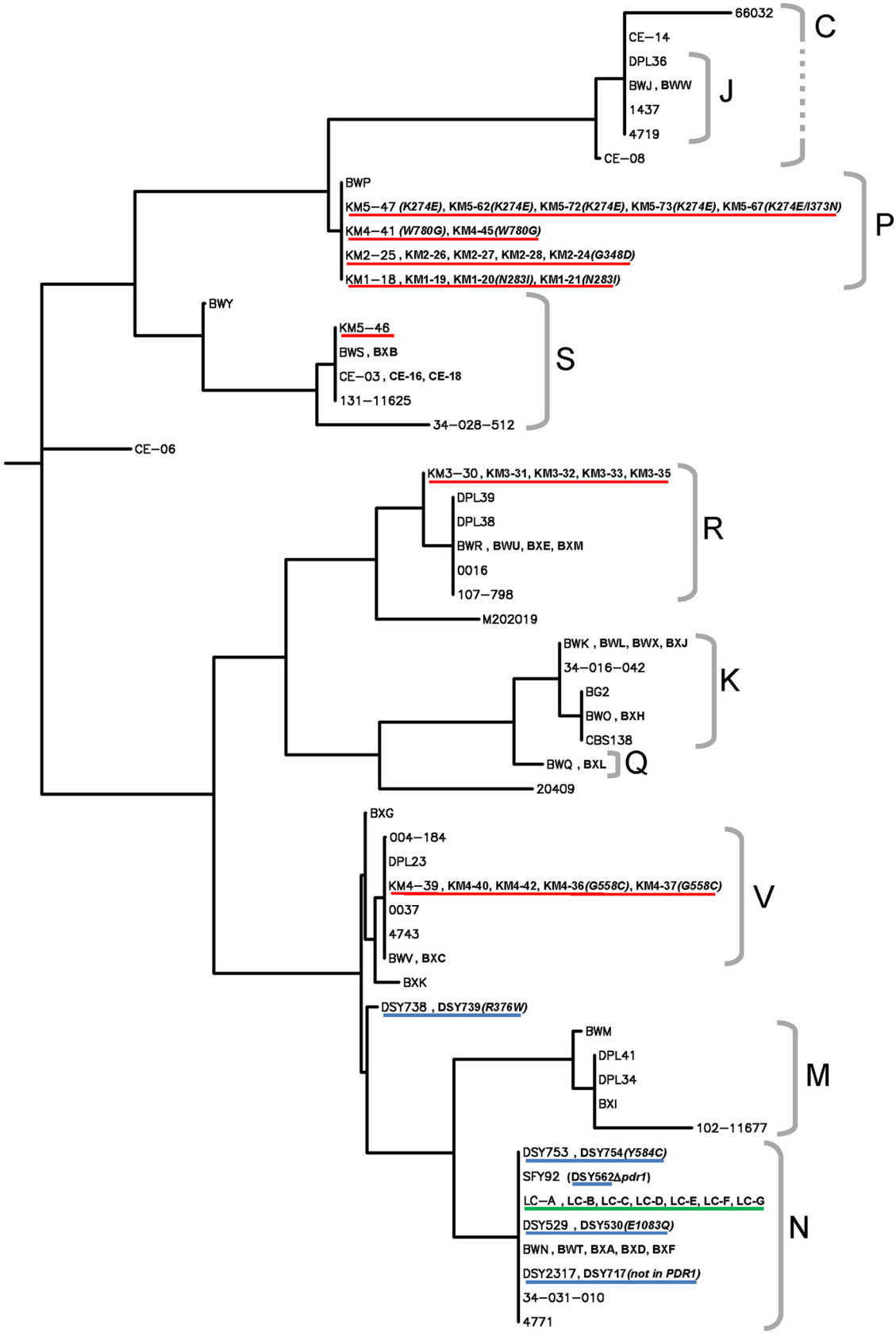

FIG 2 DNA parsimony-based dendrogram of CgMT-J sequences from *C. glabrata* strains derived from center A (KM, red underline), center B (DSY, blue underline), and center C (LC, green underline); all other strains were derived from diverse, unrelated sources. Also included are corresponding sequences from NCBI database strains CBS138 and M202019. Brackets indicate clusters with identical or nearly identical sequences, labeled according to the isolate defining that cluster (e.g., P for BWP). DSY (17) and KM (data not shown) isolates are fluconazole susceptible (no following parentheses) or resistant (following parentheses indicating the Pdr1 mutation in single letter amino acid code; e.g., *K274E* indicates a Lys to Glu mutation at Pdr1 residue 274).

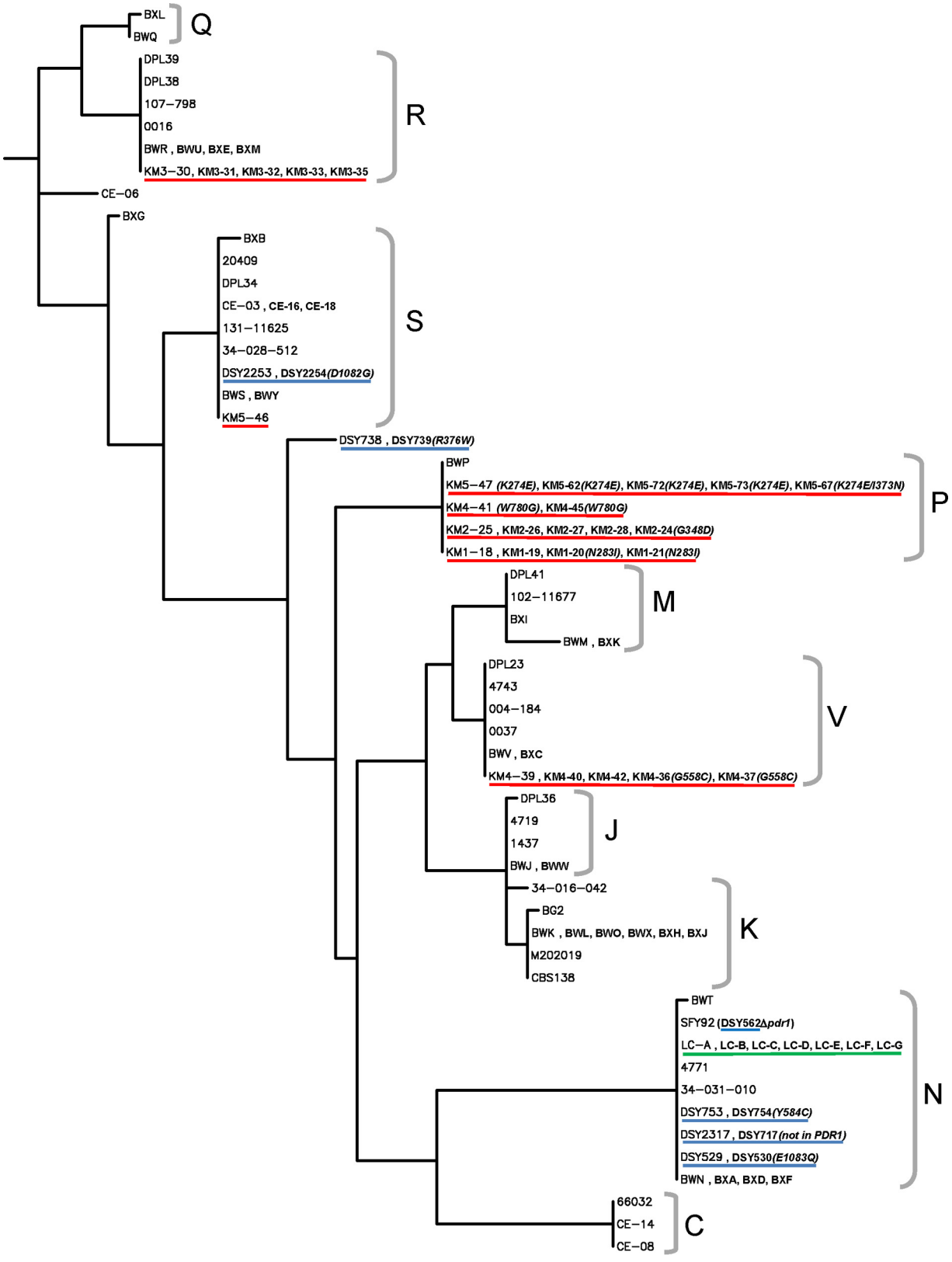

FIG 3 DNA parsimony-based dendrogram of CgMT-M sequences (see Fig. 2 legend for details).

Use of surrogate parents for mutational analysis of antifungal resistance. From center A patient KM5, there was one azole-susceptible isolate (vaginal isolate KM5-46), but CgMT-J and CgMT-M analyses showed it was unrelated to the 5 resistant KM5 isolates (from mouth, blood, and lung). Sequence analysis of the Pdr1 gene from each of these resistant KM5 isolates revealed 6 amino acid substitutions relative to Pdr1 from cluster K strain CBS138, the only strain with a completed genome. To deduce which if any of these might be responsible for resistance, the resistant KM5 Pdr1 sequence was compared to the Pdr1 sequences of susceptible isolates from patients KM1 and KM2, which were similarly typed by CgMT-J and CgMT-M to cluster P (Fig. 2 and 3). These surrogate parents shared all but one of the substitutions, specifically, Lys274Glu. In support of its role in resistance, the Lys274Glu substitution is within a Pdr1 region in which multiple other resistance-conferring mutations have been mapped (17), while the 5 other substitutions (between residues Ser76 and Thr143) are upstream of any known resistance-conferring mutation.

C. glabrata clinical isolate 34-028-512 (cluster S) is fluconazole resistant and exhibits *CDR1* upregulation (15). As is typical of other resistant strains (16, 17), resistance in 34-028-512 was reversed in a Pdr1 gene disruptant; however, sequence analysis revealed wild-type Pdr1, and resistance was also reversed by disruption of the gene encoding mitogen-activated protein (MAP) kinase Slt2 (data not shown). The latter effect, which is not observed in strains with Pdr1 mutations, suggests that resistance in 34-028-512 is conferred by a mutation activating Slt2, which then activates Pdr1. Sequencing showed that Slt2 itself was wild type, but the substitution Gly567Ala (relative to CBS138) was identified in protein phosphatase Ptp2 (CAGL0L02827g) that, in *Saccharomyces cerevisiae*, regulates Slt2 activity. As in CBS138, BLAST analysis showed that the equivalent residue is Gly in all four *C. glabrata*-related species with partially sequenced genomes (e.g., *Candida bracarensis* and *Nakaseomyces delphensis*); thus, it seemed likely that 34-028-512 had mutated Ptp2. Since its parent strain was not available, the Ptp2 gene was sequenced from cluster S surrogate parents CE-03, CE-18, and BWS (all of which are fluconazole susceptible). Ala567 was common to all, which we consequently conclude is a polymorphism rather than a resistance-conferring mutation.

As a final example of how typing can provide surrogate parents for mutational analysis of antifungal resistance, we recently described three *C. glabrata* clinical strains that exhibit caspofungin reduced susceptibility but micafungin increased susceptibility (CRS-MIS), and we subsequently identified substitutions (again relative to CBS138) in their sphingolipid biosynthesis genes (42). For two of these, complementation experiments confirmed that their Fen1 substitutions were mutations responsible for their CRS-MIS phenotype. For the third, strain 4743, tentative support for the role of an Ile339Met substitution in Ifa38 was obtained by sequencing its gene from 7 diverse *C. glabrata* strains, all of which encoded Ile339 as in CBS138 (42). However, in retrospect, none of the sequenced strains were from cluster V, which includes 4743 (Fig. 2 and 3), and so here we sequenced the Ifa38 gene from surrogate parent strains BWV, BXC, and DPL23 (none of which exhibit the CRS-MIS phenotype). All three encode Met339, and thus this substitution represents a polymorphism rather than a CRS-MIS-conferring mutation.

Conclusions. Health care-associated infections may be endogenous in origin or nosocomially transmitted, and the only way to distinguish between these two is through strain typing. Currently, however, strain typing is rarely pursued by clinical laboratories, at least in part due to the technical complexities and costs associated with established methods, including MLST, PFGE, and MLVA, or emerging methods such as whole-genome sequencing. These issues were directly addressed here, with respect to the opportunistic yeast *C. glabrata*, by using sequence-based typing services that target the polymorphic, tandem-repeat-containing loci CgMT-J and CgMT-M. The PLST protocol was robust, yielding high-quality sequencing chromatograms for nearly all of the >200 amplifications analyzed here. Furthermore, samples required minimal preparation—glass bead disruption followed by heating to 100°C sufficed—and were subsequently shipped without biohazard packaging, facilitating the commercial outsourcing of the amplification and sequencing steps. CgMT-J and CgMT-M yielded largely congruent results despite representing distinct chromosomes and coding versus noncoding loci. This congruence indicates that these single loci provide reliable phylogenetic information, and it confirms that *C. glabrata* is a predominantly clonal species in which sexual reproduction and horizontal gene transfer have contributed minimally to its evolution (30, 33, 43). On the other hand, this clonality means that strain resolution can be limiting; this is illustrated by large clusters such as N that include geographically diverse isolates (3 countries and at least 5 U.S. states). Additional typing loci that resolve these clusters are currently being sought. Nevertheless, CgMT-J and CgMT-M analyses provided strain resolution that was sufficient to strongly suggest nosocomial transmission of *C. glabrata* between patients at one center and its possible role at another. Extension of PLST to other health care-associated pathogens warrants investigation.

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