New Locus for *Candida glabrata* Sequence-Based Strain Typing Provides Evidence for Nosocomial Transmission

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Health care-associated infections with the opportunistic yeast *Candida glabrata* have become increasingly common in the fluconazole era and may be endogenous or nosocomial in origin. Effective intervention requires distinguishing between these sources, which in turn requires epidemiological analysis in conjunction with isolate typing (1–4). Multiple approaches to *C. glabrata* typing have been described, ranging from length-based analyses, including microsatellite and pulsed-field gel electrophoresis, to single nucleotide polymorphism (SNP)-based analyses, including multilocus sequence typing and whole-genome sequencing (5, 6). Issues of cost and technical complexity, however, have precluded the routine use of these approaches in clinical laboratories. Polymorphic locus sequence typing (PLST) addresses these issues by employing conventional PCR and Sanger sequencing of one or two selected tandem-repeat loci that exhibit high rates of both insertions/deletions and SNPs. In our previous study, PLST loci CgMT-J and CgMT-M resolved 104 *C. glabrata* isolates into 10 phylogenetic clusters and 20 to 24 alleles (7).

Although providing relatively modest resolution (Simpson's diversity index [DI], 0.92 to 0.94), CgMT-J/CgMT-M PLST importantly identified one cluster, labeled P, that included multiple isolates from four different patients undergoing hematopoietic stem cell transplantation at the same medical center, suggesting nosocomial transmission (7). However, this cluster also included the unrelated strain BWP, weakening this assertion. An additional CgMT-J/CgMT-M cluster, labeled N, again included isolates from three different patients at a single center, but no conclusion could be drawn regarding their epidemiological connection because cluster N included at least 7 unrelated strains.

CgMT-J/CgMT-M was identified by bioinformatic analysis of the only two *C. glabrata* genome sequences available at the time, for closely related strains CBS138 and M202019 (7). Because current GenBank databases include sequences for 15 additional strains, including 4 epidemiologically related pairs, bioinformatic analysis for new PLST loci exhibiting greater strain resolution was warranted. Tandem repeats (<http://tandem.bu.edu/>) within CBS138 chromosome sequences, plus 500 flanking nucleotides, were used as queries in BLASTN screens of GenBank databases. This identified locus CgMT-C (DI, 0.98), representing an intergenic region on chromosome C (nucleotides 514273 to 515201, GenBank accession no. [CR380949](https://doi.org/10.1128/JCM.02933-20)), which includes multiple tandem repeats and displays polymorphism in the form of both insertions/deletions and SNPs.

Phylogenetic analysis of GenBank sequences resolved 12 of 13 strains with intact CgMT-C loci (Fig. 1) (sequences for the 4 remaining strains were unassembled across the longer repeat, a not uncommon limitation of genome sequencing technologies). Epidemiologically related strains were, however, clustered, e.g., recurrent infection isolates 1A/B and 2A/B (8). In the laboratory, the CgMT-C locus was readily amplified and sequenced (see Fig. 1 legend for primer sequences and reference 7 for methods) directly from *C. glabrata* colony lysates, including those prepared from chromogenic

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FIG 1 Dendrogram of *C. glabrata* CgMT-C sequences, extracted from GenBank databases (GB/), or amplified and sequenced with primers CgMT-CF (5'-CATTTACTCAAAGAATAACAATTGCCA) and CgMT-CR (5'-CTGCGTTCCATGAACAGATAG) by previously described methods (7). Blue brackets, phylogenetic clusters; red bars, epidemiological clusters. All strains within clusters N (N/) and P (P/) along with representative strains from the remaining 8 clusters (e.g., Q/) were included in the analysis. Sequences were aligned with Clustal Omega (www.ebi.ac.uk/Tools/msa/clustalo); alignments were saved in PHYLIP format, analyzed using dnaphars with default parameters, and visualized using drawgram (<http://evolution.genetics.washington.edu/phyip.html>).

agars. Most important, sequence analysis of CgMT-C loci from the multiple KM (cluster P) and DSY (cluster N) patient isolates demonstrated center-specific identities, while resolving them from all unrelated strains (Fig. 1), consistent with nosocomial transmission.

In addition to outbreak detection and investigation, typing is required to distinguish relapse from reinfection in patients with recurrent *C. glabrata* infection (9). Our data set included multiple isolates (LC-A, LC-C, and LC-G) from a single patient collected over 10 months that varied in their antifungal susceptibility; consistent with previous studies (10), CgMT-C typing supports relapse associated with development of resistance-conferring mutations (Fig. 1).

Data availability. The DNA sequence data reported in this study have been deposited in GenBank under accession no. [MW234312](https://doi.org/10.1093/mbe/mz001) to [MW234338](https://doi.org/10.1093/mbe/mz001).

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