Fluconazole-Resistant Candida parapsilosis Bloodstream Isolates with Y132F Mutation in ERG11 Gene, South Korea

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We recently observed the emergence of fluconazole-resistant Candida parapsilosis bloodstream isolates harboring a Y132F substitution in Erg11p in South Korea. These Y132F isolates had a higher propensity to cause clonal transmission than other fluconazole-resistant isolates and persisted within hospitals for several years, as revealed by microsatellite typing.

Candida parapsilosis is the second most common species isolated from patients with Candida bloodstream infections (BSIs) in Latin America and eastern Asia (1,2). Although uncommon, fluconazole-resistant C. parapsilosis isolates harboring the Y132F substitution in Erg11p (referred to as Y132F isolates) have been reported in Brazil, the United States, and Kuwait (3–6). The precise reason for the emergence of C. parapsilosis Y132F isolates has yet to be defined; it may be related to selective drug pressure, and the mutation at position 132 may be a hot spot for resistance mediated by ERG11, a gene encoding the azole target (3). Alternatively, C. parapsilosis Y132F isolate emergence may be associated with exogenous clonal transmission (4). We recently observed the emergence and nosocomial spread of Y132F isolates in South Korea. In this study, we report a greater increase in the clonal spread of C. parapsilosis Y132F BSI isolates than of non-Y132F fluconazole-resistant isolates within hospitals during the past several years.

We assessed the first 47 C. parapsilosis BSI isolates that were fluconazole-resistant (MIC ≥8 mg/L) according to the Clinical and Laboratory Standards Institute (CLSI) species-specific clinical breakpoint (7,8). All 47 isolates were obtained from multicenter surveillance cultures from 8 university hospitals (A–H) during 2005–2016. For all fluconazole-resistant isolates, we examined genotypic relationships using microsatellite typing. We defined ≥2 isolates with identical genotypes according to microsatellite typing as clonal isolates. We sequenced the ERG11 gene and 3 transcription factor genes: TAC1, which can lead to the upregulation of CDR; MRR1, which can lead to the upregulation of MDR; and UPC2, which can lead to the upregulation of ERG11 (5); we compared the results with those of 20 fluconazole-susceptible (MIC 0.5–2 mg/L) isolates. This study was approved by the Institutional Review Board of Chonnam National University Hospital (IRB CNUH-2014-290).

Of the 47 C. parapsilosis fluconazole-resistant isolates, 30 (63.8%) had the Y132F substitution in Erg11p; however, none of the 20 fluconazole-susceptible isolates had the Y132F mutation in ERG11. Recently, 31%–57% of fluconazole-resistant C. parapsilosis isolates from different parts of the world were reported to be Y132F isolates, but the Y132F mutation was absent in all fluconazole-susceptible isolates (3–6). These data confirm that a Y132F substitution in Erg11p is the predominant fluconazole resistance mechanism for C. parapsilosis worldwide.

Microsatellite typing revealed that 4 clonal Y132F isolates (M1–4) were persistently recovered in 2 hospitals (A and B) over a period of 3–7 years, and the proportion of clonal isolates was much higher in Y132F isolates (86.7%, 26/30) than in non-Y132F fluconazole-resistant isolates (11.8%, 2/17) (Table). In a previous microsatellite study from a US surveillance study by Grossman et al. (4), no hospital specificity was detected among 13 non-Y132F fluconazole-resistant isolates; however, 2 notable clusters of isolates from 17 Y132F isolates were found over 8- or 18-month periods. The results obtained in our study and those of Grossman et al. indicate that Y132F isolates may have a higher propensity to cause clonal transmission and persist in particular hospitals than do non-Y132F fluconazole-resistant isolates. The Y132F substitution in Erg11p has also been detected in C. auris isolates from Pakistan (10/16 isolates), India (12/17 isolates), and Venezuela (5/5 isolates); these isolates are strongly associated with clonal transmission (9). Further studies are needed to determine whether the Y132F mutation in Erg11p has a direct effect on clonal transmission of C. parapsilosis or C. auris isolates.

Two previous studies conducted in the United States detected the Erg11p Y132F substitution in combination with the Erg11p R398I substitution in almost all C. parapsilosis BSI isolates (4,5). In addition, no Y132F isolates detected in a US surveillance study contained an MRR1 polymorphism, according to MRR1 sequence analysis.
Table. Molecular characterization of 47 fluconazole-resistant isolates and 20 fluconazole-susceptible isolates of *Candida parapsilosis*, South Korea*

| Microsatellite genotypes† | Hospital | No. isolates | MICs, mg/L‡ | Amino acid substitutions§ | Isolation year (no. patients) |
|--------------------------|----------|--------------|-------------|---------------------------|-----------------------------|
| **Fluconazole-resistant with Y132F in Erg11p, n = 30 isolates** | | | | | |
| M1                       | A        | 8            | FLC 8–32, VRC 0.25–0.5 | Y132F K177N               | 2006 (1), 2009 (1), 2010 (2), 2011 (2), 2012 (1), 2013 (1) |
| B                        | 3        | 16–32, 0.5   | Y132F K177N               | 2012 (2), 2013 (1)      |
| M2                       | A        | 10           | FLC 8–32, VRC 0.125–0.5 | Y132F K177N, Q1053*      | 2012 (1), 2016 (9)         |
| M3                       | A        | 3            | FLC 8–16, 0.25           | Y132F K177N, Q1053*      | 2007 (1), 2011 (1), 2012 (1) |
| M4                       | A        | 2            | FLC 8–64, 0.5–4          | Y132F K177N              | 2013 (1), 2016 (1)         |
| M5                       | A        | 1            | 32, 0.25                 | Y132F K177N              | 2012 (1)                   |
| M6                       | A        | 1            | 8, 0.5                   | Y132F K177N              | 2013 (1)                   |
| M7                       | A        | 1            | 8, 0.25                  | Y132F K177N              | 2016 (1)                   |
| M8                       | C        | 1            | 64, 2                    | Y132F                   | 2016 (1)                   |
| **Other fluconazole-resistant, n = 17 isolates** | | | | | |
| M9                       | D        | 2            | >64, 1                   | G583R                    | 2007 (1), 2009 (1)         |
| M10                      | E        | 1            | 16, 0.5                  | R398I L877P              | 2005 (1)                   |
| M11                      | E        | 1            | 8, 0.25                  | R398I L877P              | 2006 (1)                   |
| M12                      | E        | 1            | 8, 0.25                  | R398I L877P              | 2011 (1)                   |
| M13                      | F        | 1            | 16, 0.06                 | R398I L877P              | 2011 (1)                   |
| M14                      | E        | 1            | 8, 0.125                 | R398I L877P              | 2012 (1)                   |
| M15                      | G        | 1            | 8, 0.125                 | R398I L877P              | 2012 (1)                   |
| M16                      | G        | 1            | 8, 0.06                  | R398I L877P              | 2012 (1)                   |
| M17                      | E        | 1            | 8, 0.125                 | N900D                    | 2012 (1)                   |
| M18                      | C        | 1            | 8, 0.125                 | R398I P250S L877P        | 2012 (1)                   |
| M19                      | C        | 1            | 8, 0.25                  | R398I S1081P L877P       | 2012 (1)                   |
| M20                      | D        | 1            | 8, 0.125                 | R398I D394N              | 2012 (1)                   |
| M21                      | E        | 1            | 32, 0.5                  | R398I P295R L877P        | 2015 (1)                   |
| M22                      | E        | 1            | 16, 0.125                | R398I                   | 2015 (1)                   |
| M23                      | H        | 1            | 32, 0.125                | K128N W872C              | 2015 (1)                   |
| M24                      | E        | 1            | 16, 0.25                 | G927D                    | 2016 (1)                   |
| **Fluconazole-susceptible controls** | | | | | |
| M3                       | A        | 1            | 1, 0.03                  | K177N, Q1053*            | 2010 (1)                   |
| M25                      | C        | 2            | 0.5, 0.03                | R398I                   | 2012 (2)                   |
| M26                      | F        | 2            | 0.5, 0.03–0.06           | R208G                   | 2012 (1), 2013 (1)         |
| M27                      | A        | 1            | 2, 0.06                 | R208G                   | 2010 (1)                   |
| M28                      | A        | 1            | 0.5, 0.03                | K177N, Q1053*            | 2011 (1)                   |
| M29                      | A        | 1            | 1, 0.06                 | L877P                   | 2011 (1)                   |
| M30                      | A        | 1            | 1, 0.03                 | R208G                   | 2012 (1)                   |
| M31                      | A        | 1            | 0.5, 0.03               | R208G                   | 2012 (1)                   |
| M32                      | E        | 1            | 2, 0.03                 | R208G                   | 2012 (1)                   |
| M33                      | G        | 1            | 0.5, 0.03               | R208G                   | 2012 (1)                   |
| M34                      | G        | 1            | 0.5, 0.03               | R208G                   | 2012 (1)                   |
| M35                      | A        | 1            | 0.5, 0.03               | R208G                   | 2013 (1)                   |
| M36                      | A        | 1            | 1, 0.06                | R398I D394N              | 2013 (1)                   |
| M37                      | A        | 1            | 0.5, 0.03               | R398I                   | 2013 (1)                   |
| M38                      | D        | 1            | 0.5, 0.06               | R398I                   | 2014 (1)                   |
| M39                      | D        | 1            | 0.5, 0.06               | R208G                   | 2014 (1)                   |
| M40                      | E        | 1            | 0.5, 0.03               | R208G                   | 2015 (1)                   |
| M41                      | A        | 1            | 1, 0.03                | R398I L877P              | 2015 (1)                   |

*Clinical and Laboratory Standards Institute; FLC, fluconazole; VRC, voriconazole.
†For microsatellite typing, each strain was characterized by a genotype resulting from combination of the sizes of the 4 markers (CP1, CP4, CP6, and B). See the Technical Appendix Figure (https://wwwnc.cdc.gov/EID/article/24/9/18-0625-Technical.pdf) for results of microsatellite genotyping presented as an UPGMA tree.
‡Antifungal MICs were determined by the CLSI M27–A3 broth microdilution method (7). The fluconazole MICs of 30 Y132F isolates determined by Etest were ≥8 mg/L. All 67 isolates tested were susceptible to amphotericin B (MIC 0.25–1 mg/L) and micafungin (MIC 0.25–2 mg/L) according to the CLSI method.
§All were homozygote alleles except for 6 heterozygote alleles (Q1053, G583R, P250S, P295R, W872C, and G927D) in Mrr1p.

Results (4). However, a single Y132F substitution in Erg11p was found in all 30 fluconazole-resistant isolates from South Korea hospitals. The same K177N substitution in Mrr1p was found in all Y132F isolates except 1; none of the Y132F isolates showed missense mutations in Tac1p or Upc2p (Table). Taken together, these findings demonstrate low genetic diversity among Y132F isolates from the same country (the United States or South Korea). In our study, 76.7% (23/30) of patients with Y132F isolates had no antifungal exposure within 30 days before
candidemia detection, and their clonal transmission was not detected by routine hospital surveillance, partly because more than half of the patient hospitalizations did not overlap. These findings indicate that clonal Y132F isolates may be dormant over long periods and can survive and persist outside their host on hospital environmental surfaces, which may be similar to the behavior of C. auris (10). Although our study was limited by the relatively low number of isolates, our data suggest that C. parapsilosis Y132F isolates should be identified in clinical microbiology laboratories to prevent further clonal transmission of BSI caused by Y132F isolates.

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Borrelia miyamotoi Disease in an Immunocompetent Patient, Western Europe

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Borrelia miyamotoi disease is a hard tick–borne relapsing fever illness that occurs across the temperate climate zone. Human B. miyamotoi disease in immunocompetent patients has been described in Russia, North America, and Japan. We describe a case of B. miyamotoi disease in an immunocompetent patient in western Europe.

A 72-year-old woman in the Netherlands sought treatment in her third day of fever (≤38.6°C) and reported myalgia, arthralgia, headache, and a 2.5-kg weight loss. Three weeks earlier she had noticed a tick bite after gardening. Several days later, an erythematous lesion appeared, increasing to palm size within 1.5 weeks and dissolving in a similar period. Full medical history was not

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Technical Appendix

Technical Appendix Figure. Results of microsatellite genotyping presented as an unweighted pair group method with arithmetic mean (UPGMA) tree for 67 bloodstream isolates of *Candida parapsilosis* from South Korea. No., numbers of 30 Y132F isolates (Y1–Y30), other 17 fluconazole-resistant isolates (R1–R17), and 20 fluconazole-susceptible isolates (S1–S20); Hos, hospital.