Microsatellite genotyping of clinical Candida parapsilosis isolates

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Background and Purpose: Candida parapsilosis is a predominant species found in nosocomial infection, particularly in hospitalized patients. The molecular epidemiology of the clinical strains of this species has not been well studied. The present study was performed with the aim of investigating the microsatellite genotyping of Candida parapsilosis among the Iranian clinical isolates.

Materials and Methods: This study was conducted on 81 independent clinical C. parapsilosis isolates that were genotyped by using a panel of six microsatellite markers.

Results: The short tandem repeat (STR) typing of clinical C. parapsilosis isolates demonstrated 68 separate genotypes, among which 57 genotypes were observed once and the remaining 11 cases were identified for multiple times. The Simpson’s diversity index for the panel of combined six markers yielded a diversity index of 0.9951. The heterogeneity was observed among the Iranian and the Netherlands clinical C. parapsilosis isolates.

Conclusion: As the findings indicated, the clinical C. parapsilosis isolates from Iran showed a high genetic diversity. It can be concluded that molecular epidemiology could be useful for screening during outbreak investigation where C. parapsilosis is involved.

Keywords: Candida parapsilosis, Genotyping, Iran, Microsatellite

Introduction

The incidence of Candida infection associated with non-albicans Candida (NAC) species has been increasing throughout the world [1, 2]. Candida parapsilosis is one of the most frequent NAC species, which causes a broad spectrum of infections from superficial to invasive candidiasis [3, 4]. These species can lead to nosocomial infection, particularly in hospitalized neonatal and pediatric patients through catheters, intravascular devices, and hand carriage of the health care workers [5-8]. The accurate identification of C. parapsilosis strains is crucially important for the adoption of the appropriate treatment regarding the different antifungal susceptibility patterns of C. parapsilosis complex [9, 10]. C. parapsilosis has been reported to account for several cases of fungemia outbreak in hospitalized patients. Based on genotypic analysis, these outbreaks were probably due to cross-infections by health care workers [11]. Since the isolates of C. parapsilosis complex are indistinguishable based on their phenotypic features, molecular assays have been developed for distinguishing and genotyping these species [12, 13]. Among these molecular methods, microsatellite analysis has been used for molecular typing of the species belonging to C. parapsilosis and other fungi [14, 15]. Therefore, the current study aimed to apply microsatellite analysis for genotyping of clinical C. parapsilosis isolates from Iran.

Materials and Methods

Isolate identification

A total of 81 C. parapsilosis clinical isolates were
investigated using molecular typing method. All clinical isolates were grown for 48 h on Sabouraud glucose agar (Merck, Germany) at 30°C. Initial species identification was performed based on conventional tools using CHROMagar Candida medium (CHROMagar Microbiology, Paris, France) at 30°C [22]. DNA was extracted using the CinnaPure DNA isolation kit (Sinaclon, Iran) in accordance with the manufacturer’s recommendations. The DNA concentration was adjusted as approximately 25 ng/µL and stored at -20°C prior to use. The identities of C. parapsilosis isolates were confirmed by the partial sequencing of the internal transcribed spacer DNA region as previously described [16].

Microsatellite typing

A panel of six short tandem repeat (STR) markers was used for typing all clinical isolates of C. parapsilosis. Generally, three trinucleotide repeat markers and three hexanucleotide markers were amplified in a multiplex polymerase chain reaction (PCR). In addition, each amplification primers were labelled at the 5’ side with 6-carboxy-fluorescein (6-FAM), either 6-carboxy-tetramethylrhodamine (TAMRA) or hexachloro-fluorescein (HEX). The primer sequences (Table 1) and PCR amplification reactions were followed as described before [14]. Briefly, the amplification products were prepared for analysis by 100-fold dilution with distilled water. Subsequently, 1 µL of the diluted PCR product was added to 8.75 µL ddH2O and 0.25 µL internal size marker ET-ROX 400 (GE Healthcare, Diegem, Belgium). In the next step, the samples were boiled at 95°C for 1 min, subjected to rapid cooling at 4°C, and then injected and run onto AB13500xL Genetic Analyzer platform (Applied Biosystems, Foster City, CA, USA) as recommended by the manufacturer. The number of repeats in each marker was achieved by comparing the relative size of each allele with those obtained by means of the reference C. parapsilosis strain CDC317. The similarities between the genotypes were visualized by constructing a minimum spanning tree (MST) using BioNumerics, version 6.0 (Applied Math, St.-Martens-Latem, Belgium). The discriminatory power was calculated by Simpson’s index of diversity as described previously [17]. The study was approved by the Ethics Committee of Tehran University of Medical Sciences, Tehran, Iran.

Table 1. Amplification primers used for microsatellite genotyping of Candida parapsilosis isolates

| Marker | Labeled primer (5’–3’) | Unlabeled primer (5’–3’)* | Repeat Unit |
|--------|------------------------|--------------------------|-------------|
| 3A     | FAM-CCTGCGTTGCAATTTCTATT | GCTGCATCCTGTTGGAATTA     | TCT         |
| 3B     | HEX-TTGGGAAACAGCAGAAGAA | GTGCTTTGACACTTGTTGTA     | TTG         |
| 3C     | TAMRA-CAATAGCAGCCTAGGACG | GTGCTTTGGTCTGGCTTGG       | AAC         |
| 6A     | FAM-CCAGTTGGAATCATACGGT | GGGTTATTTGTTGAAAAA        | TGCTTT      |
| 6B     | HEX-CCTTTTCAAAAGACGACACA | GTTCATAGATAAAAACACCCCCATA | AGTGT       |
| 6C     | TAMRA-TGGCGTTGATATCGGCGTTA | GAATTGATACCGCGGAACCT      | TGCTGG      |

*The underlined G nucleotide in the unlabeled primer sequence is not a match to the genomic DNA, but was introduced to minimize the formation of minus-A peaks.

Results

Totally, 81 clinical C. parapsilosis isolates were identified from a variety of clinical specimens, including nail scraping (n=59), interdigital skin (n=8), groin (n=6), vaginal (n=2), ear discharge (n=2), skin (n=2), hand (n=1), and sputum (n=1). The STR typing of 81 C. parapsilosis isolates demonstrated 68 separate genotypes. The genetic relatedness of clinical C. parapsilosis isolates is depicted in figure 1. Out of all genotypes, 57 cases were observed once, and the remaining 11 genotypes were identified for multiple times. Nine genotype clusters were shared between two C. parapsilosis isolates, and also two genotypes were shared among three C. parapsilosis isolates. One cluster, including three isolates (i.e., SKCP346, SKCP368, and SKCP392), obtained from different patients of the nail samples, showed the same allelic profile at six loci in STR typing. In addition, one similar genotype was found to be related to three C. parapsilosis isolates (i.e., SKCP374, SKCP390, and SKCP389), which were obtained from various specimens of different patients. Furthermore, the same genotype was detected to be related to two C. parapsilosis isolates (i.e., SKCP311 and SKCP326) obtained from different anatomic sites. Moreover, three patients with candidiasis were sampled twice on two different days, and the same genotype was observed over the time. In Figure 2, MST represents the genotypic diversity of clinical C. parapsilosis isolates based on sample type analysis. The STR typing revealed a high genetic diversity in the Iranian C. parapsilosis isolates, compared to other clinical isolates from the Netherlands. Based on the MST (Figure 3), it was clear that two clinical isolates from Iran and two clinical isolates from Netherlands were in the same clonal cluster. The Simpson’s diversity index for the individual markers ranged within 0.2666–0.0933, and the panel of all six markers yielded a diversity index of 0.9951.

Discussion

Recently, NAC species has become an increasing prevalence among the hospitalized patients. Some of these species exhibit decreased susceptibility to the commonly used antifungal agents [7, 18, 19]. This aspect has caused a concern among the clinicians about
the potentiality for the emergence of antifungal resistance [18, 20]. *C. parapsilosis* is well known for nosocomial spread through hospital environment [6, 21, 22]. Moreover, this species has a worldwide distribution [23-25]. The discrimination of the *C. parapsilosis* isolates was accomplished by the development of the DNA-based typing methods [26-28]. Various molecular methods, including PCR-restriction fragment length polymorphism (RFLP), PCR-based random amplified polymorphic DNA (RAPD) technique, matrix assisted laser desorption ionization-time of flight mass spectrometry, and multilocus sequence typing, have been described for the identification of *C. parapsilosis* complex species in

**Figure 1.** Dendrogram showing genotypic diversity among 81 clinical *C. parapsilosis* isolates obtained from Iran. The scale bar indicates the percentage similarity between the genotypes. The columns after the short tandem repeat patterns represent the source of isolates and isolate number, respectively.
Figure 2. Minimum spanning tree showing the diversity between the genotypes based on sample type categorized analysis. Each circle represents a unique genotype, and the circle size is correlated with the number of isolates belonging to the same genotype.

Figure 3. Minimum spanning tree representing the genotypic diversity based on the origin of C. parapsilosis isolates. The green and blue circles indicate Iranian isolates (n=68) and isolates obtained from the Netherlands (n=18), respectively.
the previous studies [29-31]. Notably, STR typing assay has been reported to have a high reproducibility and discriminatory power. This assay is well utilized as a powerful tool for the specific identification of several yeast species [32-34]. Lasker et al. used a microsatellite method for the genotyping of C. parapsilosis isolates based on dinucleotide repeats; however, they reported some limitations in this regard [35]. In agreement with our results, Sabino et al. also described polymorphic microsatellite markers with higher discriminatory power (0.99) for the differentiation of C. parapsilosis isolates and reported this approach as a reliable method for molecular epidemiological studies [36]. They also asserted that microsatellite markers have high reproducibility and are potential to identify multiple genotypes for C. parapsilosis isolates. Therefore, they marked that this method has incomparable advantage over other typing methods, such as RAPD, PCR-RFLP, and internal transcribed sequence grouping [36]. This panel of six markers allow for excellent discrimination between the isolates from different origins. In the present study, we characterized clinical C. parapsilosis isolates by using six-marker microsatellite panel assay. As mentioned above, the majority of the clinical C. parapsilosis isolates had unique genotypes. In this regard, within the collection of 81 C. parapsilosis isolates obtained from clinical sources, 68 unique genotypes were observed. The pattern results of the STR analysis of two hexanucleotide markers revealed no variation between all C. parapsilosis isolates, except one isolate (SKCP313) that had diversity at these two loci, whereas high allelic variation was observed at other locus. In the present study, the isolation of the related genotypes of C. parapsilosis from multiple anatomical sites over time supported the evidence of an endogenous colonization. In line with our results, Diab-Elschahawi et al. reported 24% clonally related genotypes from multiple anatomical sites in patients [14]. In addition, similarity was observed just in a clonal cluster between the Iranian and Netherlands clinical isolates, indicating high genetic diversity of clinical C. parapsilosis isolates inside and outside Iran.

Conclusion
In conclusion, the Iranian C. parapsilosis isolates were found to have a high genetic diversity. Microsatellite genotyping method could be useful for screening during outbreak investigation, especially where C. parapsilosis complex is involved.

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Author’s contribution
S. Kh., H. B., and S. R. managed the project and wrote the first draft of the manuscript. S. Kh., F. H., S. A., K. A., and M.O. performed the tests. J.F. M. interpreted the data. M. O. and R. M. were the project partners. All the authors approved the final version of the manuscript.

Conflicts of interest
The authors declare no conflicts of interest regarding the publication of this study.

Financial disclosure
No financial interests related to the material of this manuscript have been declared.

References
1. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007; 20(1):133.
2. Laal Kargar M, Fooladi-Rad S, Mohammad Davoodi M, Khalilzadeh S, Hassanzad M, Mayahi S, et al. Fungal colonization in patients with cystic fibrosis. J Mazandaran Univ Med Sci. 2013; 22(2):204-18.
3. Bassetti M, Merelli M, Righi E, Diaz-Martín A, Rosello EM, Luzzatti R, et al. Epidemiology, species distribution, antifungal susceptibility, and outcome of candidemia across five sites in Italy and Spain. J Clin Microbiol. 2013; 51(12):4167-72.
4. Nucci M, Queiroz-Telles F, Alvarado-Mature T, Tiraboschi IN, Cortes J, Zurita J, et al. Epidemiology of candidemia in Latin America: a laboratory-based survey. PloS One. 2013; 8(3):e59373.
5. Van Asbeck EC, Clemons KV, Stevens DA. Candida parapsilosis: a review of its epidemiology, pathogenesis, clinical aspects, typing and antimicrobial susceptibility. Crit Rev Microbiol. 2009; 35(4):283-309.
6. Khodavaisy S, Nabili M, Davari B, Vahedi M. Evaluation of bacterial and fungal contamination in the health care workers’ hands and rings in the intensive care unit. J Prev Med Hyg. 2011; 52(4):215-8.
7. Pammi M, Holland L, Butler G, Gacser A, Bliss JM. Candida parapsilosis is a significant neonatal pathogen: a systematic review and meta-analysis. Pediatr Infect Dis J. 2013; 32(5):e206-16.
8. Levin AS, Costa SF, Mussi NS, Basso M, Sinto SI, Machado C, et al. Candida parapsilosis fungemia associated with implantable and semi-implantable central venous catheters and the hands of healthcare workers. Diagn Microbiol Infect Dis. 1998; 30(4):243-9.
9. Krcmery V, Barnes AJ. Non-albicans Candida spp. causing fungaemia: pathogenicity and antifungal resistance. J Hosp Infect. 2002; 50(4):243-60.
10. Trabasso P, Matsuzawa T, Fagnani R, Muraosa Y, Tominaga K, Resende MR, et al. Isolation and drug susceptibility of Candida parapsilosis sensu lato and other species of C. parapsilosis complex from patients with blood stream infections and proposal of a novel LAMP identification method for the species. Mycopathologia. 2015; 179(1-2):53-62.
11. Huang YC, Lin TY, Leu HS, Peng HL, Wu JH, Chang HY. Outbreak of Candida parapsilosis fungemia in neonatal intensive care units: clinical implications and genotyping analysis. Infection. 1999; 27(2):97-102.
12. Desnos-Ollivier M, Börnida V, Poirier P, Nourrisson C, Pan D, Bretagne S, et al. Population Structure of Candida parapsilosis: No Genetic Difference Between French and
Uruguayan isolates Using Microsatellite Length Polymorphism. Mycopathologia. 2017:1-10.

13. Hays C, Duhamel C, Cattoir V, Bonhomme J. Rapid and accurate identification of species belonging to the *Candida parapsilosis* complex by real-time PCR and melting curve analysis. J Med Microbiol. 2011; 60(4):477-80.

14. Diab-Elschahawi M, Forstner C, Hagen F, Meis JF, Lassnig AM, Presterl E, et al. Microsatellite genotyping clarified conspicuous accumulation of *Candida parapsilosis* at a cardiothoracic surgery intensive care unit. J Clin Microbiol. 2012; 50(11):3422-6.

15. Khodavaisy S, Badali H, Rezaie S, Nabil M, Moghadam KG, Afhami S, et al. Genotyping of clinical and environmental *Aspergillus flavus* isolates from Iran using microsatellites. Mycoses. 2016; 59(4):220-5.

16. Canton E, Pemán J, Quindós G, Eraso E, Miranda-Zapico I, Álvarez M, et al. Prospective multicenter study of the epidemiology, molecular identification, and antifungal susceptibility of *Candida parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis* isolated from patients with candidemia. Antimicrob Agents Chemother. 2011; 55(12):5590-6.

17. De Valk HA, Meis JF, Cursis IM, Muehlethaler K, Mouton JW, Klaassen CH. Use of a novel panel of nine short tandem repeats for exact and high-resolution fingerprinting of *Aspergillus fumigatus* isolates. J Clin Microbiol. 2005; 43(8):4112-20.

18. Arendrup MC. Epidemiology of invasive candidiasis. Curr Opin Crit Care. 2010; 16(5):445-52.

19. Lockhart SR, Iqbal N, Cleveland AA, Farley MM, Harrison LH, Bolden CB, et al. Species identification and antifungal susceptibility testing of Candida bloodstream isolates from population-based surveillance studies in two US cities from 2008 to 2011. J Clin Microbiol. 2012; 50(11):3435-42.

20. Pfaffer MA, Jones RN, Castanheira M. Regional data analysis of Candida non-albicans strains collected in United States medical sites over a 6-year period, 2006–2011. Mycoses. 2014; 57(10):602-11.

21. Trofa D, Gácser A, Nosanchuk JD. *Candida parapsilosis*, an emerging fungal pathogen. Clin Microbiol Rev. 2008; 21(4):606-25.

22. Khodavaisy S, Alialy M, Mahdavi Omran S, Habibi MR, Amri P, Monadi M, et al. The study on fungal colonization of respiratory tract in patients admitted to intensive care units of Sari and Babol hospitals. Med J Mashhad Univ Med Sci. 2011; 54(3):177-84.

23. Lofrano E, Kordbacheh P, Mirhendi H, Zaini F, Ghajari A, Mohammadi R, et al. Antifungal susceptibility analysis of clinical isolates of *Candida parapsilosis* in Iran. Iran J Public Health. 2016; 45(3):322-8.

24. Mohammadi R, Badlee P, Badali H, Abastabar M, Safa AH, Hadipour M, et al. Use of restriction fragment length polymorphism to identify *Candida* species, related to onychomycosis. Adv Biomed Res. 2015; 4:95.

25. Mohammadi R, Mirhendi H, Hedayati MT, Badali H. Caspofungin-Non-Susceptible Candida orthopsilosis isolated from onychomycosis in Iran. Iran J Public Health. 2017; 46(2):235-41.

26. Chao QT, Lee TF, Teng SH, Peng LY, Chen PH, Teng LJ, et al. Comparison of the accuracy of two conventional phenotypic methods and two MALDI-TOF MS systems with that of DNA sequencing analysis for correctly identifying clinically encountered yeasts. PLoS One. 2014; 9(10):e109376.

27. Del Pilar Vercher M, Garcia Martínez JM, Cantón E, Pemán J, Gomes García MM, Gómez EV, et al. Differentiation of *Candida parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis* by specific PCR amplification of the RPS0 intron. Int J Med Microbiol. 2011; 301(6):531-5.

28. Souza AC, Ferreira RC, Gonçalves SS, Quindós G, Eraso E, Bizerra FC, et al. Accurate identification of Candida parapsilosis (sensu lato) by use of mitochondrial DNA and real-time PCR. J Clin Microbiol. 2012; 50(7):2310-4.

29. De Carolis E, Hensgens LA, Vella A, Postaro B, Sanguinetti M, Senesi S, et al. Identification and typing of the *Candida parapsilosis* complex: MALDI-TOF MS vs. AFLP. Med Mycol. 2014; 52(2):123-30.

30. Tavanti A, Hensgens LA, Ghelardi E, Campa M, Senesi S. Genotyping of *Candida orthopsilosis* clinical isolates by amplification fragment length polymorphism reveals genetic diversity among independent isolates and strain maintenance within patients. J Clin Microbiol. 2007; 45(5):1455-62.

31. Van Asbeck EC, Clemens KV, Markham AN, Stevens DA. Correlation of restriction fragment length polymorphism genotyping with internal transcribed spacer sequence, randomly amplified polymorphic DNA and multilocus sequence groupings for *Candida parapsilosis*. Mycoses. 2009; 52(6):493-8.

32. Foulet F, Nicolas N, Eloy O, Botterel F, Gantier JC, Costa JM, et al. Microsatellite marker analysis as a typing system for *Candida glabrata*. J Clin Microbiol. 2005; 43(9):4574-9.

33. Hennequin C, Thierry A, Richard G, Lecointre G, Nguyen H, Gaillardin C, et al. Microsatellite typing as a new tool for identification of *Saccharomyces cerevisiae* strains. J Clin Microbiol. 2001; 39(2):551-9.

34. Botterel F, Desterke C, Costa C, Bretagne S. Analysis of microsatellite markers of *Candida albicans* used for rapid typing. J Clin Microbiol. 2001; 39(11):4076-81.

35. Lasker BA, Butler G, Lott TJ. Molecular genotyping of *Candida parapsilosis* group I clinical isolates by analysis of polymorphic microsatellite markers. J Clin Microbiol. 2006; 44(3):750-9.

36. Sabino R, Sampao P, Rosado L, Stevens DA, Clemens KV, Pais C. New polymorphic microsatellite markers able to distinguish among *Candida parapsilosis* sensu stricto isolates. J Clin Microbiol. 2010; 48(5):1677-82.