Genetic Variations of *Candida glabrata* Clinical Isolates from Korea using Multi-locus Sequence Typing

Min Ji Kang¹, Yoon Sung Choi¹,², Jiyoung Lee³, Kyeong Seob Shin⁴, Young Uh⁵, Young Kwon Kim⁶, Hyunwoo Jin¹,²* and Sunghyun Kim¹,²*

¹Department of Clinical Laboratory Science, College of Health Science, Catholic University of Pusan, Busan, Korea
²Clinical Trial Specialist Program for in vitro Diagnostics, Brain Busan 21 Plus Program, Graduate School, Catholic University of Pusan, Busan, Korea
³Department of Biomedical Laboratory Science, College of Health Sciences, Yonsei University, Wonju, Korea
⁴Department of Laboratory Medicine, Chungbuk National University College of Medicine, Cheongju, Korea
⁵Department of Laboratory Medicine, Yonsei University Wonju College of Medicine, Wonju, Korea
⁶Department of Biomedical Laboratory Science, College of Medical Sciences, Konyang University, Daejeon, Republic of Korea

*Corresponding author:* Hyunwoo Jin, Department of Clinical Laboratory Science, College of Health Science, Catholic University of Pusan, Busan, Korea, Tel: 82-51-510-0567; Fax: 82-51-510-0568; E-mail: jjjinhw@cup.ac.kr

Sunghyun Kim, Department of Clinical Laboratory Science, College of Health Science, Catholic University of Pusan, Busan, Korea, Tel: 82-51-510-0560; Fax: 82-51-510-0568; E-mail: shkim0423@cup.ac.kr

**Received date:** August 28, 2018; **Accepted date:** November 2, 2018; **Published date:** November 27, 2018

**Citation:** Kang MJ, Choi YS, Lee J, Shin KS, Uh Y, et al. (2018) Genetic Variations of *Candida glabrata* Clinical Isolates from Korea using Multi-locus Sequence Typing. Arch Clin Microbiol Vol No:9 Iss No:4

**Copyright:** © 2018 Kang MJ, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Abstract**

**Background:** Although *Candida albicans* is considered to be the major fungal pathogen of candidemia, severe infections by non-*albicans Candida* (NAC) spp. have been on the increase in recent years. Among NAC spp., *C. glabrata* has emerged as the second most common pathogen. Unlike other *Candida* spp., it is often resistant to variousazole antifungal agents, such as fluconazole. However, few studies have been conducted to investigate its structure, epidemiology, and basic biology. Recently, multi-locus sequence typing (MLST) has been developed as a highly useful and portable molecular biology technique.

**Methods:** In the present study, MLST was performed with a total of 102 *C. glabrata* clinical isolates that were isolated from various types of clinical specimens. The present study was performed with a total of 102 *C. glabrata* clinical isolates that were isolated from various types of clinical specimens. The fungal internal transcribed spacer (ITS) gene was amplified and sequenced to identify and confirm *C. glabrata* clinical isolates. For MLST, six housekeeping genes including 1,3-beta-glucan synthase (FKS), 3-isopropylmalate dehydrogenase (LEU2), myristoyl-CoA, protein N-myristoyltransferase (NMT1), phosphoribosyl-anthraniinate isomerase (TRP1), UTP-glucose-1-phosphate uridylyltransferase (UGP1), and orotidine-5-phosphate decarboxylase (URA3) were amplified and sequenced. The results were analyzed by using the *C. glabrata* database.

**Results:** Of a total of 3,345 base-pair DNA sequences, 49 (1.5%) variable nucleotide sites were found and the results showed that a total of 12 different sequence types (STs) were identified from the 102 clinical isolates. As classified by STs, The ST138 was the most predominant sequence type (ST) in this study as a result of 52.9% (54/102), and the following most predominant ST was the ST63 as a result of 23.5% (24/102).

**Conclusion:** In conclusion, this data demonstrated that the ST138 was the most predominant ST in Korea. Further, we found eight undetermined STs (USTs) and then seven STs among these STs were given the number by PubMLST database. The data from this study might provide a fundamental database for further studies on *C. glabrata*, including its epidemiology, and evolution. Furthermore, the data might also contribute to the development of novel antifungal agents and diagnostic tests.

**Keywords:** *Candida glabrata*; Candidiasis; Multi-locus sequence typing; Sequence types; Genetic variations

**Abbreviations:**

MLST: Multi-locus sequence typing; STs: Sequence types; AIDS: Acquired immune deficiency syndrome; NAC: Non-albicans Candida; ITS: Internal transcribed spacer; KCMF: Korean Culture Collection of Medical Fungi; Blast: Basic local alignment search tool; NCBI: National Center for Biotechnology Information; MEGA: Molecular Evolutionary Genetics Analysis; UPGMA: Unweighted Pair Group Method using Arithmetic algorithm
Introduction

Candida species belong to the normal flora of the vaginal tract, the gastrointestinal tract, and the oral cavity in human [1,2]. However, rarely, serious infections, ranging from mucosal infections to systemic infection have been caused by Candida spp. [1,2]. Fungal infections caused by Candida spp. have increased significantly, especially in acquired immune deficiency syndrome (AIDS) and immunocompromised individuals, including intensive care and, elderly patients [2,3]. Also, candidemia is associated with a high mortality rate approximately 30 to 40% in hospitalized patients and is difficult to treat, thus increasing the cost of medical care [2,3].

C. glabrata has emerged as the second or third most common Candida pathogen after C. albicans in the United States, depending on the site [3-5]. Despite its increased prevalence, there have been relatively few studies on the population structure, epidemiology, and basic biology of C. glabrata compared to those conducted on other Candida spp. [2,4,6].

As mentioned above, C. albicans is considered to be the major fungal pathogen of candidemia in the past [6,7]. However, as the number of severe infections caused by non-albicans Candida spp. (NAC) have increased, studies have shifted from C. albicans to NAC such as C. glabrata in recent years [5,8]. Furthermore, since C. glabrata infections are often resistant to azole antifungal drugs, especially fluconazole, it is important to distinguish NAC from C. albicans to ensure the appropriate antifungal therapy and clinical management [2,5,9]. Thus, the discrimination of subtypes in these species are required for investigating their epidemiology and evolutionary biology [7,10,11].

In recent years, there has been substantial progress in the development of several molecular methods for typing subspecies and strains of fungi [12]. For instance, pulsed-field gel electrophoresis (PFGE) compares total DNA band patterns with or without restriction enzyme digestion, while multilocus variable-number tandem-repeat (VNTR) analysis examines length variations in six to nine PCR-amplified loci that contain polymorphic tandem repeats. Further, the random amplification of polymorphic DNA compares banding patterns following PCR with a nonspecific primer. Finally, multilocus enzyme electrophoresis, studies the different electrophoretic mobility of multiple core metabolic enzymes. These four approaches have some limitations, such as a lower reproducibility and portability [13,14], and the results obtained in different laboratories are difficult to compare [15,16].

Among these genotyping methods, multilocus sequence typing (MLST) is a useful tool to assign single nucleotide polymorphisms as allele numbers, which are stored in a database on line (PubMLST) and determine the differences from between closely related isolates by their geographical origins, sources, and other properties [7]. Also, it is possible that database are accessed by laboratories worldwide [11,15].

In the present study, MLST targets six independent housekeeping genes including 1,3-Beta-glucan synthase (FKS), 3-isopropylmalate dehydrogenase (LEU2), myristoyl-CoA, protein N-myristoyltransferase (NMT1), phosphoribosyl-anthranilate isomerase (TRP1), UTP-glucose-1-phosphate uridylyltransferase (UGP1), and orotidine-5'-phosphate decarboxylase (URA3) was performed with a total of 102 C. glabrata clinical isolates from various clinical specimens such as blood, urine, and other body fluids in Korea and results were analyzed by using the C. glabrata MLST database (http://pubmlst.org/cglabrata/). The aim of the study is to discriminate sequence types (STs) in the same C. glabrata spp. by using common MLST and investigate the most prevalent ST from the C. glabrata in Korea.

Methods

Clinical strains

A total of 102 C. glabrata clinical isolates were provided from Korean Culture Collection of Medical Fungi (KCMF) and those isolates were collected from tertiary hospitals in Korea. Clinical isolates were isolated from a wide variety of clinical samples, including blood, catheterized urine, bile and other body fluids (Table 1).

Table 1: Details of diverse yeast isolates used in this study.

| Clinical specimens | No. of samples (%) |
|--------------------|--------------------|
| Blood              | 64 (63)            |
| Urine              | 14 (14)            |
| Bile               | 9 (9)              |
| Others<sup>a</sup> | 14 (14)            |
| Total              | 102 (100)          |

<sup>a</sup>Others : ascitic fluid, joint fluid, pleural fluid, tissue etc.
<sup>b</sup>No clinical information.

Genomic DNA extraction from fungal isolates

Genomic DNA (gDNA) of C. glabrata clinical isolates was extracted using a l-genomic BYF DNA Extraction Mini Kit (iNtRON Inc., Seongnam, Korea) according to the manufacturer’s instructions [17]. The concentration and purity of the genomic DNA were checked by 260/280 optical density using a Nanodrop 2000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The extracted gDNA was stored at 4°C until use.

Polymerase chain reaction and sequence analysis of the fungal ITS region for precise identification of C. glabrata clinical isolates

The fungal internal transcribed spacer (ITS) region, the conserved region between the 18S and 28S ribosomal RNA (rRNA) was amplified and sequenced using each primer pairs (Table 2). Target amplification was carried out in 20 μL reaction mixture containing 10 μL Prime Taq Primix (Genet Bio Inc., Daejeon, Korea), 5 μL of distilled ultra-pure water, 1 μL of each primer (10 pmol/μL), and 3 μL of genomic DNA template. The
PCR condition was: an initial denaturation at 94°C for 1 min, 30 cycles including subsequent denaturation at 94°C for 30 sec, annealing at 57°C for 30 sec and extension at 72°C for 45 sec followed by final extension at 72°C for 7 min and holding at 4°C. The amplified products were visualized by gel electrophoresis to confirm the presence of desired product.

**Table 2**: Sequences of primer pairs for fungal species identification.

| C. glabrata sequence types | No. of isolates (%) |
|---------------------------|---------------------|
| ST63                      | 24 (23.5)           |
| ST22                      | 6 (5.9)             |
| ST55                      | 3 (2.9)             |
| ST43                      | 2 (1.2)             |
| ST138                     | 54 (52.9)           |
| ST139                     | 6 (59)              |
| ST140                     | 2 (1.2)             |
| ST141                     | 1 (1)               |
| undetermined ST1          | 1 (1)               |
| ST142                     | 1 (1)               |
| ST143                     | 1 (1)               |
| ST144                     | 1 (1)               |
| Total                     | 102 (100)           |

The resulting amplicon was purified and sequenced by Macrogen Inc. (Daejeon, Korea). All sequences with low-quality bases in the chromatogram were re-sequenced for the high-quality results.

The obtained sequences were aligned with reference sequences in the Genebank database using the basic local alignment search tool (BLAST) at the National Center for Biotechnology Information (NCBI), and percent homology scores were generated to precise identification of C. glabrata clinical isolates.

**MLST analysis for identifying sequence type of C. glabrata clinical isolates**

Table 3 shows primers for the amplification and sequence analysis of C. glabrata six housekeeping gene fragments including FKS, LEU2, NMT1, TRP1, UGP1, and URA3. For the PCR amplification, 20 μL of final mixture contained 10 μL of Prime Taq Premix, 5 μL of distilled ultra-pure water, 1 μL of each forward and reverse primer (10 pmol/μL), and 3 μL of genomic DNA template.

To amplify each six gene, the PCR reaction conditions were as follows: 7 min at 94°C, 30 cycles of 1 min at the relevant annealing temperature (Table 3), and 1 min at 74°C, followed by 10 min at 74°C.

**Table 3**: List of gene fragments and primer sequences for C. glabrata MLST analysis.

| Locus | No. of polymorphic sites (%) | No. of alleles defined | Variable positions |
|-------|-------------------------------|------------------------|--------------------|
| FKS   | 5 (0.9)                       | 5                      | 403, 352, 154, 118, 43 |
| LEU2  | 6 (1.2)                       | 4                      | 392, 384, 336, 290, 111, 54 |
| NMT1  | 12 (2.0)                      | 8                      | 575, 551, 512, 480, 434, 396, 341, 328, 305, 301, 243, 155 |
| TRP1  | 13 (3.1)                      | 8                      | 387, 378, 357, 352, 333, 309, 272, 268, 255, 229, 176, 162, 158 |
| UGP1  | 4 (0.7)                       | 4                      | 585, 435, 413, 195, 99, 581, 574, 556, 549 |
| URA3  | 9 (1.5)                       | 7                      | 440, 380, 257, 164, 44 |
| Total | 49 (1.5)                      |                        |                    |

The resulting sample was analyzed by gel electrophoresis. The PCR product of all loci were purified and sequenced using reverse sequence primer at Macrogen Inc.. The obtained sequences were analyzed by using the C. glabrata MLST database (http://pubmlst.org/cglabrata/). Each unique sequence at a locus defined an allele number, and unique combinations of alleles assigned as a ST.

**Data Analysis**

The alignment of combined six target gene sequences and loci (3,345bp) was performed using the Molecular Evolutionary Genetics Analysis (MEGA) v. 7.0 software [20]. For relatedness of the same species, the phylogenetic tree was drawn with the Unweighted Pair Group Method using Arithmetic algorithm (UPGMA) with randomized 1,000 bootstrapping. And then the eBURST package (http://eburst.mlst.net/) was used to determine that all related isolates were grouped into clonal complexes.

**Results**

**Results of PCR and sequence analysis of the fungal ITS region for species identification**

1.5% TBE agarose gel DNA electrophoresis data showed that the size of amplified fungal ITS region was 978 bp, and amplicons have shown one clear band (data not shown). As a analysis result of comparison by Genebank BLAST tool for verifying the amplified PCR products, all clinical isolates used in
this study were identified as *C. glabrata* with high concordance rate (97.8% ± 2.9).

**Results of PCR and sequence analysis, and obtaining allele number of six housekeeping genes for the MLST analysis**

In order to perform the MLST analysis, six housekeeping genes of 102 *C. glabrata* clinical isolates were amplified by PCR. The size of amplified fragments of FKS, LEU2, NMT1, TRP1, UGP1, and URA3 were 589 bp, 512 bp, 607 bp, 419 bp, 616 bp, and 602 bp, respectively as the expected size and they represented clear band (data not shown).

Amplified six housekeeping gene fragments were sequenced and then sequenced data was trimmed as each length manually by using Chromas software. Trimmed sequence of six housekeeping gene fragments was analyzed at the *C. glabrata* PubMLST database then allele number of each gene was obtained. Obtained allele number of FKS gene were 3, 5, 7, 8, and 10. Those of LEU2 gene were 5, 6, 16, and 17. Those of NMT1 gene were 3, 4, 6, 8, 11, 14, 19, and 22. Those of TRP1 gene were 2, 3, 5, 7, 10, 12, 19, and 50. Those of UGP1 gene were 1, 3, 5, and 51. Those of URA3 gene were 2, 4, 6, 8, 9, 17, and 20 (Table 4).

A total of 49 (1.5%) polymorphic sites were found among six housekeeping gene fragments. The number of polymorphic site in each gene fragment were as follows: FKS (5, 0.9%); LEU2 (4, 1.2%); NMT1 (8, 2.0%); TRP1 (8, 3.1%); UGP1 (4, 0.7%); and URA3 (7, 1.5%), as shown in Table 4. Additionally, insertions, deletions, or heterozygosity were only detected in the NMT1 fragments.

**Table 4: The number of polymorphic site and different alleles, and sequence variable position of six housekeeping gene fragments in 102 *C. glabrata* clinical isolates.**

| Target region | Primer  | Nucleotide sequences (5’ to 3’) | Amplicon size (bp) | Tm (°C) | Reference |
|---------------|---------|---------------------------------|--------------------|---------|-----------|
| ITS           | pITS-F  | GTTCTAAACAGGTGTTACTGTCGG       | 970-980            | 62.4    | Pryce et al. [18] |
|               | pITS-R  | TCCTCGCCATTGATATGTC            |                    | 55.3    |           |

**Sequence type and cluster of *C. glabrata* clinical isolates**

The MLST scheme revealed a high diversity of *C. glabrata* isolates with a total of 12 STs, 8 of which were identified as undetermined STs (USTs) that were not discovered in the previous studies.

The data demonstrates that the ST138 among these USTs was the most predominant ST in this study as a total of 54 clinical isolates (52.9%) were contained in this ST, and the following most predominant ST was the ST63 as a total of 24 clinical isolates (23.5%) were contained in this ST. In addition, this study obtained the ST55, ST22, and ST43 were as a total of 6 (5.9%), and 2 (1.2%) clinical isolates were contained in respective ST and the ST139 was identified in 6 isolates (5.9%). The ST140 was identified in 2 isolates (1.2%). The remaining 5 STs (UST1, ST141, 142, 143, 144) were classified only once each (1%) (Table 5).

**Table 5: Unique sequence types determined with a combination of six loci.**

| Target gene | Gene product                      | GenBank accession no. | Primer  | Nucleotide sequences (5’-3’) | Sequenced fragment size (bp) | Annealing temp (°C) | Reference |
|-------------|-----------------------------------|-----------------------|---------|-----------------------------|-----------------------------|--------------------|-----------|
| FKS         | 1,3-Beta-glucan synthase          | AF229171              | FKS     | GTCAAATGCGCAAACAACAACACTCTGT | 589                         | 55                 |           |
|             |                                   |                       | FKS     | GCACCTCAAGCGACGACGCTGGT     |                             |                    |           |
| LEU2        | 3-Isopropylmalate dehydrogenase   | U90626                | LEU2    | TTTCTGTAATCTCCTGCTGTCG     | 512                         | 54                 |           |
|             |                                   |                       | LEU2    | ATAGAAAGGTTGCTTGGTTGCTTG   |                             |                    |           |
| NMT1        | Myristoyl-CoA, protein N-myristoyltransferase | AF073886 | NMT1    | GCCGGTGGTGTGCTGTC          | 607                         | 59                 |           |
|             |                                   |                       | NMT1    | GGTTGCGGTGGTTGGTTGCTTC     |                             |                    |           |
| TRP1        | Phosphoribosyl-anthranilate isomerase | U31471 | TRP1    | AATTGCTCCAGCTTTTG           | 419                         | 50                 | Dodgson [6] |
|             |                                   |                       | TRP1    | GACCGTTCCAGCTCTTC           |                             |                    |           |
Combined sequence (3,345 bp) of six housekeeping genes was used for the phylogenetic tree analysis. With the exception of 3 outliers, the isolates were divided into 2 major clusters: cluster 1 and cluster 2 (Figure 1). Cluster 1 consisted of the ST138 and cluster 2 consisted of the ST63 (Figures 2 and 3).

| Gene   | Description                          | Accession | Forward Primer                        | Reverse Primer                      | Length | Score |
|--------|--------------------------------------|-----------|---------------------------------------|-------------------------------------|--------|-------|
| UGP1   | UTP-glucose-1-phosphate uridylyltransferase | AB037186  | TTTCAACACCGACAAGACACAGA               | TCAGACCTCAGCAAGCACAATTCA            | 616    | 57    |
| URA3   | Orotidine-5'-phosphate decarboxylase  | L13661    | AGCGAATTGTTGAAGTTGGTGA                | GATGTTGC                            | 602    | 53    |

**Figure 1:** Evolutionary relationships of distinct sequence types-UPGMA dendrogram used to determine the pairwise differences of the concatenated sequence of C. glabrata clinical isolates.

**Figure 2:** Phylogenetic tree generated from each isolate. The MLST allele profile, sequence type (ST), and eBURST group (Cluster 1) are listed.

**Figure 3:** Phylogenetic tree generated from each isolate. The MLST allele profile, sequence type (ST), and eBURST group (Cluster 2) are listed.

**Discussion**

*C. glabrata* is a highly opportunistic pathogen of the urogenital tract and the bloodstream in humans [21]. It is especially prevalent in the elderly and within the human immunodeficiency virus positive population [22]. Although candidiasis is frequently treated with azole antifungal agents, treatment failure has become a serious concern with azole-resistant clinical isolates due to widespread and long-term use of these agents. Nevertheless, few studies have been conducted on the structure, epidemiology, and basic biology of *C. glabrata*.

Healthcare-associated infections may be endogenous in origin or nosocomially transmitted, and the only way to distinguish them is through strain typing. Recently, MLST directly investigated the DNA sequence variations in a set of housekeeping genes and characterized the strains by their unique allelic profiles. The principle of MLST is simple, involving PCR amplification followed by DNA sequence analysis. Nucleotide differences between strains can be verified at a variable number of genes depending on the desired degree of discrimination. MLST schemes now exist for a number of important bacterial pathogens including *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Campylobacter jejuni*. The technique has also been used to assess genetic relatedness among strains of *Candida spp.* including *C. glabrata*, *C. albicans*, *C. tropicalis*, and *C. krusei*. However, MLST scheme for *C.
C. glabrata has only been used by Dodgson et al. [6] and Katiyar et al. [14].

Hence, in this study, the first MLST analysis with the yeast pathogen C. glabrata was performed and evaluated in Korea. 6 loci were selected for this study, as recommended by previous studies. While a ST3 was defined as prevalent ST in Dodgson et al. [6], the data in this study demonstrates that the ST138 was the most predominant ST. Additionally, the data defined a total of 12 STs among the 102 clinical isolates, and found 8 USTs as a result and these sequence was given the number except for one ST.

**Conclusion**

In conclusion, prevalent and novel C. glabrata STs were found in the present study. The data might provide a fundamental database for further studies on C. glabrata, including its epidemiology and evolution. Furthermore, these data might also contribute to the development of novel antifungal agents and diagnostic tests. It might even be possible to discover the virulence factors associated with disease, which population genetic studies currently struggle to monitor.

**Acknowledgments**

This research was supported by a fund (2016-ER4702-00) by Research of Korea Centers for Disease Control and Prevention and Brain Busan 21 plus project.

**References**

1. Rodrigues CF, Silva S, Henriques M (2013) Candida glabrata: a review of its features and resistance. Eur J Clin Microbiol Infect Dis 33: 673-688.
2. Fidel PL Jr, Vazquez JA, Sobel JD (1999) Candida glabrata: review of epidemiology, pathogenesis, and clinical disease with comparison to C. albicans. Clin Microbiol Rev 12: 80-96.
3. Cheng MF, Yang YL, Yao TJ, Lin CY, Liu JS, et al. (2005) Risk factors for fatal candidemia caused by Candida albicans and non-albicans Candida species. J Clin Microbiol 43: 5,593-5,600.
4. Dodgson AR, Pujol C, Pfaller MA, Denning DW, Soll DR (2005) Evidence for recombination in Candida glabrata. Fungal Genet Biol 42: 233-243.
5. Spampinato C, Leonardi D (2013) Molecular fingerprints to identify Candida species. Biomed Res Int.
6. Dodgson AR, Pujol C, Denning DW, Soll DR, Fox AJ (2003) Multilocus sequence typing of Candida glabrata reveals geographically enriched clades. J Clin Microbiol 41: 5709-5717.
7. Tavanti A, Davidson AD, Johnson EM, Maiden MC, Shaw DJ, et al. (2005) Multilocus sequence typing for differentiation of strains of Candida tropicalis. J Clin Microbiol 43: 5,593-5,600.
8. Krcmery V, Barnes AJ (2002) Non-albicans Candida spp. causing fungaemia: pathogenicity and antifungal resistance. J Hosp Infect 50: 243-260.
9. Silva S, Negri M, Henriques M, Oliveira R, Williams DW, et al. (2012) Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance. FEMS Microbiol Rev 36: 288-305.
10. Odds FC, Jacobsen MD (2008) Multilocus sequence typing of pathogenic Candida species. Eukaryot Cell 7: 1,075-1,084.
11. Urwin R, Maiden MC (2003) Multi-locus sequence typing: a tool for global epidemiology. Trends Microbiol 11: 479-487.
12. Xu J (2006) Fundamentals of fungal molecular population genetic analyses. Curr Issues Mol Biol 8: 75-89.
13. Tatay-Dualde J, Prats-van der Ham M, de la Fe C, Gómez-Martín Á, Paterna A, et al. (2016) Multilocus sequence typing of Mycoplasma mycoides subsp. capri to assess its genetic variability in a contagious agalactia endemic area. Vet Microbiol 191: 60-64.
14. Katiyar S, Shifferin E, Shelton C, Healey K, Vermitksy JP, et al. (2016) Evaluation of Polymorphic Locus Sequence Typing for Candida glabrata Epidemiology. J Clin Microbiol 54: 1,042-1,050.
15. Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, et al. (1998) Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci USA 95: 3,140-3,145.
16. Bidet P, Lalande V, Salauze B, Burghoffer B, Avesani V, et al. (2000) Comparison of PCR-ribotyping, arbitrarily primed PCR, and pulsed-field gel electrophoresis for typing Clostridium difficile. J Clin Microbiol 38: 2,484-2,487.
17. Da Silva-Rocha WP, Lemos VL, Svidizinski TJ, Milan EP,ches GM (2014) Candida species distribution, genotypy and virulence factors of Candida albicans isolated from the oral cavity of kidney transplant recipients of two geographic regions of Brazil. BMC Oral Health 14: 20-28.
18. Pryce TM, Palladino S, Kay ID, Coombs GW (2003) Rapid identification of fungi by sequencing the ITS1 and ITS2 regions using an automated capillary electrophoresis system. Med Mycol 41: 369-381.
19. Hesham AEL, Wambui V, JO HO, Maina JM (2014) Phylogenetic analysis of isolated biofuel yeasts based on 5.8 S-ITS rDNA and D1/D2 265 rDNA sequences. J Genet Engineer Biotech 12: 37-43.
20. Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol 33: 1870-1874.
21. Rahimkhani M, Saberian M, Mordadi A, Varmazyar S, Tavakoli A (2015) Urinary Tract Infection with Candida glabrata in a Patient with Spinal Cord Injury. Acta Med Iran 53: 516-517.
22. Sanson GF, Briones MR (2000) Typing of Candida glabrata in a contagious area. Fungal Genet Biol. 33: 673-688.
23. Berila N, Subik J (2010) Molecular analysis of Candida glabrata clinical isolates. Mycopathologia 170: 99-105.
24. Da Matta DA, Melo AS, Guimarães T, Frade JP, Lott TJ, et al. (2010) Multilocus sequence typing of sequential Candida albicans isolates from patients with persistent or recurrent fungemia. Med Mycol 48: 757-762.
25. Meats E, Feil EJ, Stringer S, Cody AJ, Goldstein R, et al. (2003) Characterization of encapsulated and noncapsulated Haemophilus influenzae and determination of phylogenetic relationships by multilocus sequence typing. J Clin Microbiol 41: 1623-1636.
26. Wang Y, Shi C, Liu JY, Li W, Zhao Y, et al. (2016) Multilocus sequence typing of Candida tropicalis shows clonal cluster enrichment in azole-resistant isolates from patients in Shanghai, China. Infect Genet Evol 44: 418-424.