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Original Article

A rapid method for *Candida* DNA preparation from sputum, and its clinical application

Qingqing Sun[1 #], Yun Xie[2 #], Hanbing Meng[1], Lina Zhang[1], Zhengxin He[1]

[1] *Basic Medicine Laboratory, Bethune International Peace Hospital, Shijiazhuang, Hebei 050082, PR China*
[2] *Respiratory and Critical Care, Bethune International Peace Hospital, Shijiazhuang, Hebei 050082, PR China*

# Authors contributed equally to this work.

Abstract

Introduction: The analysis and identification of *Candida* in sputum specimens is of significance in clinical settings. The aim of this study was to assess the feasibility of a previously validated rapid method for preparing *Candida* DNA in conjunction with pretreatment with Sputasol (Aiyan Biotechnology, Shanghai, China) for detecting *Candida* in sputum specimens.

Methodology: The study included 181 patients with respiratory diseases who were hospitalized at the Bethune International Peace Hospital between 9 and 29 January 2020. Sputum specimens were collected retrospectively, and sputum culture results and related clinical information were extracted from electronic medical records. The sputum samples were pre-treated with Sputasol digest and subjected to rapid DNA sample preparation, and duplex polymerase chain reaction (PCR) for detecting *Candida*.

Results: Out of the 181 sputum specimens, 65 tested positive for *Candida* through PCR, and 32 were positive through fungal culture. The samples that tested positive through fungal culture were also positive through PCR, and the identified species were completely consistent. Conversely, all samples that tested negative through PCR were also negative through fungal culture. The two assays showed moderate agreement, with a Kappa value of 0.554. The false-positive results of PCR *Candida* detection may be attributed to antifungal treatment and higher sensitivity.

Conclusions: The rapid *Candida* DNA sample preparation technique combined with duplex PCR for *Candida* detection is a sensitive method for identifying *Candida* in sputum. It is necessary to combine PCR results with comprehensive clinical judgment for deriving conclusions. Further studies are needed to verify its clinical value.

Key words: *Candida*; sputum; DNA; preparation; detection; rapid.

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Introduction

Candida spp. are yeasts commonly found in the environment and human flora. It has been estimated that up to 70% of healthy individuals carry *Candida albicans* in their oral mucosa [1]. Although *Candida* is part of the normal microbiota, it can become an opportunistic pathogen, particularly in immune-compromised patients and those with severe underlying diseases [2–4]. *Candida* can cause local or disseminated infections, which can be life-threatening in susceptible individuals [5].

Although true invasive candidal pneumonia is rare in immune-competent patients [6], detecting *Candida* in the oral cavity and respiratory tract is still clinically valuable. *Candida* colonization is a prerequisite for invasive candidiasis (IC), and multi-site colonization of *Candida* is an independent risk factor for invasive infection in patients [7,8]. Thus, by analyzing *Candida* colonization in various body areas, including the oral cavity, it is well-stablished that calculating the *Candida* colonization index is crucial in predicting IC [9]. Furthermore, certain studies propose that *Candida* colonization in the respiratory tract can indirectly lead to respiratory diseases. For example, Azoulay *et al*. suggested that respiratory *Candida* colonization is linked to an extended duration of mechanical ventilation, intensive care unit (ICU), and hospital stays in critically ill patients [10]. Their research also uncovered a significant and independent correlation between respiratory *Candida* colonization and ventilator-associated pneumonia caused by *Pseudomonas aeruginosa*.

Fungal culture remains the gold standard for the identification of *Candida*. Although it provides the advantage of offering the morphology and final identification of fungi, its sensitivity is limited. Moreover, the time required for growth of the fungi is excessively long, which impacts its application [11].

The beta-(1,3)-glucan test is a commonly used test for fungal infections. It is primarily used as a screening test for invasive fungal infections in blood, although it does not distinguish between *Candida* and other fungi [12]. Furthermore, it has not been proven to be effective in identifying fungal infections in bronchoalveolar lavage (BAL) fluid and sputum [13]. Therefore, the development of a reliable and timely method for identifying *Candida* or a detection method that can differentiate colonization from infection would be a significant breakthrough in this field.

In recent years, nucleic acid detection technology, represented by polymerase chain reaction (PCR), has gained extensive attention as a crucial non-culture fungal identification tool due to its high specificity and sensitivity [14–16]. However, this technique has not been widely applied. Since fungi typically have thick cell walls, establishing a rapid and easy standardized DNA preparation method for clinical laboratories is one of the significant issues in *Candida* nucleic acid identification. In our previous study, we developed a rapid method for preparing *Candida* DNA samples that, when combined with duplex PCR, can accurately identify *Candida* [17]. In this study, we applied this technique to detect *Candida* in clinical sputum samples and explored its clinical application value.

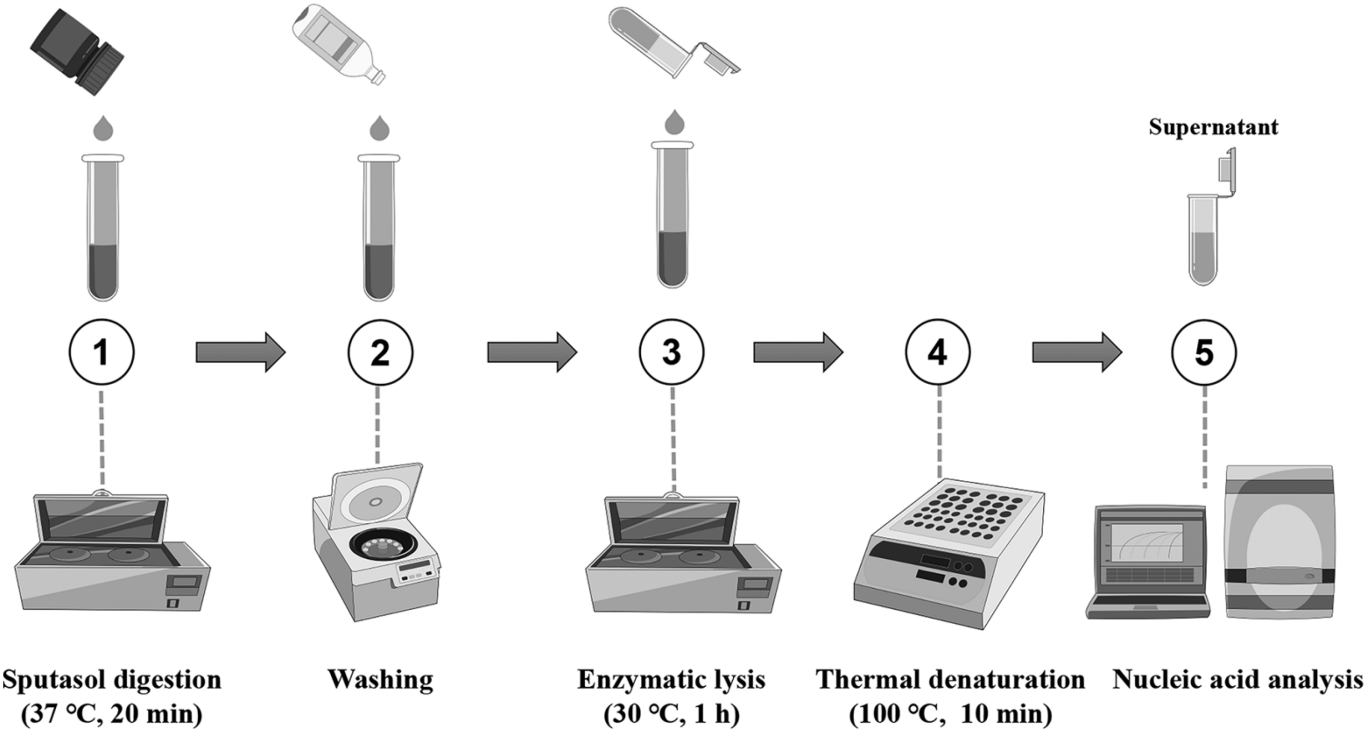
Methodology

Study design and subjects

This study was conducted on 181 inpatients with respiratory diseases admitted to the Department of Respiratory and Critical Care Medicine at the Bethune International Peace Hospital from 9 January 2020 to 29 January 2020. The principal symptoms included fever, cough, abnormal laboratory blood analysis, and pulmonary imaging abnormalities. Physicians, who were blinded to the study design, ordered sputum culture tests for clinical diagnostic purposes; thus, ensuring that the study did not interfere with clinical decision-making. Early morning deep sputum samples from the patients were collected and transported to the clinical laboratory within 30 minutes for microbial culture. The leftover specimens after sputum culture were retained for this study. Relevant clinical data of the subjects, including age, gender, primary symptoms, and results of microbial cultures, were extracted from the hospital's electronic data system.

The Medical Ethics Committee of Bethune International Peace Hospital approved this project (No. 2019-KY-23). Since the biological samples from clinical patients were collected retrospectively, and their personalized information, such as their name and ID number, was concealed, the requirement of signed informed consent form was exempted.

Figure 1. Schematic diagram of the sample preparation process for *Candida* DNA extraction from sputum specimens.



Microbial culture of sputum

Sputum samples were collected and immediately transported to the laboratory under sterile conditions. Different selective and differential media, such as MacConkey agar, sheep blood agar, chocolate agar, and Sabouraud agar, were used to streak the specimens. The morphology, color, and macroscopic characteristics of the colonies were observed following an incubation period of 24–48 hours. The bacterial colonies were sub-cultured on fresh media for further characterization. Gram staining was utilized for differentiating the bacterial colonies into Gram-positive or Gram-negative. Further characterization and identification of the bacterial species were carried out by performing various biochemical tests, such as catalase, oxidase, coagulase, indole, citrate utilization, and sugar fermentation tests. The fungi species were identified based on the growth color and colony shape on the CHROMagar plate and were further confirmed by the API 20C AUX Yeast Identification System (BioMerieux, Craponne, France).

Preparing DNA template from sputum

We utilized our previously developed technique of *Candida* fast DNA sample preparation to produce a DNA template for PCR amplification (Figure 1) from the digested sputum samples. First, 1 mL of each sample was pipetted into sterile collection tubes using a Pasteur pipette. Next, 1 mL of Sputasol sputum digest (Aiyan Biotechnology, Shanghai, China) was added to each tube and mixed thoroughly using a vortex (Dlab, Shandong, China). The tubes were then placed in a 37 ℃ water bath for 20 minutes, followed by centrifugation at 12,000 rpm for 5 minutes to discard the supernatant. The precipitate was then washed twice with 1 mL of normal saline. A lyase system was added to the washed precipitate which was then incubated at 30 ℃ for 1 hour. After that, the mixture was boiled in a metal bath (Bori Technology, Hangzhou, China) at 100 ℃ for 10 minutes. Finally, it was centrifuged again at 12,000 rpm for 5 minutes, and the supernatant was collected as the DNA template for PCR detection.

Table 1. Baseline characteristics of patients included in the study.

Characteristics	Value	Percentage (%) [a]
Gender		
Male (n)	125	69.06%
Female (n)	56	30.94%
Age (mean ± SD) years	67.46 ± 25.36	
Basic conditions		
Fever	82	45.30%
Cough	171	94.48%
WBC > 9.5×10^9	87	48.07%
Abnormal lung imaging	180	99.45%
Cultured organisms		
Bacteria species		
Pseudomonas aeruginosa	45	24.86%
Klebsiella pneumoniae	26	14.36%
Acinetobacter baumannii	25	13.81%
Stenotrophomonas maltophilia	13	7.18%
Proteus mirabilis	10	5.52%
Serratia marcescens	8	4.42%
Staphylococcus aureus	7	3.87%
Others	28	15.47%
Total	162	
***Candida* species**		
Candida albicans	23	12.71%
Candida tropicalis	5	2.76%
Candida glabrata	3	0.56%
Candida parapsilosis	2	0.56%
Candida krusei	2	0.56%
Others	3	3.87%
Total	38	

[a], The number of all included patients (181) was used as the denominator in this calculation. WBC: white blood cells.

Duplex PCR identification of Candida

The duplex PCR method for identification of *Candida* was described earlier [17]. The 50 μL dual amplification system was prepared as follows: 2× PCR mix 25 μL, Hot*Taq* polymerase 2 μL, forward primer each at 0.4 μM, reverse primer each at 0.4 μM, probe each at 0.4 μM, prepared DNA template 2 μL, and deionized water added to a final volume of 50 μL. The reaction conditions were: initial denaturation at 95 ℃ for 2 minutes; followed by 40 cycles of denaturation at 95 ℃ for 15 seconds, and annealing/extension at 60 ℃ for 1 minute. The sequences of the primers and probes are described in our previous report [17].

Statistical analysis

The data were analyzed using the statistical software SPSS 26.0 (IBM Corp., Armonk, NY, USA). Enumeration data were presented as quantity and percentage (%), while measurement data were expressed as mean ± standard deviation. The Kappa coefficient test was used to assess the agreement between different test methods. A significance level of $p < 0.05$ was used to determine statistical significance.

Results

Basic data of the patients

Each collected sputum sample was evaluated through duplex PCR and conventional culture methods to detect possible respiratory pathogens. The demographic information of the patients is detailed in Table 1. The mean age of the patients was 67.46 ± 25.36 years (range: 1–96 years). Clinical presentations were fever in 82 (45.30%) cases, cough in 171 (94.48%) cases, white blood cell (WBC) count > 9.5×10^9 in 87 (48.07%) cases, and abnormal lung imaging in 180 (99.45%) cases. A total of 162 bacterial strains were

Table 2. Results of *Candida* detection by duplex polymerase chain reaction (PCR) and fungal culture.

Fungal culture	Duplex PCR		
	Positive	Negative	Total
Positive	32	0	32
Negative	33	116	149
Total	65	116	181

isolated through microbial culture, comprising *Pseudomonas aeruginosa* (45 strains, 24.86%), *Klebsiella pneumoniae* (26 strains, 14.36%), *Acinetobacter baumannii* (25 strains, 13.81%), *Stenotrophomonas maltophilia* (13 strains, 7.18%), *Proteus mirabilis* (10 strains, 5.52%), *Serratia marcescens* (8 strains, 4.42%), *Staphylococcus aureus* (7 strains, 3.87%), and other bacterial species (28 strains, 15.47%). Additionally, 38 strains of *Candida* were isolated, including *C. albicans* (23 strains, 12.71%), *Candida tropicalis* (5 strains, 2.76%), *Candida glabrata* (3 strains, 1.66%), *Candida parapsilosis* (2 strains, 1.10%), and other *Candida* species (3 strains, 1.66%).

Comparison of fungal culture and duplex PCR findings for Candida detection

Of the 181 sputum specimens collected, 32 tested positive by culture for fungi (Table 2). A total of 65 samples tested positive by PCR, resulting in an overall PCR positive rate twice as high as that of sputum culture (35.91% versus 17.68%). The five common *Candida* species were identified using specific primers, and other unidentified *Candida* species were detected successfully by using general primers, which was in line with expectations. The results of the study indicated that all specimens with positive sputum cultures were PCR positive (32/181), all PCR negative specimens were without fungal growth in the culture (116/181), and 33 samples were negative for microbial growth in sputum cultures but detected positive with PCR. The overall agreement rate between the two methods was 81.77% (148/181), and the Kappa value of the test consistency was 0.554, indicating moderate consistency.

Characteristics of false-positive samples

Considering sputum culture as the gold standard, the PCR results for 33 samples were false-positives. We conducted a retrospective analysis of the electronic medical records of these false-positive patients, categorizing them into 3 groups based on whether there were positive records of sputum fungal culture within 3 or 7 days before or after the sample collection date: before sampling with positive records, after sampling with positive records, and no positive records before or after sampling. Among them, 3 cases (9.09%) had positive sputum cultures within 3 days before PCR sampling and received antifungal treatment, and 5 cases (15.15%) had positive sputum cultures within 7 days before PCR sampling and received antifungal treatment. There were 11 cases (33.33%) without antifungal treatment within 7 days before sampling. Post-PCR sampling, 9 cases (27.28%) had positive sputum cultures within 3 days, and 11 cases (33.33%) had positive sputum cultures within 7 days. There were 10 cases (30.30%) with negative sputum cultures within 3 days before and after PCR testing, and 6 cases (18.18%) with negative sputum cultures within 7 days before and after PCR testing (Table 3).

Clinical decisions concerning Candida detection

We analyzed the use of antifungal medications among patients to explore the correlation between PCR testing and clinical empirical treatment. Clinical doctors prescribed empirical antifungal treatment to 10 patients based on their fungal culture records and clinical characteristics. Among the samples of these patients, 7 (70.00%) fungal cultures were positive, and 3 (30.00%) fungal cultures were negative. We performed PCR testing on the remaining sputum culture samples, and the results were all positive (Table 4). Notably, the 3 negative samples originated from patients who had positive records of sputum fungal culture within the 7 days prior to sampling.

Table 3. Archival characteristics of 33 patients with false-positive polymerase chain reaction (PCR) detection.

Archival characteristics	Case number in time frame (n, %)	
	Within 3 days	Within 7 days
Prior culture positive		
with antifungal medication	3 (9.10%)	5 (15.15%)
without antifungal medication	11 (33.33%)	11 (33.33%)
Subsequent culture positive	9 (27.28%)	11 (33.33%)
Culture negative before and after PCR testing	10 (30.30%)	6 (18.18%)
Total	**33 (100%)**	**33 (100%)**

Table 4. Sputum test results of the 10 patients receiving antifungal therapy.

Sputum *Candida* tests		Patients receiving antifungal therapy (n, %)
Duplex PCR	**Fungal culture**	
Positive	Positive	7 (70.00%)
Positive	Negative	3 (30.00%)
Negative	Negative	0 (0.00%)

PCR: polymerase chain reaction.

Discussion

Preparation of high-quality DNA samples from fungal pathogens is a critical step in nucleic acid-based diagnostics [18]. Preparation of fungal DNA samples from clinical specimens can be challenging due to the presence of host DNA, inhibitors, and other contaminants [19,20]. In this study, we utilized the rapid *Candida* DNA sample preparation method and duplex PCR to analyze clinical sputum samples. This approach can sensitively identify *Candida* species in sputum and distinguish between *C. albicans*, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, and *C. krusei*. The characteristic of our previously established method is enzymatic lysis combined with thermal denaturation, which combines snailase and lyticase, achieving a higher lysis capability of *Candida* cells than using snailase, lyticase, zymolyase, and glucanase alone; while optimizing the suspension components and incubation steps [17]. Our research results indicate that sputasol sputum digestion can be combined with this method, through simple pretreatment of different types of clinical samples (such as sputum, urine, or whole blood). The precipitated *Candida* cells are digested with lysis solution for 1 hour, followed by 10 minutes of high-temperature metal bath treatment, allowing complete release of *Candida* DNA, and the supernatant can be directly used as a template for subsequent PCR amplification. Traditional culture methods take 2–5 days, while using kits to obtain *Candida* DNA and complete PCR detection takes 6 hours. The method in this study reduces the time from sample acquisition to PCR detection completion to 3 hours, making the entire process quick and simple; suitable for the processing and detection of batch samples in clinical laboratories.

Previous studies have generally acknowledged that PCR detection is more sensitive than traditional fungal culture techniques [21]. Our study revealed that the positivity rate of PCR detection of *Candida* in sputum specimens was nearly double that of fungal culture. The two detection methods exhibited moderate consistency (Kappa = 0.554), with the inconsistency mainly resulting from a significant number of samples that were PCR positive but fungal culture negative. One possible explanation is that PCR can detect both viable and non-viable fungal cells, whereas fungal culture can only detect viable cells. Additionally, PCR may produce positive results even when the fungal load is low [22]. PCR may identify fungal DNA from other sources, such as environmental contamination or commensal colonization, resulting in false-positive outcomes [23]. Furthermore, the accuracy of the results may be influenced by the sensitivity and specificity of PCR, which can vary depending on the primers and protocols employed [24]. Based on our additional analysis, among the 33 samples that exhibited false-positive results, 26 patients (78.79%) had a history of positive *Candida* culture in sputum within 7 days before or after the sample collection, and 5 of them had undergone antifungal therapy. The sensitivity and specificity of the PCR method employed have been validated in prior studies [17]; therefore, false-positives may mainly be attributed to the previous use of antifungal drugs in patients, and the sensitivity of PCR testing is significantly higher than that of fungal culture.

Although the PCR detection technology is too sensitive, it can compensate for the shortcomings of traditional culture methods and provide a basis for clinical doctors' empirical treatment. The negative result of microbial culture due to its lower sensitivity does not negate the presence of microorganisms. Similarly, a positive PCR result, due to its high sensitivity, does not confirm the causative pathogen of infection. Therefore, we believe that integrating information from both methods, in conjunction with the clinical characteristics of the patients, is more conducive to making accurate clinical decisions.

We established a rapid and accurate PCR method for detecting *Candida* in sputum samples in the laboratory and conducted preliminary preclinical research. The method requires clinical validation with a large sample size to transform the laboratory method into a clinical application product.

Empirical use of antifungal agents is frequently employed in the challenging diagnosis of invasive fungal disease and is acknowledged by multiple clinical management guidelines [25,26]. Among the patients who tested positive for *Candida* via PCR in this study, 10 were administered empirical antifungal therapy, with 7 testing positive for fungal culture and 3 testing negative. These findings suggest a greater practical clinical value of PCR *Candida* detection, although more

precise conclusions require verification through additional rigorous clinical studies.

Conclusions

We developed a novel method for the rapid preparation of *Candida* DNA from sputum and its application in PCR detection. This method offers four significant advantages over conventional fungal culture. Firstly, it has a high positive detection rate, which enables more sensitive analysis of *Candida* information in sputum and can be used as a screening method for detecting *Candida* from sputum samples. Secondly, the method allows for the rapid detection of *Candida* in sputum, with the time from obtaining the specimen to producing the detection result being controlled within 6 hours. Thirdly, the method provides *Candida* species/genus level identification using probe design, capable of detecting mixtures of up to 5 *Candida* species. Finally, although the test is more expensive than fungal culture, the more convenient and sensitive features of the test may obviate the high cost of inappropriate treatment [27].

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Authors' contributions

Conceptualization: ZH; methodology: QS, YX; formal analysis and investigation: QS, YX, HM; writing — original draft preparation: ZH, QS; writing — review and editing: ZH; funding acquisition: ZH; resources: LZ; supervision: ZH. All authors read and approved the final manuscript.

Data availability statement

The datasets generated during and/or analyzed in the current study are available from the corresponding author upon reasonable request.

Corresponding author

Prof. Zhengxin He, PhD.
Basic Medicine Laboratory, Bethune International Peace Hospital,
398 Zhongshan Road, Shijiazhuang, Hebei 050082, PR China.
Tel: +8631181568543
E-mail: hezx1979@126.com.

Conflict of interests

No conflict of interests is declared.

References

1. Millet N, Solis NV, Swidergall M (2020) Mucosal IgA prevents commensal *Candida albicans* dysbiosis in the oral cavity. Front Immunol 11: 555363. doi: 10.3389/fimmu.2020.555363.
2. Sharifi M, Badiee P, Abastabar M, Morovati H, Haghani I, Noorbakhsh M, Mohammadi R (2023) A 3-year study of *Candida* infections among patients with malignancy: etiologic agents and antifungal susceptibility profile. Front Cell Infect Microbiol 13: 1152552. doi: 10.3389/fcimb.2023.1152552.
3. Mendoza-Reyes DF, Gomez-Gaviria M, Mora-Montes HM (2022) *Candida lusitaniae*: biology, pathogenicity, virulence factors, diagnosis, and treatment. Infect Drug Resist 15: 5121–5135. doi: 10.2147/IDR.S383785.
4. Xu X, Lv H, Zhang F, Zhu H, Cai L (2021) A comparison of *Candida* detection in sputum by the conventional culture and fluorescent polymerase chain reaction methods. Med Sci Monit 27: e930293. doi: 10.12659/MSM.930293.
5. Lapaquette P, Ducreux A, Basmaciyan L, Paradis T, Bon F, Bataille A, Winckler P, Hube B, d'Enfert C, Esclatine A, Dubus E, Bringer MA, Morel E, Dalle F (2022) Membrane protective role of autophagic machinery during infection of epithelial cells by *Candida albicans*. Gut Microbes 14: 2004798. doi: 10.1080/19490976.2021.2004798.
6. Pendleton KM, Huffnagle GB, Dickson RP (2017) The significance of *Candida* in the human respiratory tract: our evolving understanding. Pathog Dis 75: ftx029. doi: 10.1093/femspd/ftx029.
7. Honore PM, Mugisha A, Kugener L, Redant S, Attou R, Gallerani A, De Bels D (2020) *Candida* infections in severe acute pancreatitis: we need to do more in order to distinguish invasive infection from simple colonization. Crit Care 24: 156. doi: 10.1186/s13054-020-02873-y.
8. Magill SS, Swoboda SM, Johnson EA, Merz WG, Pelz RK, Lipsett PA, Hendrix CW (2007) The association between anatomic site of *Candida* colonization, invasive candidiasis, and mortality in critically ill surgical patients. Diagn Microbiol Infect Dis 55: 293–301. doi: 10.1016/j.diagmicrobio.2006.03.013.
9. Caggiano G, Puntillo F, Coretti C, Giglio M, Alicino I, Manca F, Bruno F , Montagna MT (2011) *Candida* colonization index in patients admitted to an ICU. Int J Mol Sci 12: 7038–7047. doi: 10.3390/ijms12107038.
10. Azoulay E, Timsit JF, Tafflet M, de Lassence A, Darmon M, Zahar JR, Adrie C, Garrouste-Orgeas M, Cohen Y, Mourvillier B, Schlemmer B (2006) *Candida* colonization of the respiratory tract and subsequent *Pseudomonas* ventilator-associated pneumonia. Chest 129: 110–117. doi: 10.1378/chest.129.1.110.
11. Phoompoung P, Chayakulkeeree M (2016) Recent progress in the diagnosis of pathogenic *Candida* species in blood culture. Mycopathologia 181: 363–369. doi: 10.1007/s11046-016-0003-x.
12. Mikulska M, Giacobbe DR, Furfaro E, Mesini A, Marchese A, Del Bono V, Viscoli C (2016) Lower sensitivity of serum (1,3)-beta-d-glucan for the diagnosis of candidaemia due to *Candida parapsilosis*. Clin Microbiol Infect 22: 646.e5–8. doi: 10.1016/j.cmi.2016.05.020.
13. Rose SR, Vallabhajosyula S, Velez MG, Fedorko DP, VanRaden MJ, Gea-Banacloche JC, Lionakis MS (2014) The utility of bronchoalveolar lavage beta-D-glucan testing for the diagnosis of invasive fungal infections. J Infect 69: 278–283. doi: 10.1016/j.jinf.2014.04.008.

14. Rampini SK, Zbinden A, Speck RF, Bloemberg GV (2016) Similar efficacy of broad-range ITS PCR and conventional fungal culture for diagnosing fungal infections in non-immunocompromised patients. BMC Microbiol 16: 132. doi: 10.1186/s12866-016-0752-1.
15. Garcia-Salazar E, Acosta-Altamirano G, Betancourt-Cisneros P, Reyes-Montes MDR, Rosas-De-Paz E, Duarte-Escalante E, Sanchez-Conejo AR, Ocharan Hernandez E, Frias-De-Leon MG (2022) Detection and molecular identification of eight *Candida* species in clinical samples by simplex PCR. Microorganisms 10: 374. doi: 10.3390/microorganisms10020374.
16. Trovato L, Domina M, Calvo M, De Pasquale R, Scalia G, Oliveri S (2022) Use of real time multiplex PCR for the diagnosis of dermatophytes onychomycosis in patients with empirical antifungal treatments. J Infect Public Health 15: 539–544. doi: 10.1016/j.jiph.2022.03.019.
17. He Z, Huo X, Piao J (2023) Rapid preparation of *Candida* genomic DNA: combined use of enzymatic digestion and thermal disruption. AMB Express 13: 1. doi: 10.1186/s13568-022-01500-z.
18. He Z-X, Zhao H-H, Wang F-K (2020) PCR-detectable *Candida* DNA exists a short period in the blood of systemic candidiasis murine model. Open Life Sci 15: 677–682. doi: 10.1515/biol-2020-0075.
19. Khot PD, Fredricks DN (2009) PCR-based diagnosis of human fungal infections. Expert Rev Anti Infect Ther 7: 1201–1221. doi: 10.1586/eri.09.104.
20. Borman AM, Linton CJ, Miles SJ, Campbell CK, Johnson EM (2006) Ultra-rapid preparation of total genomic DNA from isolates of yeast and mould using Whatman FTA filter paper technology — a reusable DNA archiving system. Med Mycol 44: 389–398. doi: 10.1080/13693780600564613.
21. Zhang L, Lu F, Wang Y, Ji J, Xu Y, Huang Y, Zhang M, Li M, Xia J, Wang B (2023) Methodological comparison of bronchoalveolar lavage fluid-based detection of respiratory pathogens in diagnosis of bacterium/fungus-associated pneumonia in critically ill patients. Front Public Health 11: 1168812. doi: 10.3389/fpubh.2023.1168812.
22. Jones JM (1990) Laboratory diagnosis of invasive candidiasis. Clin Microbiol Rev 3: 32–45. doi: 10.1128/CMR.3.1.32.
23. Myers AN, Jeffery U, Seyler ZG, Lawhon SD, Hoffmann AR (2021) Diagnostic accuracy of a direct panfungal polymerase chain reaction assay performed on stained cytology slides. Vet Pathol 58: 542–548. doi: 10.1177/0300985821991562.
24. McKeating C, White PL, Posso R, Palmer M, Johnson E, McMullan R (2017) Diagnostic accuracy of fungal PCR and β-D-glucan for detection of candidaemia: a preliminary evaluation. J Clin Pathol 71: 420–424. doi: 10.1136/jclinpath-2017-204692.
25. Pappas PG, Kauffman CA, Andes D, Benjamin DK, Jr., Calandra TF, Edwards JE, Jr., Filler SG, Fisher JF, Kullberg BJ, Ostrosky-Zeichner L, Reboli AC, Rex JH, Walsh TJ, Sobel JD, Infectious Diseases Society of America (2009) Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis 48: 503–535. doi: 10.1086/596757.
26. Chinese Association of Hematologists, Chinese Invasive Fungal Infection Working Group (2020) The Chinese guidelines for the diagnosis and treatment of invasive fungal disease in patients with hematological disorders and cancers (the 6th revision). Zhonghua Nei Ke Za Zhi 59: 754–763. [Article in Chinese]. doi: 10.3760/cma.j.cn112138-20200627-00624.
27. Camacho-Cardoso JL, Martínez-Rivera MÁ, Manzano-Gayosso P, Méndez-Tovar LJ, López-Martínez R, Hernández-Hernández F (2023) Molecular detection of *Candida* species from hospitalized patients' specimens. Gac Med Mex 153: 581–589. doi: 10.24875/GMM.M18000045.