Case Report

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# Comparison between *Candida Guillermondii* and *Exophiala Dermatitidis* Identifications from MALDI-TOF MS (Autobio Autof ms1000) as Part of a Surveillance Program in a Medical Microbiology Laboratory: Case Report

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**ABSTRACT**

**Background:** *Candida guillermondii (Pichia guillermondii)*, also called *Meyerozyma guillermondii*, is a rare opportunistic human pathogen, said to cause "deep-seated "infections in immunocompromised hosts, just like Exophiala dermititidis (40% of fatality rate). The first pathogen is said to be an emerging infectious yeast, and the second is a rare cause of fungal infections. *C. guillermondii* is of clinical interest since species have resisted antifungal agents such as polyenes, azoles, flucytosine, and echinocandins. Because of its complex phenotypic group of origin, C. guillermondii's accurate and rapid identification in the microbiology laboratory is difficult but essential in drug administration. The newest technologies perfectly demonstrate how easy it is to misidentify both fungal strains in routine analysis. In addition, how important, in terms of life-saving, optimum turn-around time and accurate therapy, is MALDITOF-MS in an antimicrobial stewardship program?

**Results:** Mounted on MALDI TOF-MS, the specimens yielded an exact and concise "detailed results" pattern for *C. guillermondii* and favourable results for *E. dermatitidis* but with less confidence. Up to 15 different isolate types were registered for the latter. Its best MALDITOF-MS identification score is lower than the lowest identification score of the Candida.

**Discussion/ Conclusion:** MALDITOF-MS is reliable for diagnosing both fungi. Therefore, MALDITOF-MS is highly important in the diagnostic process of microorganisms of this category. The Autof MS 1000 (Autobio) is almost 100% sure of its *C. guillermondii* and strives to achieve the same with *E. dermatitidis*. However, MALDI TOF-MS cannot yet tell whether one or the other is a resistant strain to an antifungal drug.

**Keywords:** MALDI-TOF MS; Antimicrobial Stewardship; AMR, Fungal Identification; Surveillance

## Introduction

Timely identification of black yeasts in the laboratory is challenging, mainly because they lack reproductive cells. Fungal reproductive cells are essential in black yeasts' final biochemical, physiological and morphological identification. Candidemia is the cause of up to 30% of annual death and increased medical costs in most healthcare settings and systems [1], apparently due to emerging resistance and delays in the administration of antifungal drugs [1,2] in infected patients and lack of precise and rapid identification of the pathogen. Nosocomial infection caused by yeasts is said to have severely increased mortality and morbidity in immunocompromised patients in recent years [3]. The current situation of candidiasis in paediatrics is quite complicated to evaluate. Still, most pediatric infections with candida species are suspected to be associated with hospitalisations and immune depression [1,2,4]. Therefore, candidal species like *C. guillermondii* are of health concern in most control and antimicrobial stewardship programs. *Candida guillermondii (Pichia guillermondii)*, also called *Meyerozyma guillermondii*, is an opportunistic human pathogen, still considered an emerging infectious yeast, but of clinical interest since species have shown resistance to fluconazole and other antifungal drugs, and higher Minimal Inhibitory Index (MIC) to echinocandins, in comparison to other candida species [5]. Echinocandins are recommended as the target treatment in candidemia, though their routine use is suspected to induce selective pressure for resistance and alteration of species distribution [6].

Because they share similar phenotypic profiles, *C. guillermondii* is often misidentified as Candida famata [5] or Candida's sake or parapsilosis [7]. E. dermititidis, on the other hand, is very difficult to identify dark yeasts [8]. It is a dimorphic fungus distributed all over the world, causing subcutaneous infections as well as invasive infections in immunocompromised patients [4], and its disorders range from cases of association with cystic fibrosis, some respiratory infections with mild symptoms to brain infection [9]. This evidence renders early enough identification essential to control mortality and morbidity. Previous research [7] has demonstrated how MALDI-TOF MS replaces conventional phenotypic identification techniques and procedures in the clinical laboratory. It has also been proven more efficient than traditional methods in identifying *C. guillermondii* and other uncommon pathogens [10]. Most automated clinical microbiology laboratories use MALDI-TOF MS to identify pure cultures, including fungal growth cultures. The use of these newest diagnostic technologies in crowded to more contained health facilities has yielded significant results in precision, concision, and turn-around time. Innovative versions of MALDI TOF-MS are highly creative in approaching bacterial identification, especially the newest models, using mass ionisation techniques [3,10-15]. The present paper aims to report the accuracy and precision of MALDI-TOF MS (Autof ms1000 Autobio) in identifying yeasts.

By presenting two cases of profilation of fungal species, C. guillermondii, from a standard national control sample in a pediatric hospital in Italy. In addition, the precise duplicated ability of recognition of E. dermititidis, despite providing several misleading results on the same identification profile. Fungemia is a life-threatening infection with significant morbidity and mortality among pediatric patients, especially those subjected to intravenous catheters for a long time, hematopoietic stem cell transplantation, immunosuppressive therapy, or patients with severe immunodeficiency and cancers [16].

## Case Report

The control lot was received in the laboratory as part of the routine regional/national laboratory control plan. The sample was cultured in appropriate growth media for fungal agents. After three days, colonies were exclusively identified as white round, creamy colonies-like growth on Sabouraud dextrose agar media. Fungal identification on Autobio (Biotyper/Autof ms1000) was performed by directly spotting the sample on the target plate in duplicate, per the laboratory's standard preparation of fungal isolates. After extraction and installation of the fungal reactant on the scale, 1uL of matrix solution was applied on top of the dry spot and re-allowed to air dry at room temperature before introduction inside the instrument. The mass spectra profiles were acquired from the instrument library. The accuracy of the Mass spectrum detailed results was undeniable. *C. guillermondii* was picked up 10 times and scored from 9.627 to 8.894 in the first sample spot (Figure 1: D11) and 9.529 to 9.103 in the second sample spot (Figure 2: D12), according to the spectral quantification. Though the highest-grade score was achieved from the sample spot D11 (9.627: Detailed results NO. 1), slight differences are perceptible on the mass spectrums. Four characteristics picks are noticeable. First between 0 to 4000 mz/Da (43,766 and 3365, 617), for the highest of them all, at 6727.579 (Sample spot D11: Figure 1), then at 6728.322 (Sample spot D12: Figure 2; 9.529: Detailed results NO. 1), between 4000 and 8000 mz/Da, and finally between 12000 and 16000 mz/Da at 12592.183 (D11) and 12593.041 (D12).

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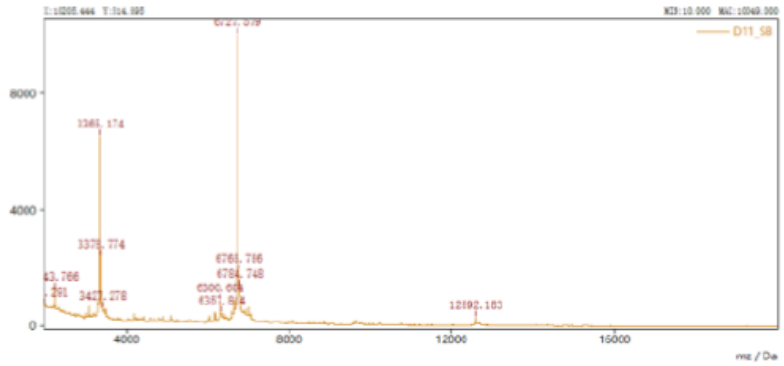
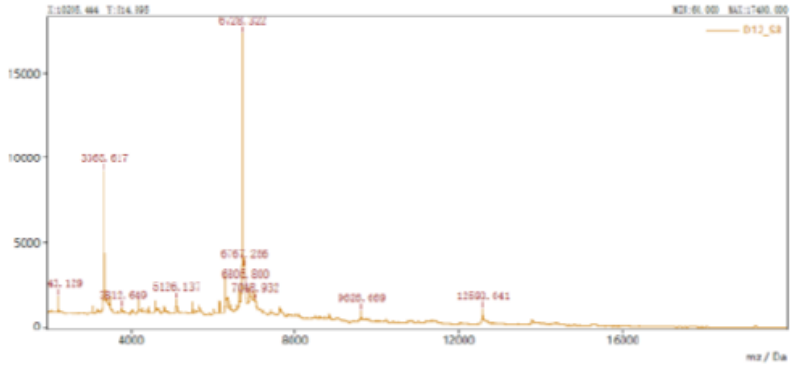
Mass Spectrum




Detailed Results:

| NO. | Result | Score |
|---|---|---|
| 1 | Candida guilliermondii(Pichia guilliermondii) | 9.627 |
| 2 | Candida guilliermondii(Pichia guilliermondii) | 9.519 |
| 3 | Candida guilliermondii(Pichia guilliermondii) | 9.167 |
| 4 | Candida guilliermondii(Pichia guilliermondii) | 9.099 |
| 5 | Candida guilliermondii(Pichia guilliermondii) | 9.085 |
| 6 | Candida guilliermondii(Pichia guilliermondii) | 9.081 |
| 7 | Candida guilliermondii(Pichia guilliermondii) | 9.060 |
| 8 | Candida guilliermondii(Pichia guilliermondii) | 9.026 |
| 9 | Candida guilliermondii(Pichia guilliermondii) | 8.928 |
| 10 | Candida guilliermondii(Pichia guilliermondii) | 8.894 |

**Figure 1:** Autof ms1000 Identification Result: Candida guilliermondii (Pichia guilliermondii) 9.627; Generated by Autobio, on the 02/14/2022 at 17:29.




Detailed Results:

| NO. | Result | Score |
|---|---|---|
| 1 | Candida guilliermondii(Pichia guilliermondii) | 9.529 |
| 2 | Candida guilliermondii(Pichia guilliermondii) | 9.515 |
| 3 | Candida guilliermondii(Pichia guilliermondii) | 9.446 |
| 4 | Candida guilliermondii(Pichia guilliermondii) | 9.269 |
| 5 | Candida guilliermondii(Pichia guilliermondii) | 9.251 |
| 6 | Candida guilliermondii(Pichia guilliermondii) | 9.213 |
| 7 | Candida guilliermondii(Pichia guilliermondii) | 9.157 |
| 8 | Candida guilliermondii(Pichia guilliermondii) | 9.135 |
| 9 | Candida guilliermondii(Pichia guilliermondii) | 9.128 |
| 10 | Candida guilliermondii(Pichia guilliermondii) | 9.103 |

**Figure 2:** Autof ms1000 Identification Result: Candida guilliermondii (Pichia guilliermondii) 9.529; Generated by Autobio, on the 02/14/2022 at 17:29.

## Discussion

The applicability of MALDITOF-MS as a reliable instrument of fungal identification has already been demonstrated. Both highlighted species are rare and difficult to isolate by "conventional techniques". The ability of *C. guillermondii* to grow resistance mechanisms is of concern, while the situation about *E. dermatitidis* remains at the level of its most accurate and precise identification. Thus, it is a problem to encounter one or both microorganisms in the laboratory since one is liable to be a resistant strain and the other to be easily misidentified (Figure 3: Detailed results NO. 3 to NO. 10 and Figure 4: Detailed results NO. 2 to NO. 4 and from NO. 6 to NO. 10). *C. guillermondii* was ideally identified. Detailed result spots showing ten out of ten results scores, attributed to the same microorganism, ranging from 9.627 to 8.894 (detailed results scores of samples spot D11) and from 9.529 to 9.103 (detailed results scores of sample spot D12) in both reports (Figure 1 & 2). Although mass spectrometry in the microbiology laboratory is the most reliable antimicrobial identification route, this paper presents a case of 15 potential isolate types appearing on results profiles of *E. dermatitidis* 's identification in both reports (Figures 3 & 4). The highest identification scores were in both spot cases: 6.765 (Sample Spot C9) and 6.702 for sample Spot C10. This evidence means that more traditional identification techniques for the same pathogen (*E. dermatitidis*) are even more liable to failures and mistakes and, subsequently, to failure of the therapy.

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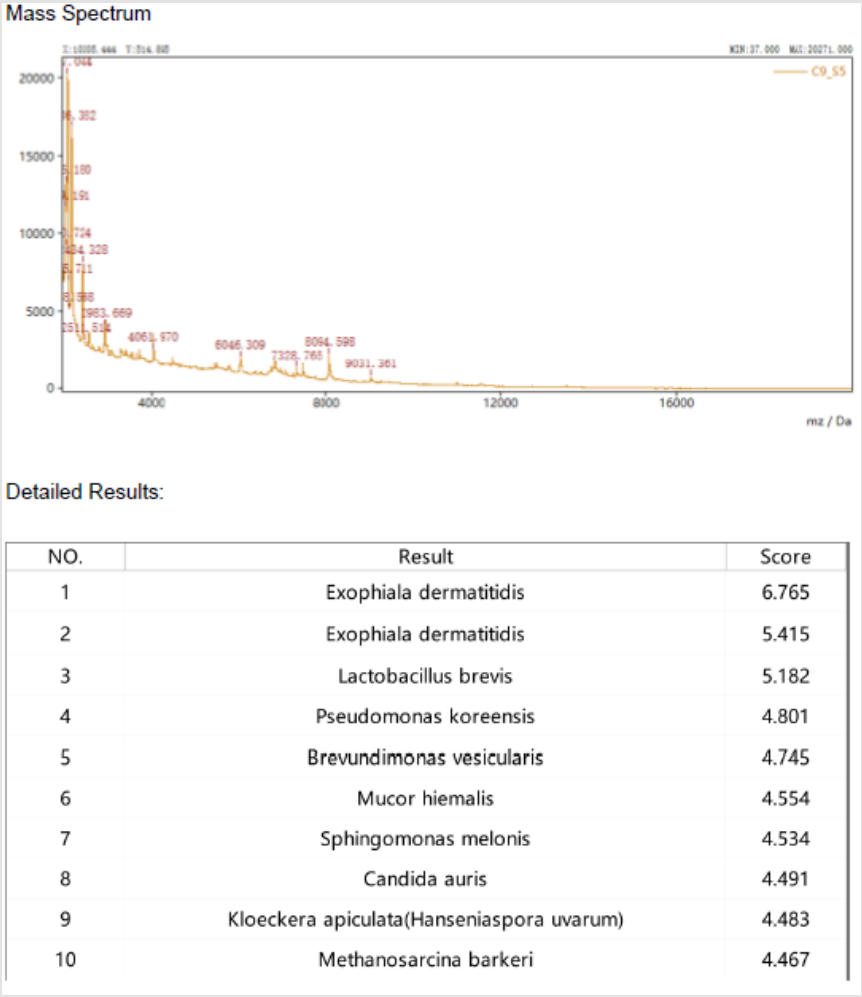
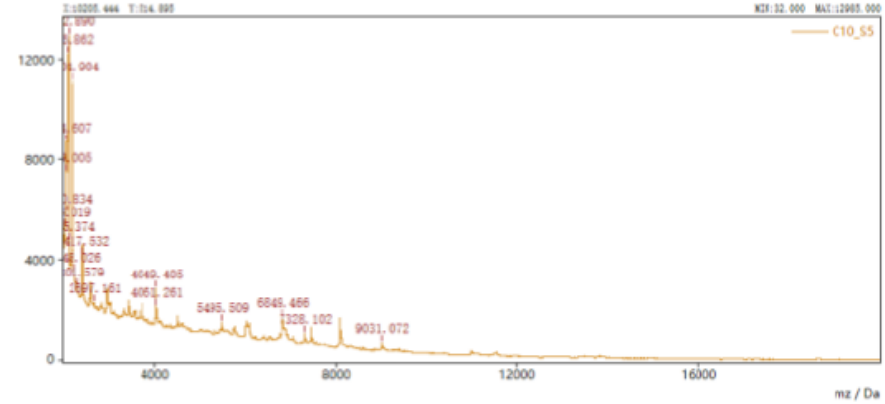



Detailed Results:

| NO. | Result | Score |
|---|---|---|
| 1 | Exophiala dermatitidis | 6.765 |
| 2 | Exophiala dermatitidis | 5.415 |
| 3 | Lactobacillus brevis | 5.182 |
| 4 | Pseudomonas koreensis | 4.801 |
| 5 | Brevundimonas vesicularis | 4.745 |
| 6 | Mucor hiemalis | 4.554 |
| 7 | Sphingomonas melonis | 4.534 |
| 8 | Candida auris | 4.491 |
| 9 | Kloeckera apiculata(Hanseniaspora uvarum) | 4.483 |
| 10 | Methanosarcina barkeri | 4.467 |

**Figure 3:** Autof ms1000 Identification Result: Exophiala dermatitidis 6.765; Generated by Autobio, on the 02/14/2022 at 17:28.




Detailed Results:

| NO. | Result | Score |
|---|---|---|
| 1 | Exophiala dermatitidis | 6.702 |
| 2 | Aspergillus inflatus(Penicillium inflatum) | 4.884 |
| 3 | Pseudomonas koreensis | 4.541 |
| 4 | Kloeckera apiculata(Hanseniaspora uvarum) | 4.494 |
| 5 | Exophiala dermatitidis | 4.432 |
| 6 | Sulfitobacter donghicola | 4.323 |
| 7 | Trichoderma afroharzianum | 4.293 |
| 8 | Marinobacter salarius | 4.283 |
| 9 | Streptomyces cacaoi subsp. cacaoi | 4.247 |
| 10 | Trichoderma harzianum | 4.244 |

**Figure 4:** Identification Result: Exophiala dermatitidis 6.702; Generated by Autobio, on the 02/14/2022 at 17:28.

Wrong microbial identification is among the leading causes of antibiotic resistance, and it is crucial to strive to avoid generating some more, curing inappropriate pathogens with the wrong antibiotics. Despite the knowledge of the existing possibilities to make routine laboratory identification mistakes such as contamination and other reckless manual errors that may lead to diagnostic bugs, this report highlights that MALDI-TOF MS will probably no longer produce identification mistakes. This analytic technique could be the undeniable technique of microbial identification, for sure. Thus, although it is associated with expensive maintenance contracts with producers, using this instrument in the laboratory could be recommended and financed in and for remote areas or crowded hospital laboratories of Low to Middle-Income Countries (LMIC) to sustain the fight against AMR. It could be the subtractable laboratory instrument in settings adhering to global antimicrobial stewardship programs. Further analysis of the same cultures could have been performed on other MS instruments to assess the other instrument's abilities to identify these fungal isolates.

The findings will have probably helped in understanding if the problem is at the level of the MALDI-TOF databases that are not yet well updated as far as fungal agents are concerned or if it is a matter of the genuine ability of E. dermititidis to bypass most detection mechanisms due to its phylogenic structure. Overcoming this problem in the microbiology laboratory will be highly helpful since most fungal pathogens deemed to create antifungal drug resistances are phylogenic ones.

## Conclusion

E. dermititidis and *C. guillermondii* are both very rare fungal pathogens, but in the rise of infective fungal agents. They surely need to be monitored, just like in the abovementioned case. Accurate and precise identification of both species cannot be underestimated nor undermined in a controlled system, as risks of misidentifications, even with the most recent technologies, still bear some critical risks. *E. dermatitidis*, more significantly, has to benefit from careful identification techniques. MALDI-TOF MS yet struggles to provide the laboratory with precise grade scores upon its identification, and several research reports on its identification with molecular technique do admit limitations on the possibility of providing the right diagnostic at first try. It is, therefore, usual that most routine laboratories would instead go for several conventional identification techniques to confirm their primary result or rule out suspects.

## Conflict of Interest

There is no conflict of interest between the authors.

## Acknowledgement

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