Comparative Study of Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry and Culture Test for *Candida* Identification

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Abstract

Background: A new microorganism identification method using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) has been developed, however, reports on its use for delineating *Candida* spp. are scarce. **Objectives:** The purpose of this study was to compare the identification accuracy of mixed infection between culture test and MALDI-TOF MS. **Materials and Methods:** Eighty-nine denture wearers (average 74.0 ± 9 years) were selected. Specimens were immediately inoculated onto selective medium for CHROMagar™ Candida, and were also carried out using MALDI-TOF MS. The distribution frequencies of them were analyzed. **Results:** The numbers and rates of detection/non-detection by MALDI-TOF MS of genus *Candida* were 58/31 (65.2%/34.8%), respectively. Infection types were single infection in 34 (38.2%), mixed infection in 24 (27.0%), and non-infection in 31 (34.8%) cases. Concerning the single infection, *C. albicans* was the most predominant (58.8%), followed by *C. parapsilosis* (17.6%), *C. glabrata* (14.7%), *C. tropicalis* (5.9%), and *C. krusei* (2.9%). As for the mixed infection, the most frequent combination was *C. albicans* and *C. glabrata* (50.0%), followed by *C. albicans* and *C. parapsilosis* (29.2%), *C. albicans* and *C. tropicalis* (8.3%), *C. glabrata* and *C. tropicalis* (4.2%), *C. albicans*, *C. glabrata*, and *C. parapsilosis* (4.2%), and *C. albicans*, *C. parapsilosis*, and *C. glabrata* (4.2%). There were four MALDI-TOF MS positive results that were negative by the culture test. Conversely, there were six MALDI-TOF MS negative results that were positive by the culture test. The sensitivity and specificity of MALDI-TOF MS were 0.929 and 0.840,

respectively. The concordance rate of genus *Candida* was 0.644, indicating substantial agreement. **Conclusion:** *Candida* infection is complicated by disease type and oral cavity environment changes due to aging. A rapid microorganism detection method, such as MALDI-TOF MS, will be helpful to quickly determine the causative pathogen in dental infections.

Keywords

Candida, Culture Test, MALDI-TOF MS, Non-C. Albicans *Candida* (NCAC)

1. Introduction

Candida is a large genus of ascomycetous yeast, consisting of about 150 species, and more than 20 species have clinical importance [1]. *Candida* spp. are found in the oral cavity of 25% - 50% of healthy individuals, including adults and children. When only denture wearers are considered, these frequencies increase to 60% - 100% [2]. Moreover, wearing a dental prosthesis such as a removable denture [3] or removable orthodontic appliance [4] enhances oral candidal colonization and predisposes the wearer to oral candidiasis.

Candida albicans is the most frequently isolated species and the causative species of almost 70% of *Candida* infections [5] [6]. Although *C. albicans* is a well-known colonizer and pathogen of the oral mucosa, non-*C. albicans Candida* (NCAC) is increasingly encountered [7] [8] [9] and its emerging role in human infections has gained attention [10] [11]. This rising number in infections caused by NCAC species may reflect both the improvement in diagnostic methodologies and the superior capability of NCAC species to persist in the host compared with *C. albicans*. Moreover, in human mixed candidiasis, NCAC species demonstrate high antifungal resistance profiles 10).

CHROMagar™ *Candida* is a very useful medium to distinguish *Candida* spp. such as *C. albicans*, *C. tropicalis*, *C. krusei*, and *C. glabrata*, which account for almost 90% of all clinical yeastisolates, including dental samples [12] [13]. However, CHROMagar™ *Candida* cannot definitely distinguish *C. albicans* from *C. dubliniensis* [14] [15], which is more often isolated from HIV-positive patients. In addition, the accuracy of CHROMagar™ to distinguish particular *Candida* spp. from mixed infection is poorly examined. Namely, insufficient evidence is available about the quality of CHROMagar™ *Candida* testing in patients with various underlying conditions. A new microorganism identification method using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) has been developed, however, reports on its use for delineating *Candida* spp. are scarce [16] [17] [18].

Therefore, the purposes of this study were to compare the identification accuracy of mixed infection between CHROMagar™ *Candida* and MALDI-TOF MS in older denture wearers, and to highlight gaps in knowledge and the justification for the present work about candidal distribution.

2. Material and Methods

2.1. Subjects

Eighty-nine denture wearers (49 males, 40 females) who had visited Kubota Dental Hospital for denture adjustment/checkup from August 2017 to September 2018 were selected for inclusion in this study. The mean age and standard deviation of subjects were 74.0 ± 9 years (range, 43 - 99 years). Inclusion criteria were individuals who: 1) were wearing full/partial dentures; 2) had data for oral examination and microbiological tests; 3) had agreed to the study. Exclusion criteria were individuals who: a) were taking antifungal agents or using antiseptic mouthwashes; b) were taking any medication known to predispose them to oral candidiasis; c) had a medical history that revealed any disease or medical condition (such as diabetes mellitus or anemia) that predisposed them to oral candidiasis or promoted candidal carriage in the 6 weeks before the study; or d) for whom necessary data were lacking. None of the included subjects had signs or symptoms suggestive of candidal paronychia or dermatologic fungal infection.

2.2. Microbiological Examination

1) Sample collection

To reduce the bias in sampling procedures, microbiological samples were collected from all patients by swabbing denture mucosal surfaces 10 times with a cotton swab dipped in sterile purified water approximately 2 hours or more after breakfast eating.

2) Culture test using CHROMagar™

Specimens were then immediately inoculated onto selective medium for *Candida*, CHROMagar™ *Candida* (KANTO KAGAKU, Tokyo, Japan). After aerobic incubation for 24 - 48 hours at 25°C, *Candida* spp. were manually measured and enumerated by colony morphology and color. According to the manufacturer's instructions, *C. albicans* colonies are distinguished by a distinctive greencolor, *C. glabrata* colonies exhibit a purple to pale pink color, *C. tropicalis* colonies have a dark blue color with pink edge, *C. krusei* colonies are rough with pale pink centers and white edges, and *C. parapsilosis* colonies present a white or pale pink color. After the 48-hour incubation on CHROMagar™, incubation was continued for more than 2 weeks at 37°C to confirm not to culture.

3) MALDI-TOF MS analysis

Species determination of all samples was also carried out using MALDI-TOF MS (BD Bruker MALDI Biotyper™; BD). Fungal colonies were thinly applied to the target plate using a toothpick, 1 μl of matrix was added, and samples were measured according to the manufacturer's protocol. Application of a sample is one time, and MALDI Biotyper measures the strain several times and calculates average value of each peak of a mass spectrum and the strength. Acquired spectrum were expressed as a score value by phylogenetic tree analysis using a pattern matching spectral library. The case where the score value was 2.0 or more was taken as the identification result at the bacterial species level.

2.3. Statistical Analysis

The distribution frequencies of *Candida* spp. of culture test and MALDI-TOF MS were analyzed. The coincidence rate of culture testing and MALDI-TOF MS was examined using the kappa coefficient with 95 confidence interval [CI]. The software used by statistical analysis of this research was Bell Curve for Excel.

2.4. Compliance with Ethical Standards

Informed consent was obtained from all individuals included in the study. All procedures in studies involving human participants were conducted in accordance with the ethical standards of the Committee on Studies Involving Human Beings of Nihon University School of Dentistry at Matsudo (EC-15-14-033-1) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

3. Results

3.1. Subject Characteristics

Characteristics of subjects in the present study were shown in Table 1. Subjects consisted of 49 males and 40 females (average age 74.1 ± 9.1). Denture types included upper full denture (n = 25), lower full denture (n = 2), upper partial denture (n = 47), and lower partial denture (n = 15). The mean number of artificial teeth indentures was 9.88. Gross findings of mucosal surfaces of the denture base identified 18 cases (20.2%) of denture stomatitis (DRS) and 71 cases (79.8%) with no evidence of DRS. Concerning DRS, 13 cases (72.2%) had reddish mucosa, 3 cases had (16.7%) whitish mucosa, and 2 cases (11.1%) had mixed red and white mucosa.

Table 1. Characteristics of subjects.

Item	Character	
	Male	49
Sex	Female	40
	Total	89
	Male	74.1 ± 9.0
Ave. ± SD[*]	Female	74.1 ± 9.0
	Total	74.1 ± 9.1
	Upper full denture	25
	Lower full denture	2
Denture types	Upper partial denture	47
	Lower partial denture	15
Artificial teeth	Average number	9.88
	Reddish mucosa	13
	Whitish mucosa	3
Denture stomatitis	Mixed red and white	2
	Total	18

*: Average age ± standard deviation.

3.2. Microbiological Distribution

The results of microbiological distribution were listed on the cross tabulation in Table 2.

1) Culture test using CHROMagar™

Candida colonies were detected from the denture mucous membrane of 59 (66.3%) subjects. Infection types included single infection in 34 (57.6%) subjects and mixed infection in 25 (42.4%) subjects. Concerning single infection, *Candida* distribution by colony morphology and color were as follows: *C. albicans* in 24 (70.6%), *C. parapsilosis* in 4 (11.8%), *C. glabrata* in 4 (11.8%), and *C. tropicalis* in 2 (5.9%) cases. As for the 25 cases of mixed infection, *C. albicans* and *C. glabrata* were identified in 15 (60.0%) cases, *C. albicans* and *C. parapsilosis* were identified in 5 (20.0%) cases and *C. albicans* and *C. tropicalis* were identified in 3 (12.0%) case, *C. albicans* and *C. krusei* in 1 (4.0%) and *C. albicans*, *C. parapsilosis*, and *C. tropicalis* were identified in 1 (4.0%) case.

2) MALDI-TOF MS analysis

The numbers and rates of detection/non-detection by MALDI-TOF MS of genus *Candida* were 58/31 (65.2%/34.8%), respectively. Infection types were single infection in 34 (38.2%), mixed infection in 24 (27.0%), and non-infection in 31 (34.8%) cases. Concerning the 34 cases of single infection, *C. albicans* was the

Table 2. Cross tabulation between culture test (CHROMagar™) and MALDI-TOF MS analysis.

							MALDI-TOF MS									
		C.a*	C.g**	C.k***	C.p†	C.t††	C.a, C.g	C.a, C.k	C.a, C.p	C.a, C.t	C.g, C.t	C.a, C.g, C.p	C.a, C.p, C.t	Non-detection	Total	
CHROMagar™	C.a	17	1	0	0	1	0	0	0	0	0	0	0	5	24	70.8
	C.g	0	4	0	0	0	0	0	0	0	0	0	0	0	4	100.0
	C.k	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
	C.p	0	0	1	3	0	0	0	0	0	0	0	0	0	4	75.0
	C.t	0	0	0	0	1	0	0	1	0	0	0	0	0	2	50.0
	C.a, C.g	0	0	0	0	0	11	0	2	0	1	1	0	0	15	73.3
	C.a, C.k	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0.0
	C.a, C.p	0	0	0	1	0	0	0	2	0	0	1	0	1	5	40.0
	C.a, C.t	0	0	0	1	0	0	0	0	1	1	0	0	0	3	33.3
	C.g, C.t	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
	C.a, C.g, C.p	0	0	0	0	0	0	0	0	0	0	0	0	0	0	-
	C.a, C.p, C.t	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0.0
	Non colonies	3	0	0	1	0	1	0	0	0	0	0	0	25	30	83.3
	Total (n)	20	5	1	6	2	12	0	7	1	2	2	0	31	89	
	Concordance rate (%)	85.0	80.0	0.0	50.0	50.0	91.7	-	28.6	100.0	0.0	0.0	-	80.6	64	71.9

C.a*: *Candida albicans*; *C.g***: *Candida glabrata*; *C.k****: *Candida krusei*; *C.p*†: *Candida parapsilosis*; *C.t*‡: *Candida tropicalis*.

most predominant (n = 20, 58.8%), followed by *C. parapsilosis* (n = 6, 17.6%), *C. glabrata* (n = 5, 14.7%), *C. tropicalis* (n = 2, 5.9%), and *C. krusei* (n = 1, 2.9%). As for the 24 cases of mixed infection, the most frequent combination was *C. albicans* and *C. glabrata* (n = 12, 50.0%), followed by *C. albicans* and *C. parapsilosis* (n = 7, 29.2%), *C. glabrata* and *C. tropicalis* (n = 2, 8.3%), *C. albicans*, *C. glabrata*, and *C. parapsilosis* (n = 2, 8.3%), and *C. albicans* and *C. tropicalis* (n = 1, 4.2%).

3) Comparison of culture test and MALDI-TOF MS results

There were four MALDI-TOF MS positive results (*C. albicans*, n = 2; *C. albicans* and *C. glabrata*, n = 1; and *C. albicans* and *Penicillium* spp., n = 1) that were negative by the culture test. Conversely, there were six MALDI-TOF MS negative results (sample degeneration) by drying, n = 4; *Aspergillus spp.*, n = 1; and *C. albicans* and *Penicillium* spp., n = 1) that were positive by the culture test.

3.3. Statistical Results

The sensitivity and specificity of MALDI-TOF MS were 0.929 and 0.840, respectively. The concordance rate (kappa coefficient) of genus *Candida* was 0.644, indicating substantial agreement [19]. Table 1 shows all concordance rates for single and mixed infections of all *Candida* spp. and combinations of mixed infection. Concordance rates of single infection were as follows: *C. albicans* 85.0%, *C. glabrata* 80.0%, *C. parapsilosis* 50.0%, *C. tropicalis* 50.0%, and *C. krusei* 0.0%. Furthermore, concordance rates of mixed infection were as follows: *C. albicans* and *C. tropicalis* 100.0%, *C. albicans* and *C. glabrata* 91.7%, *C. albicans* and *C. parapsilosis* 28.6%, *C. glabrata* and *C. tropicalis* 0.0%, and *C. albicans*, *C. glabrata*, and *C. parapsilosis* 0.0%. In addition, the concordance rate of *Candida* negative was 80.6%.

4. Discussion

The elderly are known to be more vulnerable to fungal infections because of underlying conditions, such as chronic diseases, medications, poor oral hygiene, reduced salivary flow, and immune system impairment [20] [21] [22]. Furthermore, fungi are isolated not only from the oral cavity but also from tissue-fitting surfaces and outer surfaces of dentures [21]. Therefore, in this study, we evaluated two *Candida* spp. detection methods, culture test and MALDI-TOF MS, in older denture wearers (mean age, 74 years) without subjective symptoms.

C. albicans is the most frequently isolated *Candida* spp. as a colonizer and pathogen of the oral mucosa but reports on the appearance of NCAC are increasing [23] [24] [25]. This shift towards NCAC species is mainly due to severe immunosuppression, use of broad-spectrum antibiotics, and empirical use of antifungal drugs [7] [8] [9]. Nevertheless, NCAC infections are usually indistinguishable based on symptoms alone due to similarities in clinical presentations. Consequently, the need for improvement of NCAC mixed infection diagnostic methodologies has increased.

The present study detected 66.3% and 65.2% of NCAC by culture test and MALDI-TOF MS, respectively, and these values were higher than that of a previous study (58.5%) [26]. CHROMagar™ is a commercial product that was developed for the isolation and presumptive identification of specimens containing mixtures of yeast species [27]. CHROMagar™ *Candida* is a very useful medium to distinguish *Candida* spp. with different morphologies and colors, which account for almost 90% of all clinical yeastisolates, including dental samples [12] [13]. The specificity and sensitivity of *C. albicans*, *C. tropicalis*, and *C. krusei* identification exceed 99% [27]. However, changes in the oral environment, such as during severe immunosuppression, increase the number of NCAC species, and the sensitivity of CHROMagar™ *Candida* in detecting mixed infections with NCAC has not been sufficiently examined.

Bacterial identification by MALDI-TOF MS in outsourced inspection has gained attention as a rapid diagnostic method for clinical use. Advantages of MALDI-TOF MS included small bacterial sample needed, easy preprocessing, and high spectral analyses. Numerous species have been identified at the genus level by MALDI-TOF MS; particularly, the same number of species have been identified for genera *Candida* and *Cryptococcus* [28] [29]. However, MALDI-TOF MS data on *Candida* genus remain scarce.

In the present study, MALDI-TOF MS analyses demonstrated that *C. albicans* (58.8%) remained the predominant species in oral candidiasis patients respectively, followed by *C. parapsilosis* (17.6%), *C. glabrata* (14.7%), *C. tropicalis* (5.9%), and *C. krusei* (2.9%). Although the species distribution was similar to those of other reports [5] [6], the proportion of NCAC was higher [26] [30] [31]. In the previous two decades, *C. albicans* represented over 80% of all human candidiasisisolates [32]. In this study, *C. glabrata* and *C. parapsilosis* proportions were particularly high. *C. glabrata* and *C. parapsilosis* have emerged as significant nosocomial pathogens. *C. glabrata* is associated with immunosuppressive and antimicrobial treatments [10] [33], while *C. parapsilosis* is associated with invasive procedures [11]. The higher proportions of NCAC species observed in the present study may be explained by the study cohort characteristics (older subjects with chronic diseases) and increased immunosuppression by advanced medical treatment. Thus, the oral cavities of hospitalized older adults contain greater species diversity than previously thought and is not limited to typical *Candida* species.

Although most *Candida*-positive carriers (62.9%) possessed only one *Candida* spp., 37.1% of subjects harbored more, which was similar to the report by Lockhart *et al.* [22]. Although *Candida* spp. are generally not very aggressive, they may cause infection if the immune system is impaired or if an environmental niche becomes available. Mixed infection involving NCAC has been reported in recent years. Specifically, 36.6% of cases were *C. parapsilosis* species mixed. Furthermore, *C. tropicalis*, *C. glabrata* species mixed, and *C. krusei* comprised 35.4%, 24.3%, and 3.7% of all isolates, respectively [34]. In the present study, the

most frequent combination was *C. albicans* and *C. glabrata* (50.0%), and 87.5% of mixed infection was a combination of *C. albicans* and NCAC. Accordingly, we focused the results of our study on the distribution of *Candida* species. The microbiological examination showed that the most frequently isolated species were *C. albicans*, *C. glabrata*, and *C. parapsilosis*. The latter species belong to NCAC species that have recently caused systemic candidiasis, and fatal cases of *C. glabrata* infection have been reported [35] [36].

The kappa coefficient between culture test and MALDI-TOF MS was 0.644, which indicated substantial agreement 19). The highest concordance rate of single infection was *C. albicans* (85.0%), followed by *C. glabrata* (80.0%). These high concordance rates may be explained by the ease of distinguishing *C. albicans* and *C. glabrata* colonies by color (green and deep purple, respectively). Conversely, *C. parapsilosis*, *C. tropicalis*, and *C. krusei*, which have similar colony colors, presented low concordance rates. In addition, the concordance rate of *Candida* negative was 80.6%. Furthermore, concerning mixed infections, *C. albicans* and *C. parapsilosis*, *C. glabrata* and *C. tropicalis*, and *C. albicans*, *C. glabrata*, and *C. parapsilosis* showed low or non-concordance rates. We speculate that the number of fungi on agar plates was large, colonies were fused due to mixed infection, and identification by colony color is subjective. However, these issues do not affect identification by MALDI-TOF MS. Furthermore, our findings suggested that degeneration due to fungal death leads to overdiagnosis by the culture test. The sensitivity and specificity rates of MALDI-TOF MS were high, even in cases of mixed infection. It was speculated that the reason for the very few misidentifications was caused by spectral pattern matching for each *Candida* species. More than one strain by the same species was registered in the spectral library, so even if diversity with the spectral pattern existed between strains, it should be possible to correctly identification. With the increase in NCAC and multiple infections, the value of MALDI-TOF MS application is recognized for *Candida* identification. In addition, there is a need to improve the criteria for simple CHROMagar™ culture testing for diagnosis of oral infections.

In conclusion, *Candida* mixed infection was observed in 27.0% of older Japanese denture wearers. *Candida* infection is complicated by disease type and oral cavity environment changes due to aging. A rapid microorganism detection method, such as MALDI-TOF MS, will be helpful to quickly determine the causative pathogen in dental infections.

5. Conclusions

The following could be concluded in the present study:

1) The numbers and rates of detection by MALDI-TOF MS of genus Candida were 65.2% of elder denture wearers.

2) The rates of single and mixed infection were 38.2/27.0%, respectively.

3) *C. albicans* (58.8%) of single and *C. albicans* and *C. glabrata* (50.0%) of mixed infection were the most predominant.

4) The sensitivity and specificity of MALDI-TOF MS were 0.929 and 0.840, respectively.

Acknowledgements

Conflicts of Interest

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

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