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Digestive fungal flora in asymptomatic subjects in Bobo-Dioulasso, Burkina Faso

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PEER REVIEW

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Comments

The paper describes several *Candida* species and their percentages found in fecal and urine samples of asymptomatic voluntary donors in a town of Burkina Faso. The authors used MALDI-TOF MS method which is supposed to be one of the most accurate and succinct methods for identification of fungi and other microorganisms. Although the present work is performed on asymptomatic donors, the work would help in future diagnosis of patients with candidiasis.
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ABSTRACT

Objective: To identify *Candida* species in asymptomatic subjects in Bobo-Dioulasso (Burkina Faso) by the matrix-assisted laser desorption ionization-time of flight mass spectrometry.
Methods: A cross-sectional study was conducted from January to February 2013 in Bobo-Dioulasso to collect fecal and urine specimens from voluntary donors. Fungal strains were isolated on Sabouraud dextrose agar and analyzed using matrix-assisted laser desorption ionisation-time-of-flight mass spectrometry.
Results: A total of 135 samples including stools (78.5%, 106/135) and urine (21.5%, 29/135) were analyzed. The results revealed that fecal specimens contained mainly *Candida krusei* (*C. krusei*) (42.5%) followed by *Candida albicans* (29.3%), *Candida glabrata* (18.0%) and *Candida tropicalis* (*C. tropicalis*) (4.7%). *C. krusei* (34.6%) was also found to be the most frequently identified in urine samples followed by *Candida albicans* (27.0%), *C. tropicalis* (15.4%) and *Candida parapsilosis*. However, uncommon species such as *Candida nivariensis*, *Candida kefyr*, *Candida norvegensis*, *Candida parapsilosis*, *Candida lusitaniae* and *Candida robusta* were also identified from fecal and urines samples.
Conclusions: This study noted the emergence of species such as *C. krusei*, *Candida glabrata*, *Candida parapsiolosis*, *C. tropicalis*, *Candida nivariensis*, *Candida norvegensis*, and others. It is an imperative to take into account the existence of these species in the therapeutic management of patients in Bobo-Dioulasso.

KEYWORDS
Candida, Mass spectrometry, Matrix-assisted laser desorption ionization-time of flight, Bobo-Dioulasso

1. Introduction

Invasive fungal infections caused by *Candida* spp. remain a major cause of morbidity and mortality in the immunocompromised individuals, and more than 150 species of yeast have now been associated with human pathologies[1-3]. Although *Candida albicans* (*C. albicans*) remained the predominant agent of nosocomial infections, an increasing number of infections are being attributed to non-albicans species, such as *Candida glabrata* (*C. glabrata*), *Candida parapsilosis* (*C. parapsilosis*), *Candida tropicalis* (*C. tropicalis*),

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Candida lusitaniae, and *Candida krusei* (*C. krusei*) that emerged over recent years as significant opportunistic pathogens[4,5].

Recent studies concerning the epidemiology of invasive mycoses showed a significant change in the profile of *Candida* species involved in human pathology[2]. Many strains of *C. albicans* are still susceptible to antifungals; however species such as *C. krusei*, *C. glabrata*, *Candida bracarensis*, *Candida nivariensis* (*C. nivariensis*), *C. parapsilosis* and *Candida guilliermondii* have developed intrinsic resistance to triazoles, amphotericin B or to echinocandins[6,7]. It is therefore essential to consider these changes in fungal ecology upon the therapeutic management of patients, particularly for empirical prescription.

Given the inherently variable antifungal susceptibility profiles of *Candida* spp., the correct identification of the species is often critical for efficient therapeutic decisions. The identification of the species therefore remains a crucial element in prediction of the response to antifungal treatment[8]. The main *Candida* spp. associated with human disease are readily identified by conventional mycological methods, which rely upon a combination of morphological features coupled with the abilities of the organisms to metabolize selected sugars or assimilate a variety of carbon and nitrogen sources. This requires two to five days or more in the case of uncommon species[9]. These phenotypic methods can sometimes lead to misidentification or imprecise identification, especially for species phylogenetically proximate, such as *C. albicans*/*Candida dubliniensis*, *Candida inconspicua*/*Candida norvegensis* (*C. norvegensis*) or *C. glabrata*/*C. nivariensis*/*Candida bracarensis*[9,10]. Thus, the adequate identification of these species often requires the combination of several phenotypic and molecular approaches to increase the discrimination level. The molecular approaches are based on the analysis of the genes encoding the ribosomal RNA. More recently, molecular methods, including restriction fragment length polymorphism, sequencing of internal transcribed spacer regions, multilocus sequence typing, and barcoding have been used to identify clinical and nonclinical isolates. All these methods are relatively expensive and time-consuming[11,12]. Recent studies report the use of mass spectroscopy for the identification of yeasts as a serious alternative for conventional identification methods[13,14].

Burkina Faso is a sub Saharan country, with endemic HIV infection and fungal infections. Despite this, there is no coordination on the question of HIV and opportunistic mycoses. In the country, even though the data exist on the identification and management of therapeutics bacteria, there is limited data on fungal infections. The present study was conducted in Bobo-Dioulasso, a town located in the south-western of Burkina Faso. The main objective was to identify *Candida* species by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (MS) in asymptomatic individuals.

2. Materials and methods

2.1. Yeast collection strains

A cross-sectional study was conducted to collect fecal and urine samples from voluntary donors for a total period of two months in the laboratory of Parasitology-Mycology in the university hospital of Bobo-Dioulasso. The collected samples included urines and stool. Fungal strains were isolated from these samples by cultivation on Sabouraud dextrose agar and frozen in sterile water at -20 °C until used[9].

2.2. Sample preparation

Pure fungal strains from frozen stocks were obtained by 24 to 48 h incubation on BBL-CHROME agar™ or CandiSelect4™ or on Sabouraud medium supplemented with antibiotics[15,16]. For the MALDI-TOF assay, the on-target extraction method was used. In brief, microorganisms from a colony were applied directly to a disposable target slide using 1 μL loop[15,17]. They were lysed with 1.2 μL of 70% formic acid and dried at room temperature. Subsequently, each sample was overlaid with 1.2 μL of matrix solution, a saturated solution of α-cyano-4-hydroxycinnamic acid (CHCA), and air dried at room temperature.

The CHCA matrix co crystallized with the sample and dried on a metal plate (96-AnchorChip™ spot or spots Polished Steel™ 384, Bruker Daltonics) on the site of a spot. The reading of the plate was made in 24 h by MALDI-TOF system (Bruker Daltonics GmbH, Bremen, Germany).

3. Results

A total of 135 fecal samples (78.5%, 106/135) and urine (21.5%, 29/135) from voluntary donors were analyzed by the MALDI-TOF assay. *Candida* was the only genus of yeast identified by the MALDI-TOF. A total of 132 samples (97.8%, 132/135) were successfully identified through direct deposit on the target. Three urine samples (2.2%, 3/135) were neither identified after direct deposit nor after complete extraction of proteins. Thus success rate of species identification in MALDI-TOF was 97.8% (132/135).

From 106 species identified from fecal samples, *C. krusei* (42.5%) was the most frequent followed by *C. albicans* (29.3%), *C. glabrata* (18.0%) and *C. tropicalis* (4.7%) (Figure 1). Uncommon species such as *C. nivariensis*, *Candida kefyr*, *C. norvegensis*, *C. parapsilosis*, and *Candida robusta* were also identified.

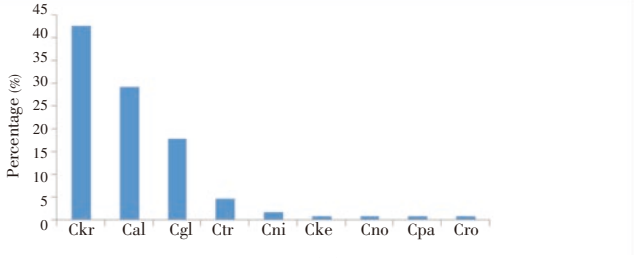

Figure 1. *Candida* species identified in stools by MALDI-TOF assay.
Ckr: *C. krusei*, Cal: *C. albicans*, Cgl: *C. glabrata*, Ctr: *C. tropicalis*, Cni: *C. nivariensis*, Cke: *Candida kefyr*, Cno: *C. norvegensis*, Cpa: *C. parapsilosis*, Cro: *Candida robusta*.

For urine, a total of 26 samples were successfully analyzed by MALDI-TOF system. *C. krusei* (34.6%) was the most frequent followed by *C. albicans* (27%), *C. tropicalis* (15.4%), and *C. parapsilosis* (Figure 2). *C. glabrata*, *Candida lusitaniae* and *Candida robusta* were the uncommon species identified from urines.

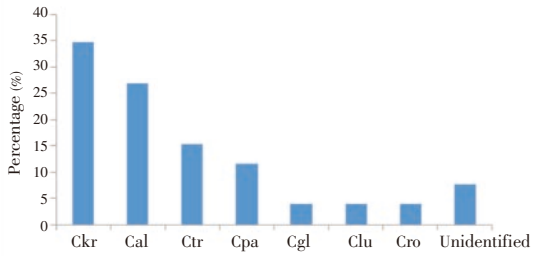

Figure 2. *Candida* species identified in urine by MALDI-TOF assay.
Ckr: *C. krusei*, Cal: *C. albicans*, Ctr: *C. tropicalis*, Cpa: *C. parapsilosis*, Cgl: *C. glabrata*, Clu: *Candida lusitaniae*, Cro: *Candida robusta*.

4. Discussion

The recent evolution of epidemiology of invasive fungal infections is marked by a spectacular increase in non-albicans species. Diversity of non-albicans species prompted biologists to develop new tools for molecular and immunological characterization of some unusual species whose identification by phenotype methods has proven difficult. More recently, MALDI-TOF MS has been proposed as alternative conventional methods[18]. The present study was designed as identifying some *Candida* species isolated from stool and urine samples in Bobo-Dioulasso by using MALDI-TOF system. According to our results, *C. krusei* prevailed in fecal samples and urine samples. Usually, this species is non-pathogenic in the absence of subjacent immunosuppression; this justifies this high prevalence in asymptomatic voluntary donors. In contrast, previous studies showed the predominance of *C. albicans* in 50% of pathological samples beside the other species emerging such as *C. krusei*, *C. glabrata*, *C. parapsiolosis*, *C. tropicalis*, *C. nivariensis*, *C. norvegensis*, and others[2,4].

Candida albicans and most of non-albicans yeasts identified in this study such as *C. krusei*, *C. glabrata*, *C. parapsiolosis*, *C. tropicalis*, *C. nivariensis*, *C. norvegensis* are the saprophytes of the digestive tract of humans and many animals. These yeasts are opportunistic which become pathogenic under the influence of general risk factors notably, imbalance of intestinal flora after taking antibiotics or local risk factors including diabetes and deficit of immunity. Identification of these yeasts in this study corroborates their emerging nature in human fungal infections. However, the emergence requires a correct phenotypic and molecular identification for adequate therapeutic management of patients. In our context, further large-scale study in all health regions of Burkina Faso taking into account pathological samples would allow better understanding of their epidemiology.

Furthermore, the identification of some species such as *C. nivariensis*, *C. glabrata*, *C. parapsilosis* which showed intrinsic resistance to usual antifungal should be investigated for antifungal susceptibility[19]. In fact, these strains are from Bobo-Dioulasso, where the prescription of antifungals is almost inexistent compared to antibiotics which are widely prescribed against any infectious. This low usage of antifungals could give another behavior of isolates from Bobo-Dioulasso for the use of antifungal agents. The investigation for antifungal susceptibility would permit a better understanding of the susceptibility of these isolates which showed intrinsic resistance to conventional antifungal such as triazole and amphotericin[19].

Moreover, three strains (2.2%) from this study could not be identified by MALDI-TOF system. In fact, uncommon yeast species are emerging as human pathogens and their identification may pose a challenge when the type strains have not yet been included into the diagnostic databases of identification system. This can lead to misidentification of species. In addition, it is essential that databases designers provide regular updates. This update is major advantage for identification by MALDI-TOF. In fact, two common causes of identification failures may be related to the content of the database[20].

Mass spectrometry MALDI-TOF allows rapid identification of microorganisms isolated in a routine laboratory. The present study is the first conducted in Bobo-Dioulasso. Some emerging species of *Candida* such as *C. krusei*, *C. glabrata*, *C. parapsiolosis*, *C. tropicalis*, *C. nivariensis*, *C. norvegensis*, have been recorded. These species being isolated from asymptomatic subjects could become pathogenic at any time; it is imperative to take into account the existence of these species in the therapeutic management of patients in Bobo-Dioulasso. However, further large-scale study in all health regions of Burkina Faso taking into account pathological samples would allow better understanding of the epidemiology of *Candida* yeasts and establish the cartography of these species.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Invasive infections by *Candida* spp. are one of the major threads particularly for immuno-compromised patients. In candidiasis, *Candida albicans* has long been a major pathogen, but clinical reports of infections of other *Candida* species are rapidly increasing in late years. Because

different *Candida* species show different antifungal drug susceptibility, accurate and succinct methods for species identification are required.

Research frontiers

The authors determined *Candida* species cultured from fecal and urine specimens corrected from asymptomatic subjects in a town of Burkina Faso, using MALDI-TOF method.

Related reports

Recent studies report the use of mass spectroscopy for the identification of yeasts as a serious alternative for conventional identification methods.

Innovations and breakthroughs

Candida krusei and some other *Candida* species which are not usual main human pathogens are found in fecal and urine samples from asymptomatic voluntary donors. The authors are warning and insisting that establishment of proper and simple method for identifying *Candida* species are required, because susceptibility of antibiotics is different among *Candida* species.

Applications

Diagnosis, epidemiology, and preventive measures are applied against candidiasis. The identification of some species such as *C. nivariensis*, *C. glabrata*, *C. parapsilosis* which showed intrinsic resistance to usual antifungal should be investigated for antifungal susceptibility.

Peer review

The paper describes several *Candida* species and their percentages found in fecal and urine samples of asymptomatic voluntary donors in a town of Burkina Faso. The authors used MALDI-TOF MS method which is supposed to be one of the most accurate and succinct methods for identification of fungi and other microorganisms. Although the present work is performed on asymptomatic donors, the work would help in future diagnosis of patients with candidiasis.

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