Medical Mycology Case Reports 2 (2013) 156–158

Contents lists available at ScienceDirect

Medical Mycology Case Reports

journal homepage: www.elsevier.com/locate/mmcr

Candida nivariensis isolated from a renal transplant patient with persistent candiduria—Molecular identification using ITS PCR and MALDI-TOF ☆

Rebecca L. Gorton [a,*], Gareth L. Jones [b], Christopher C. Kibbler [a], Sophie Collier [a]

[a] Department of Microbiology, Royal Free Hospital, Pond Street, London NW3 2QG, United Kingdom
[b] UCL Centre for Nephrology, Royal Free Hospital, Pond Street, London NW3 2QG, United Kingdom

ARTICLE INFO

Article history:
Received 30 July 2013
Received in revised form
16 October 2013
Accepted 17 October 2013

Keywords:
C. nivariensis
MALDI-TOF
ITS
Candiduria

ABSTRACT

We report on the isolation of *Candida nivariensis* from a renal transplant patient with persistent candiduria. Biochemical profiling misidentified isolates as *Candida glabrata* (3/5) and *Candida inconspicua* (2/5). All isolates produced white colonies on CHROMagar™ Candida medium. Internal transcribed spacer (ITS) ribosomal gene sequence analysis and MALDI-TOF-MS analysis (Bruker Biotyper™ 2.0) identified all isolates as *C. nivariensis*, demonstrating the utility of MALDI-TOF as a rapid, accurate approach for the identification of cryptic Candida species.

1. Introduction

Invasive Candida infections are opportunistic, primarily affecting immunocompromised hosts [1,2]. Whilst *Candida albicans* remains the leading aetiological agent, infections with 'non-albicans' species represent a significant burden [1–3]. Candiduria is often thought to mainly represent contamination or colonisation. However, in immunocompromised patients, especially renal transplant patients, it is important to rule out invasive disease, such as pyelonephritis. From limited data *Candida glabrata* has been associated with candiduria in the renal setting [4–6]. This is important clinically as *C. glabrata* is associated with decreased susceptibility to fluconazole [7]. Furthermore in cases of autopsy proven invasive candidiasis where blood cultures have remained negative other samples, such as urine, have been positive, thus providing evidence of the organism causing the invasive infection [8]. It is therefore necessary to fully identify all yeasts from urine in renal transplant patients to guide targeted antifungal therapy when required [9,10].

The emergence of cryptic *Candida spp* has created a new challenge for the routine diagnostic laboratory. Cryptic species may be misidentified as commercially available assays have not yet incorporated *de novo* species within assay databases. *Candida nivariensis,* first described in 2005, is a rare emerging pathogen closely related to *C. glabrata* [11] sharing an almost identical biochemical phenotype, leading to misidentification using commercially available assays. Importantly, recent reports have described *C. nivariensis* isolates with increased resistance to azole antifungal agents when compared with *C. glabrata* [12]. In this paper we report the isolation of *C. nivariensis,* from a renal transplant patient suffering from persistent candiduria in which routine biochemical identification methods misidentified all Candida isolates. For the purpose of this report the initial biochemical identifications are given first and the retrospective *C. nivariensis* identifications are indicated in brackets for isolates that were re-investigated.

2. Case report

The case involves a 27 year old renal patient with a history of chronic renal failure, secondary to vesicoureteric reflux. Following her 3rd renal transplant in 2007, complications included renal artery stenosis, acute cellular and vascular rejection, obstruction with a lower ureteric stricture, poor bladder function and a large pelvic lymphocele. Six months post-transplant she underwent the insertion of a JJ stent, which was subsequently removed after urine cultures became positive with yeasts in February 2008. A left native nephrectomy was performed in August 2008, leaving the

* Corresponding author.
E-mail address: rebecca.gorton@nhs.net (R.L. Gorton).

2211-7539/$ - see front matter © 2013 The Authors. Published by Elsevier B.V. on behalf of International Society for Human and Animal Mycology. All rights reserved.
http://dx.doi.org/10.1016/j.mmcr.2013.10.001

right native kidney and left transplanted kidney *in situ*. Ten episodes of candiduria were recorded by the microbiology laboratory over a 19 month period, Table 1. For the purpose of this report day 0 is defined as the first known isolation of *C. nivariensis*.

Despite yeasts being observed by direct microscopy in some instances almost all cultures were reported either as 'yeasts' or 'no significant growth' with two exceptions. On day 0 the first yeast isolate to be fully investigated was identified as *Candida inconspicua* (*C. nivariensis* isolate URC*n*1) susceptible to fluconazole *in vitro* with an MIC equal to 8 mg/L (Table 1). On day 214 a second isolate was fully identified as *C. inconspicua* (*C. nivariensis* isolate URC*n*2) susceptible to fluconazole *in vitro* with an MIC equal to 8 mg/L (Table 1). Following this fluconazole antifungal therapy was initiated on day 228 at a daily dose of 200 mg (Table 1). On day 274 after 6 weeks of fluconazole therapy a further episode of Candiduria was recorded. The isolate was identified as *C. inconspicua* (*C. nivariensis*, isolate URC*n*3) and was resistant to fluconazole *in vitro* with an MIC > 256 mg/L. On day 309 fluconazole therapy was discontinued after a total of 12 weeks.

Following outpatient fluconazole therapy our patient was re-admitted on day 321 to the renal unit presenting with haematuria, dysuria and lower abdominal pain. On examination she was apyrexial with generalised tenderness over her lower abdomen. Urine analysis indicated the presence of blood, leucocytes and protein. On admission she was considered stable with a creatinine of 103 and eGFR of 58. Her immunosuppressive agents were tacrolimus 3 mg bd, mycophenolate mofetil 750 mg bd, and prednisolone. Cephradine 5 mg nocte was being administered as prophylaxis against bacterial urinary tract infections. During her admission, as her most recent *C. inconspicua* isolate (*C. nivariensis*, isolate URC*n*3) was fluconazole resistant, she was treated empirically with liposomal amphotericin B but suffered related adverse effects so antifungal therapy was discontinued. Her symptoms partially responded to treatment with co-amoxiclav although cultures remained negative for bacteria. Problems with ongoing urinary symptoms continued including cloudy urine, dysuria and abdominal pain. Four further episodes of candiduria were documented between December 2009 and February 2010. On day 393 and 407 two further isolates were fully investigated and both identified as *C. glabrata* (*C. nivariensis* isolates URC*n*4 and URC*n*5). The respective isolates measured susceptible to fluconazole *in-vitro* with an MIC of 4 (Table 1).

To investigate the source of infection, urodynamic studies were performed and showed reflux of urine back up a large dilated ureter on the right. She was admitted for an elective nephrectomy of the native right kidney with 1 week of IV caspofungin and 6 weeks of oral fluconazole as cover as her isolates were found to be susceptible at this point. Although caspofungin is not excreted well into the urine this was to cover possible renal parenchymal infection. Histopathology showed patchy interstitial inflammation but fungal stains were negative. Of note the patient's blood cultures remained negative throughout. Since this operation her candiduria has resolved.

The correct identification of *C. nivariensis* was first indicated in a reference laboratory report for isolate URC*n*3 in November 2009, as this isolate was sent for confirmation of fluconazole resistance. The reference laboratory had investigated the original biochemical identification as isolate URC*n*3 colonies appeared white on chromogenic agar, which is characteristic of several Candida species within the *C. glabrata* clade [13]. This report initiated further investigation at the RFH NHS UKCMN laboratory with retrospective analysis of the five isolates URC*n*1, URC*n*2, URC*n*3, URC*n*4 and URC*n*5. All isolates formed white colonies on chromogenic media (CHROMagar Candida, Becton Dickenson). Using genomic sequencing and MALDI-TOF analysis the isolates were further identified as *C. nivariensis*. DNA amplification of the ribosomal ITS1-5.8S-ITS2 rRNA fragment was performed using primers ITS1 (5' TCC GTA GGT GAA CCT GCG G 3') and ITS4 (5' TCC TCC GCT TAT TGA TAT GC 3') [14] Nucleotide sequencing of the amplicons region was conducted using Big Dye Terminator Cycle Sequencing kit, Version 3.1 (Applied Biosystems, CA, USA) and sequence analysis was performed on an ABI 3130 Genetic Analyser (Applied Biosystems). Consensus sequences were generated from 2 forward and 2 reverse strands using BioNumerics software version 6.5 (Applied Maths, Ghent, Belgium). The sequences obtained were compared with those in the GenBank DNA database. For MALDI-TOF analysis conventional formic acid protein extraction was performed on all isolates. Mass spectra acquisition and analysis was performed on a Bruker Microflex platform using MALDI Biotyper™ 2.0 software. All

Table 1
Specimens from which yeasts were isolated during hospital admission including direct microscopy results for urine specimens and phenotypic identification reported for Candida isolates.

	Date of sample	Specimen	Direct microscopy			Microbiology report	MALDI-TOF/ITS	Isolate	Flu MIC
			WBC		Yeasts (cfu/mL)				
	15/02/08	MSU	10–40	–	10^4–10^5	Yeasts			
	12/09/08	MSU	40–200	–	$>10^5$	Yeasts			
	19/09/08	MSU	>200	–	$>10^5$	Yeasts			
Day 0	01/12/08	MSU	>200	–	$>10^5$	*C. inconspicua*	*C. nivariensis*	URC*n*1	8
	28/01/09	MSU	>200	Yeasts	–	NSG			
	11/02/09	MSU	10–40	Yeasts	–	NSG			
	13/02/09	MSU	40–200	Yeasts	–	NSG			
	01/06/09	MSU	>200	Yeasts	–	NSG			
Day 214	03/07/09	MSU	>200	Yeasts	$>10^5$	*C. inconspicua*	*C. nivariensis*	URC*n*2	8
Day 228	17/07/09	Fluconazole antifungal therapy initiated							
Day 274	01/09/09	MSU	Nil	–	10^3	*C. inconspicua*	*C. nivariensis*	URC*n*3	>256
Day 309	06/10/09	Fluconazole antifungal therapy discontinued							
Day 393	29/12/09	MSU	40–200	Yeasts	$>10^5$	*C. glabrata*	*C. nivariensis*	URC*n*4	4
Day 407	12/01/10	MSU	>200	Yeasts	10^4–10^5	*C. glabrata*	*C. nivariensis*	URC*n*5	4
	16/02/10	MSU	40–200	–	$>10^5$	*C. inconspicua*	–		
	26/02/10	MSU	40–200	–	10^3	*C. glabrata*	–		
	08/04/10	MSU	10–40	–	–	NSG			
	11/01/11	MSU	>200	–	–	NSG			

MSU = mid stream urine, NSG = no significant growth, LVS = lower vaginal swab, cfu = colony forming units, Mod = moderate, Occ = occasional, Flu = fluconazole.

isolates were identified as *C. nivariensis* in less than 15 min with confidence log scores ranging between 2.193 and 2.226 indicating secure genus probable species identification.

3. Discussion

This case highlights the difficulty of interpreting the clinical significance of yeasts isolated from urine, especially in high risk patients such as renal transplant recipients. A recent retrospective study including 996 renal patients, indicated a cumulative candiduria incidence of 3.4% over 2 years [15]. However, not all of these episodes required treatment and no adverse events were noted in those patients who were not treated. Another nested case control study, which looked at 1738 renal transplant patients over an 8 year period found that candiduria was associated with a worse outcome [4]. Unfortunately, treatment did not improve survival rates. This supports the view that candiduria is a marker of those patients who are more severely unwell rather than directly affecting mortality. Risk factors for candiduria were similar to those in the non-transplant population and included female sex, ITU admission, antibiotics in the previous month, presence of an indwelling catheter, diabetes and neurogenic bladder. Despite the fact that candiduria may represent contamination, in immunocompromised patients, especially renal transplant patients it is important to rule out invasive disease. Although unproven the likely source of candiduria in our patient was the right native kidney, supported by the fact that post nephrectomy the candiduria resolved.

Current guidelines from the British Society of Medial Mycology strongly recommend that all Candida isolates from urine in the transplant setting are fully identified [8]. Biochemical profiling is currently the primary method for yeast identification in most diagnostic laboratories. Cryptic *de novo* species such as *C. nivariensis* are not incorporated into databases for commercially available assays and are mis-identified. The confusing scenario of mixed candiduria in this case further complicated the clinical relevance of candiduria. Repeat cultures of a single species would have more strongly indicated an ongoing source of infection and would possibly have brought forward the date of the patients native nephrectomy. Until recently genomic analysis by PCR and sequencing was the only method of yeast identification for species such as *C. nivariensis*, and such methods are time consuming and expensive. In recent years MALDI-TOF has been introduced into diagnostic laboratories as a bench top technology for the identification of microorganisms. To our knowledge this is the first report detailing the use of MALDI-TOF to identify *C. nivariensis*. In a similar report Arendrup et al. [7] utilised MALDI-TOF to correctly identify *Candida palmioleophila*, a species commonly misidentified biochemically as *Candida famata* or *Candida guillermondii* [16]. As MALDI-TOF analysis is rapid, under 15 min, and inexpensive at approximately 0.30 pence per test, it has provided the routine diagnostic laboratory with a new means of identifying cryptic *Candida spp.*

Several non-albicans species have reduced susceptibility to azole antifungal agents. Recent data describes *C. nivariensis* isolates with varying susceptibility to the azoles. Of particular concern, the largest review found that *C. nivariensis* isolates exhibited significant *in vitro* resistance to itraconazole, voriconazole, and fluconazole when compared with representative *C. glabrata* isolates [12]. After repeatedly isolating *Candida spp* from urine, in the presence of symptoms, our patient was treated with fluconazole initially as this drug reaches excellent concentrations in the urine. During the fluconazole therapy window *C. nivariensis* strain URC*n*3 was isolated and demonstrated a high level of *in-vitro* resistance to Fluconazole. Without strain typing data, it is impossible to confirm induced resistance but this does seem the most likely explanation. However, upon discontinuation of fluconazole therapy subsequent *C. nivariensis* isolates were found to be susceptible to fluconazole.

4. Conclusion

Although *C. nivariensis* is rare, correct identification is clinically important as this newly described species may be capable of rapidly acquiring *in-vitro* resistance to azoles upon *in-vivo* exposure, as suggested in our case report. The limitations of the current routine diagnostic methods for speciation of novel pathogenic *Candida spp* was demonstrated in this case and has highlighted the requirement for alternative diagnostic approaches such as ribosomal rRNA sequencing and MALDI-TOF mass spectroscopy. In our laboratory we have implemented a new algorithm identifying white colonies on CHROMagar as isolates that require further investigation for correct identification when clinically relevant; we have enhanced surveillance of urine from renal transplant patients for the presence of *Candida spp* and have introduced MALDI-TOF for the routine identification of yeasts.

Conflict of interest

C.C. Kibbler has received honoraria from Astellas, Gilead, MSD and Pfizer.

References

[1] Ortega M, Marco F, Soriano A, Almela M, Martinez JA, Lopez J, et al. Candida species bloodstream infection: epidemiology and outcome in a single institution from 1991 to 2008. J Hosp Infect 2011;77(2):157–61.
[2] Tortorano AM, Dho G, Prigitano A, Breda G, Grancini A, Emmi V, et al. Invasive fungal infections in the intensive care unit: a multicentre, prospective, observational study in Italy (2006–2008). Mycoses 2012;55(1):73–8.
[3] Marr KA. Fungal infections in hematopoietic stem cell transplant recipients. Med Mycol 2008;46(4):293–302.
[4] Safdar N, Slattery WR, Knasinski V, Gangnon RE, Li Z, Pirsch JD, et al. Predictors and outcomes of candiduria in renal transplant recipients. Clin Infect Dis 2005;40(10):1413–21.
[5] Gokulnath Satish R. Intractable urinary tract infection in a renal transplant recipient. Saudi J Kidney Dis Transplant 2009;20(3):458–61.
[6] Wasi N, Reddivari V, Salinas-Madrigal L, Garvin P, Bastani B. A rare case of acute renal failure due to massive renal allograft infiltration with *Candida glabrata*. Nephrol Dial Transplant 2008;23(1):374–6.
[7] Arendrup MC. Epidemiology of invasive candidiasis. Curr Opinion Crit Care 2010;16(5):445–52.
[8] Denning DW, Kibbler CC, Barnes RA. British Society for Medical Mycology proposed standards of care for patients with invasive fungal infections. Lancet Infect Dis 2003;3(4):230–40.
[9] Schelenz S, Barnes RA, Kibbler CC, Jones BL, Denning DW. Standards of care for patients with invasive fungal infections within the United Kingdom: a national audit. J Infect 2009;58(2):145–53.
[10] Standards Unit HPA. UK Standards for Microbiology Investigations B41 Issue 7.1; 2012.
[11] Alcoba-Florez J, Mendez-Alvarez S, Cano J, Guarro J, Perez-Roth E, del Pilar AM. Phenotypic and molecular characterization of *Candida nivariensis* sp. nov., a possible new opportunistic fungus. J Clin Microbiol 2005;43(8):4107–11.
[12] Borman AM, Petch R, Linton CJ, Palmer MD, Bridge PD, Johnson EM. *Candida nivariensis*, an emerging pathogenic fungus with multidrug resistance to antifungal agents. J Clin Microbiol 2008;46(3):933–8.
[13] Bishop JA, Chase N, Lee R, Kurtzman CP, Merz WG. Production of white colonies on CHROMagar Candida medium by members of the *Candida glabrata* clade and other species with overlapping phenotypic traits. J Clin Microbiol 2008;46(10):3498–500.
[14] Innis MA, Gelfand DH, Ninsky JJ. PCR protocols—a guide to methods and applications. In: White TJ, Bruns T, Lee S, Taylor J, editors. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics, PCR Protocols. Massachusetts, United States: AP Press; 1990. p. 315–22.
[15] Delgado J, Calvo N, Gomis A, Perez-Flores I, Rodriguez A, Ridao N, et al. Candiduria in renal transplant recipients: incidence, clinical repercussion, and treatment indication. Transplant Proc 2010;42(8):2944–6.
[16] Jensen RH, Arendrup MC. *Candida palmioleophila*: characterization of a previously overlooked pathogen and its unique susceptibility profile in comparison with five related species. J Clin Microbiol 2011;49(2):549–56.