Identification of *Candida dubliniensis* in a diagnostic microbiology laboratory

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استعراف المبيضَّات الدَّبْلينيَّة في مختبر ميكروبيولوجي تشخيصي

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الخلاصة: المبيضَّات الدَّبْلينيَّة *Candida dubliniensis* نوعٌ من الفطريات الخميرية المستجدَّة يمكن استفراده بصفة أساسية من المرضى الـمُنْتَقَصِي المناعة. وبالنظر إلى أن الاختبارات التي تعتمد فحص الجزيئات لم تعد ملائمة في الوقت الحاضر للاستخدام في المختبرات التشخيصية، فقد قام الباحثان بمقارنة طائفة من الطرق التي تعتمد على النمط الظاهري phenotype للتفريق بين المبيضَّات البيضاء والمبيضَّات الدَّبْلينيَّة. وقد شملت هذه الاختبارات لون المستعمرات على مستنبت الكروماغار للمبيضَّات، وملاحظة النمو في درجتَيْ حرارة 37 مئوية و45 مئوية، وكذلك القدرة على إنتاج أنابيب إنتاشيَّة وأبواغ متدثرة، إضافة إلى الاختبار بجهاز "أوكساكولور". وقد تضمنت الكائنات الدقيقة التي أمكن استفرادُها 105 مستفرَدَةً سَبَقَ تصنيفُها على أنها مبيضَّات بيضاء و10 ذَرَار مرجعية من المبيضَّات البيضاء وذُرِّيَّتَيْن مرجعيَّتين من المبيضَّات الدَّبْلينيَّة، و102 مستفرَدَةً سريرية جديدة صُنفت على أنها مبيضَّات بيضاء. ولم يكن أي من هذه الاختبارات على حِدَة كافياً للتوصُّل إلى نتيجة يُعتمد عليها، ولكنَّ إشراك الاختبارات الثلاثة يمكن أن يكون ملائماً لأغراض الاستعراف الظنِّي للمبيضَّات الدَّبْلينيَّة.

ABSTRACT ***Candida dubliniensis*** is an emerging yeast pathogen isolated mainly from immunocompromised patients. As molecular tests are currently unsuitable for use in routine diagnostic laboratories, we compared a variety of phenotypic techniques for differentiating ***C. albicans*** and ***C. dubliniensis***. The tests included: colony colour on CHROMagar™ Candida medium; growth at 37 °C and 45 °C; ability to produce germ tubes and chlamydospores; and the Auxacolor® system. The organisms included 105 isolates previously identified as ***C. albicans***, 10 reference strains of ***C. albicans***, 2 reference strains of ***C. dubliniensis*** and 102 fresh clinical isolates identified as ***C. albicans***. None of the tests alone was satisfactory but a combination of 3 tests may be suitable for presumptive identification of ***C. dubliniensis***.

Identification de *Candida dubliniensis* dans un laboratoire de microbiologie diagnostique

RÉSUMÉ ***Candida dubliniensis*** est un nouvelle levure pathogène isolée principalement chez des patients immunodéprimés. Les tests moléculaires ne convenant pas actuellement pour être utilisés dans les laboratoires de diagnostic de routine, nous avons comparé diverses techniques phénotypiques pour différencier ***C. albicans*** et ***C. dubliniensis***, dont : la couleur des colonies sur milieu CHROMagar™ Candida ; la culture à 37 °C et 45 °C ; la capacité de produire des tubes de germes et des chlamydospores ; et le système Auxacolor®. Les micro-organismes comprenaient 105 isolats identifiés auparavant comme ***C. albicans,*** 10 souches de référence de ***C. albicans***, 2 souches de référence de ***C. dubliniensis*** et 102 isolats cliniques frais identifiés comme ***C. albicans.*** Aucun des tests seul n'était satisfaisant mais une association de trois tests peut convenir pour une identification présomptive de ***C. dubliniensis.***

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Introduction

Over the past 10 years there have been frequent reports describing "atypical" isolates of *Candida albicans* [*1–5*]. In 1995, Sullivan et al. suggested that these isolates comprised a new species and named it *C. dubliniensis*, after Dublin, the capital city of Ireland, where the new species was first identified [*2,6*].

The organism has been recovered from the oral cavity in healthy people, HIV-infected patients and AIDS patients [*7,8*]. The greatest concern about *C. dubliniensis* is the potential for development of antifungal drug resistance, especially in HIV-infected patients [*9,10*].

Candida dubliniensis shares many phenotypic similarities with *C. albicans*, resulting in significant problems in differentiation between the 2 species. Currently, there is a real need for a rapid and simple test for use in routine clinical laboratories to distinguish isolates of *C. dubliniensis* and *C. albicans*. Definitive identification of *C. dubliniensis* currently relies on molecular methods; however, these have high running costs and require well-trained laboratory personnel, who might not be available in routine diagnostic laboratories.

Several methods have been proposed for differentiating between the strains. The use of CHROMagar™ chromogenic culture medium has been proposed as a means of recognizing colonies of *C. dubliniensis* [*11,12*]. Several researchers have conducted carbohydrate assimilation studies [*2,13, 14*]; some kits contain individual tests that can be utilized for *C. dubliniensis*. Pinjon et al. suggested culture at 45 °C, concluding that *C. dubliniensis* is unable to grow at such high temperatures while *C. albicans* grows well [*5*]. A recent study however showed that some *C. albicans* strains are unable to grow at 45 °C [*11*].

The aim of this study was to assess the usefulness of different phenotypic techniques for differentiating between isolates of the 2 species, *C. albicans* and *C. dubliniensis*.

Methods

Organisms used

Two *C. dubliniensis* reference strains were used in this study. One strain (coded NCPF3108) was originally identified as *C. stellatoidea* and deposited in the British collection of pathogenic fungi; it has since been re-identified as *C. dubliniensis* [*6*]. The other strain (the Bristol strain) was supplied by Dr Colin Campbell from the Bristol Mycology Reference Laboratory at the Public Health Laboratory Service, Bristol, United Kingdom. The 2 strains were identified by molecular methods.

Ten *C. albicans* reference strains were used: 324/94RA, WK1, 122/94Rgl, 684/93, 455/94rgh, 455/94sm, ATCC 3516, Y01.544, LSHTM3153 and 91L.

Stock *Candida* spp. strains supplied by the mycology laboratory of the Department of Microbiolgy, University of Wales College of Medicine were used (n = 105). These had previously been identified as *C. albicans* using the germ tube test, chlamydospore formation and Auxacolor® yeast identification system.

A total of 158 clinical specimens were studied, comprising 91 urine samples, 66 genital samples (high vaginal, penile and vulvo-vaginal swabs) and 1 blood culture sample, resulting in the identification of 102 isolates of *C. albicans* by the germ tube and Auxacolor® tests.

Culture media

CHROMagar™ Candida medium (CHROMagar Microbiology, Paris, France) was

prepared according to the manufacturer's instructions.

Sabouraud agar + chloramphenicol and corn meal agar were supplied by the Department of Microbiolgy, University of Wales College of Medicine. Sabouraud agar + chloramphenicol was supplied as ready-to-use agar plates in packs of 15 plates. Corn meal agar was supplied ready made as 100 mL prepared solid medium in a flask. The agar was melted and distributed into 5 sterile Petri dishes.

The germ tube test for the production of germ tubes was done using horse serum (TCS Biologicals Ltd, Buckingham, UK).

Organisms were cultured on CHROMagar™ Candida medium and Sabouraud agar + chloramphenicol for 48 hrs at 37 °C and 45 °C to determine the ability to grow at both temperatures and to study colony appearance and colour. To study the formation of chlamydospores, cultures on corn meal agar were incubated at room temperature. Identification was carried out using the Auxacolor® system (Sanofi Diagnostics Pasteur, Marnes La Coquette, France). The Auxacolor® test procedures were done in accordance with the manufacturer's instructions.

Results

Six of the 105 stock strains of *C. albicans* failed to grow on both the Sabouraud and the CHROMagar™ media and 1 showed mould contamination. These were excluded from the study. The remaining 98 grew equally well on both media.

On CHROMagar™ Candida medium, all 10 references strains of *C. albicans* grew well, forming medium-sized, 3–5 mm smooth, entire colonies. The colony colour ranged from light green to green, most often with lighter edges, sometimes with the intense colour at the edge of the colony and sometimes in the centre. The 2 *C. dubliniensis* reference strains formed colonies similar to those of *C. albicans*; 1 was light green and the other dark green.

The conventional method using the germ tube test and chlamydospore formation identified 9 of the 10 *C. albicans* reference strains, and misidentified the *C. dubliniensis* strains as *C. albicans*; these were also misidentified by the Auxacolor® system. The Auxacolor® system correctly identified the 1 strain of *C. albicans* that was germ-tube negative.

Of the 219 cultures investigated, all remaining 212 which were viable grew well at 37 °C on both CHROMagar™ and Sabouraud agar + chloramphenicol. Growth was consistent at 48 hours.

Table 1 shows a summary of colony colours and growth at 45 °C of the strains investigated. None of the *C. albicans* stock culture strains produced a deep green colour on CHROMagar™ but 15 clinical isolates as well as the Bristol strain of *C. dubliniensis* produced colonies of a deep bluish-green colour. The germ tube test was positive for these strains, as was chlamydospore formation. The deep green clinical isolates were identified by the Auxacolor® system as *C. albicans* with biocodes of 7145207 or 7143207. The Bristol reference strain had a biocode of 7141207 and the NCPF3108 reference strain had a biocode of 7143207.

All reference strains of *C. albicans* grew at 45 °C. The 2 *C. dubliniensis* strains did not grow at all at 45 °C on either CHROMagar™ or Sabouraud agar + chloramphenicol. Among the stock culture strains, only 2 failed to grow at 45 °C. Among the clinical isolates, 1 from a urine sample and 1 from a genital sample did not grow at this temperature.

Specimens that did not grow at 45 °C and/or showed dark green colonies on

Table 1 **Summary of colony colour and growth at 45 °C of *Candida albicans* and *Candida dubliniensis* strains**

Strain	Total tested	Colour on CHROMagar[a]		Growth at 45 °C	
		Dark green	Green or light green	Yes	No
C. albicans reference strains	10	0	10	10	0
C. dubliniensis reference strains	2	1	1	0	2
Stock ***C. albicans***	98[b]	0	98	96	2
Clinical isolates (***C. albican***s)	102	15	87	100	2

[a]***CHROMagar™ Candida medium.***
[b]***Total viable.***

CHROMagar™ and showed a biocode of 7143207 or 714107 were re-identified as *C. dubliniensis.*

Discussion

Over the past 2 decades, there has been a rapid growth in clinical microbiology technology. In the past, test results were available only after several days owing to the labour-intensive methods. With the emergence of new pathogens, especially the drug-resistant *Candida* spp., it has become necessary for laboratories to seek more efficient and cost-effective methods of identification. Therefore, it is essential that the available diagnostic tools be constantly modified to keep abreast of the ever-changing spectrum of pathogens.

A new emerging yeast pathogen, *C. dubliniensis,* was the focus of this study. It is closely related to *C. albicans*. Microbiological information about *C. dubliniensis* shows similarities with *C. albicans*; using the currently available conventional methods it is difficult to discriminate between them. The colony appearance on CHROMagar™ Candida medium makes it extremely useful for rapid presumptive identification of the most common *Candida* spp. As reported originally by Odds and Bernaert, the green colour is unique to *C. albicans* [*15*]. Casal described the deep green colour as characteristic of *C. zeylanoides*, a species not mentioned in the study of Odds and Bernaert [*16*]. In our study, *C. dubliniensis* could show the same colour as *C. albicans*. However, the colour was less intense in older cultures and in freshly cultured clinical specimens it appeared dark green. Sullivan and Coleman described this phenomenon as a way of differentiating between the 2 species on primary culture. In our study, 2 of the stock cultures and 1 *C. dubliniensis* reference strain appeared light green and failed to grow at 45 °C while 2 of the fresh clinical isolates and the Bristol reference strain (all dark green) failed to grow at 45 °C. Thirteen *C. albicans* strains appeared deep green on primary isolation.

These findings suggest that CHROMagar™ medium alone is not suitable for discriminating between *C. albicans* and *C. dubliniensis* on primary isolation.

Pinjon et al. reported that *C. dubliniensis* could be discriminated from *C. albicans* by the ability of the latter to grow at 45 °C [*5*]. This test is unsatisfactory because some *C. albicans* strains are unable to grow at this elevated temperature and this cannot, therefore, be used as a discriminatory test [*11*]. The results obtained in our study are consistent with Pinjon's criterion, as the 2 reference strains of *C. dubliniensis* failed to grow at 45 °C. Two stock culture strains and 2 clinical isolates failed to grow at this temperature, although all 10 *C. albicans* reference strains did grow. Those strains are considered suspect for *C. dubliniensis.*

Gales et al. studied the use of xylose and α-methyl-glucose as determined with the API 20C AUX and Vitek YBC systems for the identification of *C. dubliniensis* [*17*], The Auxacolor® system used in this study contained xylose. The inability of *C. dubliniensis* to utilize xylose is reflected by specific biocodes.

Conclusion

None of the tests alone—CHROMagar™ Candida media, growth at 45 °C or Auxacolor®—was a satisfactory discriminatory test. A combination of the 3 tests used in this study may be a good tool for presumptive identification of *C. dubliniensis*. The 4 strains (2 stock culture strains and 2 clinical isolates) that appeared to be presumptive of *C. dubliniensis* need to be confirmed by further testing. Recognition of *C. dubliniensis* provides valuable information regarding its epidemiology to help establish its clinical significance.

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