



Note

Simple low cost differentiation of *Candida auris* from *Candida haemulonii* complex using CHROMagar Candida medium supplemented with Pal's medium



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ABSTRACT

Background: *Candida auris* is unique due to its multidrug resistance and misidentification as *Candida haemulonii* by commercial systems. Its correct identification is important to avoid inappropriate treatments.

Aims: To develop a cheap method for differentiating *C. auris* from isolates identified as *C. haemulonii* by VITEK2.

Methods: Fifteen *C. auris* isolates, six isolates each of *C. haemulonii* and *Candida duobushaemulonii*, and one isolate of *Candida haemulonii* var. *vulnera* were tested using CHROMagar Candida medium supplemented with Pal's agar for better differentiation.

Results: On CHROMagar Candida medium supplemented with Pal's agar all *C. auris* strains showed confluent growth of white to cream colored smooth colonies at 37 °C and 42 °C after 24 and 48 h incubation and did not produce pseudohyphae. The isolates of the *C. haemulonii* complex, on the contrary, showed poor growth of smooth, light-pink colonies at 24 h while at 48 h the growth was semiconfluent with the production of pseudohyphae. *C. haemulonii* complex failed to grow at 42 °C.

Conclusions: We report a rapid and cheap method using CHROMagar Candida medium supplemented with Pal's agar for differentiating *C. auris* from isolates identified as *C. haemulonii* by VITEK2.

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Diferenciación sencilla y de bajo coste de *Candida auris* del complejo *Candida haemulonii* con el empleo del medio CHROMagar Candida enriquecido con medio de Pal

RESUMEN

Antecedentes: *Candida auris* es una especie única debido a su resistencia a múltiples fármacos y a la identificación errónea por sistemas comerciales como *Candida haemulonii*. Su correcta identificación es importante para evitar un tratamiento inadecuado.

Objetivos: Desarrollar un método de bajo coste para diferenciar *C. auris* de aislamientos identificados como *C. haemulonii* por el método VITEK® 2.

Métodos: Quince aislamientos de *C. auris*, seis de *C. haemulonii*, seis aislamientos de *Candida duobushaemulonii* y un aislamiento de *Candida haemulonii* var. *vulnera* se sembraron en medio CHROMagar Candida enriquecido con medio de Pal para una mejor diferenciación.

Palabras clave:

Candida auris

Complejo *Candida haemulonii*

CHROMagar Candida

Medio de Pal

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Resultados: En el medio CHROMagar Candida enriquecido con medio de Pal, todos los aislamientos de *C. auris* presentaron un crecimiento confluyente con colonias lisas de color blanco a blanco amarillento a 37 y 42 °C tras 24 y 48 h de incubación; no hubo producción de seudohifas. Los aislamientos del complejo *C. haemulonii*, en cambio, mostraron un crecimiento menor. Las colonias eran lisas y de un color rosa claro a las 24 h; a las 48 h el crecimiento fue semiconfluente y se observó producción de seudohifas. No hubo crecimiento de ninguno de los aislamientos a 42 °C.

Conclusiones: El medio CHROMagar Candida enriquecido con medio de Pal es rápido y de bajo coste, y resulta efectivo para identificar *C. auris* entre cepas previamente identificadas como *C. haemulonii* por el método VITEK® 2.

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The first reports about *Candida auris* were exclusively from external ear infections and since then this yeast has been isolated from a wide variety of samples like blood, tissue diabetic foot infections, nails and broncho-alveolar lavage.^{2,7,8,14} The resistance of *C. auris* to fluconazole, as well as the reduced susceptibility to voriconazole and itraconazole and its ability for clonal transmission, is worrisome.^{3,10} Automated identification systems like VITEK2 (bioMérieux, Marcy l'Etoile, France) are known to misidentify *C. auris* as *Candida haemulonii* or *Candida famata*, while API20C AUX (bioMérieux) misidentifies it as *Rhodotorula glutinis*.^{2,3,6,7} Reliable identification of these phylogenetically closely related species can be achieved using DNA sequencing and Matrix-Assisted Laser Desorption Ionization-Time Of Flight Mass spectrometry (MALDI-TOF MS) assays, which unfortunately are not available in most clinical microbiology laboratories. Since the treatment strategies for invasive candidiasis is based on the correct species identification, a wrong identification coupled with misleading high minimum inhibitory concentrations (MIC) limits the therapeutic options. Pal's agar, which was developed for the identification of *Cryptococcus neoformans*, has been previously used in combination with CHROMagar Candida for differentiating between *Candida albicans* and *Candida dubliniensis*.^{11,12} Therefore we evaluated the use of CHROMagar Candida medium supplemented with Pal's agar to differentiate between *C. auris* and *C. haemulonii* complex for all isolates identified as *C. haemulonii* with VITEK2 system.

Fifteen *C. auris* (CBS 12822, 12883, 12886, 12885, 12876, 12879, 12881, 12880, 12889, 12875, 12874, 12877, 12878, 12887), 6 isolates each of *C. haemulonii* (VPCI-1158/P/13, 1164/P/13, 1165/P/13, 716/P/14, 1161/P/13, 1163/P/13) and *Candida duobushaemulonii* (VPCI-713/P/14, 1160/P/13, 1157/P/13, 11470/P/15, 1159/P/13, 1162/P/13), and one solitary isolate of *C. haemulonii* var. *vulnera* (VPCI 715/P/14) from our previous study were used.⁶ All isolates' identification were confirmed by sequencing the ITS region of the ribosomal subunit and D1/D2 region of the large ribosomal subunit (LSU) respectively.^{2,3,6} All our clinical isolates were initially identified as *C. haemulonii* by VITEK2 system. Pal's agar was prepared by autoclaving the mixture containing agar, creatinine,

glucose, KH₂PO₄ and sunflower seed extract.¹¹ CHROMagar (Becton Dickinson, Sparks, MD) supplemented with Pal's medium was prepared as described previously by mixing equal volumes of both the medium at their normal strength.¹² Test isolates were inoculated on the CHROMagar-Pal's medium after being grown on Sabouraud dextrose agar for 48 h. The plates were incubated at 37 °C and 42 °C and colony characteristics were recorded at 24 and 48 h by two different observers. Pseudohyphae formation was detected microscopically using lactophenol cotton blue stain.

The colony color, morphology, growth at 37 °C and 42 °C and pseudohyphae formation when grown in CHROMagar Candida medium and CHROMagar Candida medium supplemented with Pal's agar have been listed in Table 1. On CHROMagar Candida medium supplemented with Pal's agar all *C. auris* strains showed confluent growth of white to cream colored smooth colonies at 37 °C and 42 °C after 24 and 48 h incubation and did not produce pseudohyphae. *C. haemulonii* complex, on the contrary, showed poor growth of smooth light pink colonies at 24 h while at 48 h the growth was semiconfluent (Fig. 1A and B) with production of pseudohyphae. When incubated at 42 °C in CHROMagar Candida supplemented with Pal's medium all *C. haemulonii* complex isolates failed to grow (Fig. 1C).

A false impression of high incidence of *C. haemulonii* infections in India has been created due to studies based on the identification using VITEK2 system.^{9,13,15} A recent study reported that in addition to misidentifying these isolates, VITEK2 system also reports higher MIC values for amphotericin B, resulting in inappropriate treatment.⁷ *C. auris* is phylogenetically very closely related to *C. haemulonii* complex, the latter comprising the genotypically distinguishable species *C. haemulonii*, *C. duobushaemulonii* and *C. haemulonii* var. *vulnera*.^{1,13} Differential media like CHROMagar Candida can presumptively identify *Candida albicans*, *Candida tropicalis* and *Candida krusei*. Colony morphology on CHROMagar Candida is not discriminatory as both *C. auris* and *C. haemulonii* complex grew well, producing light pink colonies.^{2,3,6} Many non-*C. albicans* *Candida* species like *C. krusei*, *Candida glabrata*, *Candida kefyr*, *Candida guilliermondii* and *Candida parapsilosis* also produce light pink colonies on CHROMagar Candida and CHROMagar

Table 1

Colony color and growth characteristics of *C. auris* and *C. haemulonii* complex isolates on different culture media at different incubation temperatures.

Parameters (Culture media, incubation temperature and duration)	Characteristics (Colony color/growth)	No. of isolates	
		<i>C. auris</i> (n = 15)	<i>C. haemulonii</i> complex (n = 13)
CHROMagar Candida medium (37 °C for 24 and 48 h).	Light pink Cream/white	15 (100%) 0	13 (100%) 0
CHROMagar Candida medium supplemented with Pal's agar (37 °C for 24 and 48 h).	Light pink Cream-white	0 15 (100%)	13 (100%) 0
CHROMagar Candida medium supplemented with Pal's agar (42 °C for 24 and 48 h).	Confluent growth of cream-white colonies No growth	15 (100%) 0	0 13 (100%)

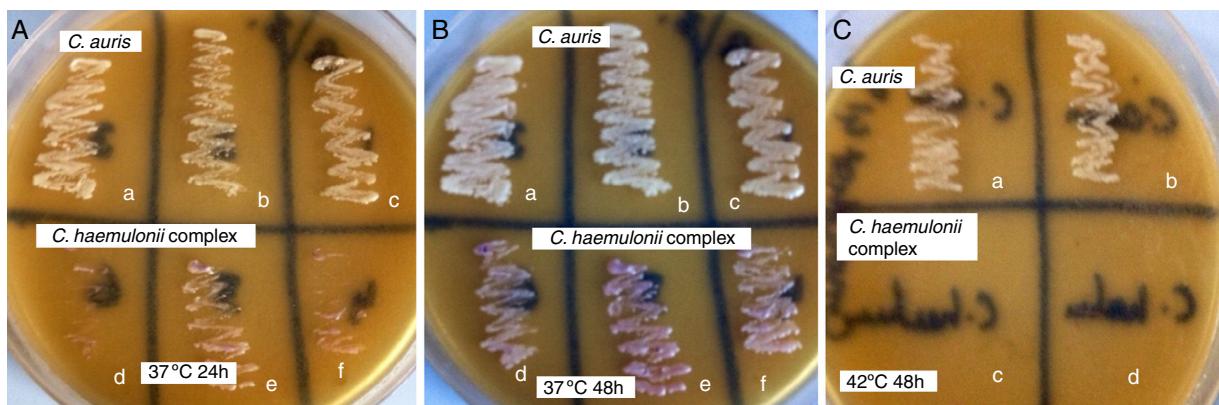


Fig. 1. Plates of CHROMagar Candida supplemented with Pal's medium showing confluent growth of white colored colonies of *C. auris* (a, b and c) and poor growth of light pink colored colonies *C. haemulonii* var. *vulnera* (d), *C. duobushaemulonii* (e) and *C. haemulonii* (f) after incubation at (A) 37 °C for 24 h, (B) 37 °C for 48 h and (C) white colored colonies of *C. auris* (a, b) and no growth of *C. duobushaemulonii* (c) and *C. haemulonii* (d) after incubation at 42 °C for 48 h.

Candida supplemented with Pal's medium.^{4,5} *C. auris* grows well at 37 °C and 42 °C and does not produce pseudohyphae on corn meal agar after 4–8 days incubation at 28 °C.⁶ In contrast *C. haemulonii* complex produces pseudohyphae and failed to grow at 42 °C.⁶ The differentiation between *C. auris* and *C. haemulonii* complex was achieved by observing the colonies color and their ability to grow when incubated at 42 °C in CHROMagar Candida supplemented with Pal's medium with 100% sensitivity and specificity. It is a cheap and rapid method to differentiate these two phylogenetically closely related species, as the alternative methods are DNA sequencing and MALDI-TOF MS assays, which are prohibitively expensive, technically challenging and not available in most clinical microbiology laboratories. The main limitation of this study is that preliminary identification needs to be done by an automated identification system like VITEK2 to rule out other non-*C. albicans* *Candida* species that can produce pink colonies on CHROMagar Candida medium.

Conflict of interest

We certify that there are no entities with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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References

- Cendejas-Bueno E, Kolecka A, Alastruey-Izquierdo A, Theelen B, Groenewald M, Kostrzewa M, et al. Reclassification of the *Candida haemulonii* complex as *Candida haemulonii* (*C. haemulonii* group I), *C. duobushaemulonii* sp. nov. (*C. haemulonii* group II), and *C. haemulonii* var. *vulnera* var. nov.: three multiresistant human pathogenic yeasts. *J Clin Microbiol.* 2012;50:3641–51.
- Chowdhary A, Anil Kumar V, Sharma C, Prakash A, Agarwal K, Babu R, et al. Multidrug-resistant endemic clonal strain of *Candida auris* in India. *Eur J Clin Microbiol Infect Dis.* 2014;33:919–26.
- Chowdhary A, Sharma C, Duggal S, Agarwal K, Prakash A, Singh PK, et al. New clonal strain of *Candida auris*, Delhi, India. *Emerg Infect Dis.* 2013;19:1670–3.
- Daef E, Moharram A, Eldin SS, Elsherbiny N, Mohammed M. Evaluation of chromogenic media and semistemmed PCR in the identification of *Candida* species. *Braz J Microbiol.* 2014;45:255–62.
- Jain N, Mathur P, Misra MC, Behera B, Xess I, Sharma SP. Rapid identification of yeast isolates from clinical specimens in critically ill trauma ICU patients. *J Lab Physicians.* 2012;4:30–4.
- Kathuria S, Singh PK, Sharma C, Prakash A, Masih A, Kumar A, et al. Multidrug-resistant *Candida auris* misidentified as *Candida haemulonii*: characterization by matrix-assisted laser desorption ionization-time of flight mass spectrometry and DNA sequencing and its antifungal susceptibility profile variability by Vitek 2, CLSI Broth Microdilution, and Etest method. *J Clin Microbiol.* 2015;53:1823–30.
- Kim MN, Shin JH, Sung H, Lee K, Kim EC, Ryoo N, et al. *Candida haemulonii* and closely related species at 5 university hospitals in Korea: identification, antifungal susceptibility, and clinical features. *Clin Infect Dis.* 2009;48:e57–61.
- Lee WG, Shin JH, Uh Y, Kang MG, Kim SH, Park KH, et al. First three reported cases of nosocomial fungemia caused by *Candida auris*. *J Clin Microbiol.* 2011;49:3139–42.
- Oberoi JK, Wattal C, Goel N, Raveendran R, Datta S, Prasad K. Non-albicans *Candida* species in blood stream infections in a tertiary care hospital at New Delhi, India. *Indian J Med Res.* 2012;136:997–1003.
- Oh BJ, Shin JH, Kim MN, Sung H, Lee K, Joo MY, et al. Biofilm formation and genotyping of *Candida haemulonii*, *Candida pseudoaemulonii*, and a proposed new species (*Candida auris*) isolates from Korea. *Med Mycol.* 2011;49:98–102.
- Pal M. Natural occurrence of *Cryptococcus neoformans* var. *neoformans* in wooden canary cages. *Rev Iberoam Micol.* 1995;12:93–4.
- Sahand IH, Moragues MD, Eraso E, Villar-Vidal M, Quindós G, Pontón J. Supplementation of CHROMagar Candida medium with Pal's medium for rapid identification of *Candida dubliniensis*. *J Clin Microbiol.* 2005;43:5768–70.
- Sarma S, Kumar N, Sharma S, Govil D, Ali T, Mehta Y, et al. Candidemia caused by amphotericin B and fluconazole resistant *Candida auris*. *Indian J Med Microbiol.* 2013;31:90–1.
- Satoh K, Makimura K, Hasumi Y, Nishiyama Y, Uchida K, Yamaguchi H. *Candida auris* sp. nov., a novel ascomycetous yeast isolated from the external ear canal of an inpatient in a Japanese hospital. *Microbiol Immunol.* 2009;53:41–4.
- Xess I, Jain N, Hasan F, Mandal P, Banerjee U. Epidemiology of candidemia in a tertiary care centre of north India: 5-year study. *Infection.* 2007;35:256–9.