



Nota

Gentamycin inhibits the growth of *Malassezia pachydermatis* in culture

Carmen Aspíroz^{a,*}, Yolanda Gilaberte^b, Antonio Rezusta^c, Teun Boekhout^d and M^a Carmen Rubio^e

^a Servicio de Microbiología, Hospital Royo Villanova, Zaragoza, Spain

^b Servicio de Microbiología, Hospital Miguel Server, Zaragoza, Instituto de Ciencias de la Salud de Aragón, Spain

^c Servicio de Dermatología, Hospital San Jorge, Huesca, Spain

^d Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands

^e Servicio de Microbiología, Hospital Clínico Universitario, Zaragoza, Spain

ARTICLE INFO

Article history:

Received 10 August 2009

Accepted 6 November 2009

Available online 10 de febrero de 2010

Keywords:

Malassezia pachydermatis

Gentamycin

External otitis

Dogs

ABSTRACT

Background: *Malassezia pachydermatis* is a yeast of importance in both veterinary and human medicine.

Aims: To know if *M. pachydermatis* grow on micological media with high concentrations of gentamycin.

Methods: Twenty *M. pachydermatis* strains were streaked on Sabouraud Dextrose Agar plates with different concentrations of gentamycin.

Results: All isolates were inhibited when high concentrations of gentamycin were added.

Conclusions: The use of plates with high concentrations of gentamycin can lead to some important misdiagnoses: firstly, false-negative cultures, and secondly, an erroneous classification of *M. pachydermatis* as a lipid-dependent species. Moreover, all of this could be useful in two therapeutic fields: i) in animals, topical gentamycin could be an efficacious treatment for a disease such as external otitis in dogs; ii) in humans, we hypothesize that gentamycin could be regarded as a possible therapy ("antibiotic-lock") for catheter-associated *Malassezia* spp. infections.

© 2009 Revista Iberoamericana de Micología. Published by Elsevier España, S.L. All rights reserved.

La gentamicina inhibe el crecimiento de *Malassezia pachydermatis* en cultivo

RESUMEN

Antecedentes: *Malassezia pachydermatis* es una levadura de gran interés tanto en medicina humana como en veterinaria.

Objetivos: Conocer si la adición de gentamicina a los medios micológicos más comunes era capaz de inhibir el crecimiento de *M. pachydermatis*.

Métodos: Se estudiaron 20 cepas de *M. pachydermatis* en medios micológicos con gentamicina a diferentes concentraciones.

Resultados: Todos los aislamientos se inhibieron con altas concentraciones de gentamicina.

Conclusiones: El uso de placas con altas concentraciones de gentamicina puede llevar a resultados falsamente negativos en cultivo y a una errónea clasificación de *M. pachydermatis* como especie lípido-dependiente. Estas observaciones podrían llegar a tener dos aplicaciones hipotéticas: i) en veterinaria, la gentamicina tópica podría ser eficaz en procesos como la dermatitis o la otitis externa, y ii) en humanos, podría ser una terapia ("antibiotic-lock") para las infecciones por *Malassezia* relacionadas con el uso de catéteres.

© 2009 Revista Iberoamericana de Micología. Publicado por Elsevier España, S.L. Todos los derechos reservados.

Palabras clave:

Malassezia pachydermatis

Gentamicina

Otitis externa

Perros

Malassezia pachydermatis is a lipophilic yeast of importance in both veterinary and human medicine.⁷ *M. pachydermatis* has been rarely associated with systemic infections of humans, although fungemia has been reported in patients receiving parenteral nutrition.^{4,2} However, infections associated with *M. pachydermatis*

in animals are frequent and include mainly dermatitis and otitis in dogs.⁷

Canine external otitis (OE) is a disease of multifactorial aetiology, and the microorganism most frequently isolated is *M. pachydermatis*, often in combination with *Staphylococcus intermedius* bacteria.⁸ Additionally, *Pseudomonas aeruginosa* is the organism most frequently isolated from dogs with suppurative EO.⁸ External otitis related to *M. pachydermatis* is usually secondary to underlying problems.⁷ Although the evidence for a pathogenic role for the yeast remains

* Corresponding author.

E-mail address: carmenaspiroz@gmail.com (C. Aspíroz).

Table 1
Growth of *M. pachydermatis* on different mycological media (5 days, 35 °C).

	<i>M. pachydermatis</i> CBS 1879	<i>M. pachydermatis</i> (20 clinical strains)
mDixon	+++	+++
LNA	+++	+++
SDA	+++	+++
SDA-C	+++	+++
SDA-C-Cy	+++	+++
SDA-Gm 12.5 µg/ml	+++	+++
SDA-Gm 25 µg/ml	±	+ / ±
SDA-Gm 50 µg/ml	–	– / ±
SDA-Gm 100 µg/ml	–	–
SDA-Gm-c	–	–

mDixon: modified Dixon. LNA: Leeming and Notman medium [Leeming JP, Notman FH. Improved methods for isolation and enumeration of *Malassezia furfur* from human skin. J Clin Microbiol 1987; 25: 2017–9]. SDA: Sabouraud dextrose agar (Bio-Mérieux, Marcy l'Etoile, France). SDA-C: SDA-chloramphenicol (Bio-Mérieux). SDA-C-Cy: SDA with cycloheximide and chloramphenicol commercialized medium/gelose (Bio-Mérieux). SDA-Gm: SDA supplemented with gentamycin. SDA-Gm-c: Sabouraud commercialized plates (Sabouraud glucose agar with gentamycin, Bio-Mérieux, Marcy l'Etoile, France). +++ Growth > 50 CFU, large colonies, > 2 mm. ++ Growth > 30 CFU, large colonies. + / ± Sparse growth ≤ 30 CFU, ≤ 2 mm. ± / – No growth or sparse colonies (< 5) and very small (< = 1 mm). – No growth.

circumstantial, the best therapeutic response is achieved when both *M. pachydermatis* and bacteria are removed by topical therapy.⁷ Concerning bacteria, both *S. intermedius* and *P. aeruginosa* show good susceptibility to gentamycin.⁸

In order to examine the ear exudate in dogs with ceruminous or exudative EO, rolling of exudate in a thin layer on glass slides with a cotton-tipped swab may be the preferred method.⁹ However, culture may be useful to study the yeast, either on normal skin and mucosae or on the otitis or dermatitis skin. Sabouraud dextrose agar (SDA) with a 27–35 °C incubation temperature allows the isolation of most fungal species responsible for cutaneous diseases in mammals (*Microsporum canis* and other dermatophytes, *M. pachydermatis* and *Candida* spp.) However, specific media with lipid supplements, such as Leeming and Notman medium or modified Dixon's medium⁶, may be appropriate to isolate all *Malassezia* species. Some key characteristics of the different species of *Malassezia* to routinely facilitate their identification have been reported. These characteristics include growth on SDA, tolerance to high temperatures, catalase activity and growth in Tween as the sole lipid supplement in a regular medium.⁵ The first step in this identification scheme of *Malassezia* yeasts is making a new culture on SDA (at 32 °C). If growth on SDA is observed, the organism is the non-lipid-dependent strains of *M. pachydermatis*.⁵

During a survey of the appearance of strains of *M. pachydermatis*, we observed a systematic absence of growth on several media, mainly on commercialized Sabouraud medium (Sabouraud Gentamycin chloramphenicol, BioMérieux, Marcy l'Étoile, France).¹ To assess in detail these culture characteristics and the probable existence of an inhibitor substance in the composition of some of these media we performed this study.

Twenty *M. pachydermatis* strains obtained by swabbing the external ear canals of dogs and CBS 1879 *M. pachydermatis* as the control strain were used for the study. A suspension of yeasts adjusted to about 10⁵ cells/ml was cultured on lipidic medium (modified Dixon) and on non-lipidic media containing Sabouraud agar with other ingredients and antimicrobial agents (Table 1).

Plates were read after 5 days of incubation at 35 °C. The cultures were examined every 24 h for 7 days when the results were obtained.

Results are summarized in Table 1. Colonies consistent with *M. pachydermatis* were visible at 48 h on modified Dixon and at 72 h on SDA and SDA-C-Cy plates. No colonies or quite small colonies were observed on SDA-Gm. Gentamycin at a concentration of 100 mg/L inhibited all strains tested on SDA.

Gentamycin at high concentrations used in commercialized media (100 mg/ml) effectively inhibits the growth of *M. pachydermatis* on SDA. Growth is also inhibited at other concentrations used in the market (40 mg/L). This fact can lead to some important misdiagnoses if commercialized plates with gentamycin are employed: firstly, false-negative cultures, and secondly an erroneous classification of *M. pachydermatis* as a lipid-dependent species. According to our findings, high concentrations of gentamycin (> 25 mg/L) have a deleterious effect against *M. pachydermatis* and other species of *Malassezia* (personal observation). These findings could be useful in two therapeutic fields: (i) in animals, topical gentamycin could be an efficacious treatment for disease related to *M. pachydermatis*, and this can be especially convenient in infections where this yeast appears together with bacteria (such as EO in dogs); (ii) in humans, we hypothesize that gentamycin could be regarded as a possible therapy (“antibiotic-lock” or “antifungal-lock”) for catheter-associated *Malassezia* spp. infections.^{1,3,10} For the latter, gentamycin has important advantages, namely its wide action spectrum; its reputation and acceptance as one of the more well-known antimicrobial agent in this type of therapy, and finally its low cost.

Acknowledgement

We would like to thank Nick Thompson for his assistance in translating this manuscript into English.

Author's disclosure

Authors have nothing to declare.

References

- Aspíroz C, Rezusta A, Rubio MC, Gómez-Lus R. *Malassezia pachydermatis* failure to grow on a commercial Sabouraud medium with gentamycin, in: Proceedings of XIVth Congress of the International Society of Human and Animal Mycology, May 8–12, 2000, Buenos Aires, Argentina, PO97/ P-97, 2000.
- Cannizzo FT, Eraso E, Ezkurra PA, Villar-Vidal M, Bollo E, Castellá G, et al. Biofilm development by clinical isolates of *Malassezia pachydermatis*. Med Mycol. 2007;45:357–61.
- Fortún J, Grill F, Martín-Dávila P, Blázquez J, Tato M, Sánchez-Corral J, et al. Treatment of long-term intravascular catheter-related bacteraemia with antibiotic-lock therapy. J Antimicrob Chemother. 2006;58:816–21.
- Guého E, Simmons RB, Pruitt WR, Meyer SA, Ahearn DG. Association of *Malassezia pachydermatis* with systemic infections of humans. J Clin Microbiol. 1987;25:1789–90.
- Guillot J, Guého E, Lesourd M, Midgley G, Chévrier G, Dupont B. Identification of *Malassezia* species. A practical approach. J Mycol Méd. 1996;6:103–10.
- Guillot J, Bond R. *Malassezia pachydermatis*: a review. Med Mycol. 1999;37:295–306.
- Guillot J, Breugnot C, de Barros M, Chermette R. Usefulness of modified Dixon's medium for quantitative culture of *Malassezia* species from canine skin. J Vet Diagn Invest. 1998;10:384–6.
- Kiss G, Radványi S, Szigeti G. New combination for the therapy of canine otitis externa. I. Microbiology of otitis externa. J Small Animal Pract. 1997;38:51–6.
- Morris DO. *Malassezia* dermatitis and otitis. Vet Clin North Am Small Anim Pract. 1999;29:1303–10.
- Shuford JA, Rouse MS, Piper KE, Steckelberg JM, Patel R. Evaluation of caspofungin and amphotericin B deoxycholate against *Candida albicans* biofilms in an experimental intravascular catheter infection model. J Infect Dis. 2006;194:710–3.