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Gentamycin inhibits the growth of *Malassezia pachydermatis* in culture

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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.

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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.

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Palabras clave:

*Malassezia pachydermatis*

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

in animals are frequent and include mainly dermatitis and otitis in dogs.<sup>7</sup>

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.<sup>8</sup> Additionally, *Pseudomonas aeruginosa* is the organism most frequently isolated from dogs with suppurative EO.<sup>8</sup> External otitis related to *M. pachydermatis* is usually secondary to underlying problems.<sup>7</sup> Although the evidence for a pathogenic role for the yeast remains

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**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.<sup>7</sup> Concerning bacteria, both *S. intermedius* and *P. aeruginosa* show good susceptibility to gentamycin.<sup>8</sup>

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.<sup>9</sup> 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 medium<sup>6</sup>, 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.<sup>5</sup> 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*.<sup>5</sup>

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).<sup>1</sup> 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 10<sup>5</sup> 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.<sup>1,3,10</sup> 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.

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## Author's disclosure

Authors have nothing to declare.

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