



## Original article

## Antimicrobial activity of [2-(methacryloyloxy)ethyl]trimethylammonium chloride against *Candida* spp.

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## ABSTRACT

**Background:** *Candida*-associated denture stomatitis is the most common manifestation of oral candidal infection, caused mainly by *Candida albicans*. Several authors have attempted to add antifungal agents or antiseptics to denture temporary soft lining materials or to denture acrylic resins, without relevant results. Therefore, the investigation of a quaternary ammonium functionalized compound [2-(methacryloyloxy)ethyl]trimethylammonium chloride (MADQUAT), which copolymerizes with methacrylates and which could act as a fungal inhibitor, is of paramount importance.

**Aims:** To evaluate the in vitro activity of MADQUAT against *Candida* species.

**Methods:** Thirty-one *Candida* strains were used to determine the in vitro antifungal activity of this compound. The minimum inhibitory concentrations and minimum fungicidal concentrations of MADQUAT and nystatin were determined.

**Results:** MADQUAT showed antifungal properties at concentrations of 6.25 to > 100 mg/ml, and fungicidal activity between 25 and > 100 mg/ml. The quantitative determinations of the fungistatic and fungicidal activity of MADQUAT showed fungistatic activity against all *Candida albicans*, *Candida krusei* and *Candida parapsilosis* strains, revealing fungicidal activity against some strains of the other species.

**Conclusions:** MADQUAT has antifungal activity against *Candida* spp. Moreover, the sensitivity to this substance varies across the different species in terms of MIC values and fungicidal or fungistatic activity.

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### Actividad antimicrobiana del cloruro de 2-metacrilóil oxietil trimetilamonio contra *Candida* spp.

## RESUMEN

**Antecedentes:** La estomatitis protética es la forma más común de infección bucal producida por especies de *Candida*, siendo *Candida albicans* el agente etiológico más común. Diversos autores han intentado asociar agentes antifúngicos o antisépticos a los materiales de revestimiento blando o a las resinas acrílicas de las prótesis dentales, pero sin éxito. Por ello, se ha investigado un compuesto de amonio cuaternario (2-metacrilóil oxietil trimetilamonio [MADQUAT]), que copolimeriza con los metacrilatos y que podría actuar como inhibidor de levaduras.

**Objetivos:** El objetivo de este estudio fue evaluar la actividad in vitro del MADQUAT contra especies de *Candida*.

**Métodos:** Se utilizaron 31 cepas de *Candida* para determinar la actividad antifúngica in vitro. Se determinó la concentración mínima inhibitoria (CMI) y la concentración mínima fungicida del MADQUAT, así como de la nistatina.

## Palabras clave:

2-metacrilóil oxietil trimetilamonio  
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**Resultados:** El MADQUAT presentó propiedades antifúngicas en las concentraciones entre 6,25 y > 100 mg/ml y actividad fungicida entre 25 y > 100 mg/ml. Los estudios cuantitativos de la actividad fungistática y fungicida del MADQUAT demostraron actividad fungistática contra todas las cepas de *Candida albicans*, *Candida krusei* y *Candida parapsilosis*, revelando actividad fungicida contra algunas cepas de otras especies.

**Conclusiones:** El MADQUAT presenta actividad antifúngica contra *Candida* spp. Además, la sensibilidad a dicho compuesto es distinta entre las diferentes especies considerando los valores de la CMI y la actividad fungicida o fungistática.

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*Candida* is both a normal commensal and opportunistic pathogen found in warm-blooded animals, including humans. It colonizes the mucosal surfaces of the oral cavity, digestive or genitourinary tract of healthy individuals and causes a variety of infections depending on host susceptibility.<sup>6</sup> The prevalence of candidosis has increased due to the larger number of immunocompromised patients, including those on broad-spectrum antibacterial drugs, transplant recipients, and HIV-infected individuals<sup>21</sup> and, therefore, fungal infections have been given a lot of attention. One of the first clinical manifestations of candidosis occurs in the oral cavities of prosthesis (acrylic denture) wearers.

*Candida* species are found in the oral cavity of 25–50% of healthy individuals, in both adults and children. In denture wearers, these rates climb to 60–100%. *Candida albicans* is the most common species, accounting for almost 70% of the isolates. In addition to *Candida albicans*, other species including *Candida tropicalis*, *Candida glabrata*, *Candida krusei*, *Candida guilliermondii*, and *Candida parapsilosis* are also usually isolated from denture and non-denture wearers.<sup>27</sup>

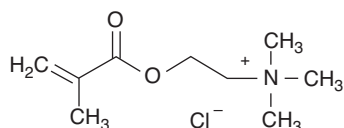
*Candida* adheres directly or via an intermediate layer of plaque-forming bacteria to denture acrylic resin (polymethylmethacrylate).<sup>8</sup> Despite antifungal treatment for denture stomatitis, infection recurs soon afterwards, suggesting that denture plaque may serve as a protected reservoir for *C. albicans*.<sup>7</sup> Several antifungal substances, such as triclosan, nystatin<sup>3</sup> and zeolite,<sup>23</sup> have been added to denture acrylic resins in order to avoid *Candida* proliferation on prosthetic device surfaces.

However, the addition of these substances to acrylic resins leads to short-term efficacy due to the leachability of the antifungal agent from the bulk of the polymer. Therefore, the investigation of a quaternary ammonium functionalized compound [2-(methacryloyloxy)ethyl]trimethylammonium chloride –MADQUAT– (fig. 1), which copolymerizes with methacrylates and which could act as a fungal inhibitor, is of paramount importance.

The aim of this study was to evaluate the activity of MADQUAT against *Candida* species, determining the minimum inhibitory concentration (MIC) and the minimum fungicidal concentration (MFC).

## Material and methods

Thirty-one *Candida* strains were used to determine the in vitro antifungal activity: *C. albicans* (American Type Culture Collection – ATCC 10231, ATCC 18804, ATCC 28367, 0050-L, 0051-L, MG), *C. dubliniensis* (22, 23, 25, 27, 28, 29, ATCC 7987), *C. glabrata* (0030-L,



**Figure 1.** Chemical structure of [2-(methacryloyloxy)ethyl]trimethylammonium chloride.

993, ATCC 2001, MG), *C. krusei* (ATCC 6258, ATCC 20298, 0037-L, 219, 990, MG), *C. parapsilosis* (ATCC 22019, 0052-L, 0053-L, 0054-L, MG) and *C. tropicalis* (0056-L, ATCC 750, 0055-L).

Minimum inhibitory concentrations of MADQUAT and nystatin were determined using the *Clinical and Laboratory Standards Institute* M27-A3 methodology.<sup>4</sup> The strains were subcultured onto Sabouraud dextrose agar at 35 °C for 24 h. The inoculum was suspended in saline solution and adjusted to a final concentration of  $0.5 \times 10^3$ – $2.5 \times 10^3$  in RPMI 1640 medium (Sigma, St Louis, MO, USA) buffered to pH 7.0 with 165 mmol l<sup>-1</sup> morpholinopropanosulfonic acid (MOPS; Sigma).

Nystatin (Jansen-Cilag) was used as positive control. Stock solutions of nystatin and MADQUAT were prepared in dimethyl sulfoxide (DMSO; Vetec) and diluted in RPMI 1640 medium. The final concentrations of the antifungal agents ranged from 0.0312 to 16 µg/mL for nystatin and from 0.20 to 100 mg/ml for MADQUAT.

Sterilized round-bottomed 96-well microtiter plates (Cral Plast) were used, with addition of 100 µl of each drug to columns 1 to 10; 100 µl of RPMI 1640 medium were added to columns 11 and 12, which were used as growth positive and medium sterile controls, respectively. Aliquots of 100 µl of the standardized inoculum were added to the wells and the microtiter plates were incubated at 35 °C for 24 h. After incubation, the MIC was determined visually by comparison with the drug-free growth control well. The MIC was defined as the lowest concentration of the antifungal agent preventing visible fungal growth.

In order to determine the MFC, 100 µl of all wells with 100% of growth inhibition were seeded into culture tubes with 2 ml of Sabouraud dextrose broth medium. The tubes were incubated at 35 °C for 3 days to determine fungal growth. The MFC was the minimum fungistatic concentration that prevented fungal growth.<sup>10</sup>

## Results and discussion

According to table 1, MADQUAT showed antifungal activity with a geometric mean (GM) MIC of 48.9 mg/ml (230 µM) whereas nystatin had a GM antifungal activity of 1.8 µg/ml. The quantitative determinations of the fungistatic and fungicidal activity of MADQUAT showed fungistatic activity against all *C. albicans*, *C. krusei* and *C. parapsilosis* strains, revealing fungicidal activity against some microorganisms of the other species. The nine reference strains of *Candida* spp. used in the present study demonstrated fungistatic activity (table 2). Nystatin showed fungicidal activity against all strains (GM 4.0 µg/ml).

*Candida* species frequently cause oral infections, including denture-related stomatitis, a chronic inflammatory condition associated with the oral mucosa and that affects 30–60% of patients wearing removable dental prostheses.<sup>28</sup> While several factors have been implicated in the etiology of denture stomatitis, such as poor denture hygiene, mechanical irritation, diet, use of antibiotics or allergic reaction to denture base material, *Candida* species have been recognized as the primary agents.<sup>18,34</sup>

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