Mycetoma is a chronic infectious disease of the skin that can affect subcutaneous tissues, bones, and organs. The disease is characterized by the triad of firm tumefaction of the affected site, the formation of abscesses, and the presence of granules or microcolonies of the etiologic agents in the pus (14). The causative agents are introduced in the skin through minor traumas with wood splinters or other materials containing these microorganisms, which normally inhabit the soil. Myce- toma can be produced either by true fungi (eumycetoma) or by aerobic actinomycetes (14). Worldwide, around 60% of the mycetoma cases are caused by aerobic actinomycetes, but in México, actinomycetoma predominates, constituting about 98% of the cases (5). In this country, a single agent, Nocardia brasiliensis, produces about 86% of the cases; the rest of the cases are produced either by other aerobic actinomycetes (12%) or by eumycetes (2%).

Sulfonamides, particularly the combination sulfamethoxazole-trimethoprim (SXT), are the treatment of choice for actinomycetoma (13). However, this combination has to be given for months, even years, and the cure rate achieved is only 70% (14). In 1987, Welsh et al. described the use of amikacin-SXT to treat patients with disseminated or recalcitrant actino- mycetoma cases (12). By using this therapeutic approach, a higher cure rate was obtained in a shorter period of time than when using SXT alone.

Since then, we have used the combination amikacin-SXT in our dermatology clinic for the therapy of severe mycetoma cases or those resistant to the SXT treatment. However, in a few cases, even with the use of amikacin, the patient’s condition does not improve; also, amikacin, as with the rest of aminoglycosides, can produce unwanted secondary affects, such as ototoxicity or nephrotoxicity, and when they appear the patient has to abandon the treatment. The sulfonamides can also produce secondary effects, such as hemolytic and aplastic anemia, urticaria, photosensitivity, etc. Therefore, there is a need to search for new more-potent and less-toxic drugs to treat actinomyetoma caused by N. brasiliensis.

In the present study we utilized 30 N. brasiliensis strains isolated from mycetoma cases that had been referred to our dermatology department and identified by conventional biochemical methods, including the hydrolysis of casein, hipoxanthine, xanthine, tyrosine, esculin, and adenine. The susceptibilities of these strains to DA-7867 (a new oxazolidinone), gatifloxacin, moxifloxacin, and a recently described quinolone (11), garenoxacin, was determined using the broth microdilution method. The activities of these dugs were compared to those of the antimicrobials used in the conventional treatment of actinomyetoma caused by N. brasiliensis, SXT and amikacin. Linezolid was also assayed to compare its activity with that of DA-7867.

Linezolid was obtained from its manufacturers (Pharmacia and Upjohn, Kalamazoo, Mich.); SXT and amikacin were obtained from Sigma Chemical Products (St. Louis, Mo.). DA-7867 was provided by Dong-A Pharm., Yongin, Korea. Garenoxacin and gatifloxacin were obtained from Bristol-Myers Squibb (Princeton, N.J.), and moxifloxacin was obtained from commercial sources (Bayer AG, Leverkusen, Germany). The broth microdilution method that we utilized has been used with modifications for the tests against N. brasiliensis. The results obtained showed that linezolid is also active against these strains.

### Table 1. Activity of DA-7867 and other antimicrobial agents against clinical isolates of Nocardia brasiliensis.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Range (MIC μg/ml)</th>
<th>50%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>0.125–4</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>SXT</td>
<td>2.3/0.12–19/1</td>
<td>4.75/0.25</td>
<td>9.5/0.5</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0.12–2</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>DA-7867</td>
<td>0.015–0.12</td>
<td>0.03</td>
<td>0.06</td>
</tr>
<tr>
<td>Garenoxacin</td>
<td>0.25–2</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.25–2</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>0.12–1</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

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* 50% and 90%, MICs at which 50 and 90% of the N. brasiliensis strains are inhibited, respectively.
described previously (1, 10). Briefly, we utilized fresh colonies on Sabouraud agar (7 days old) to prepare the inoculum. Since *N. brasiliensis* grows as firm mycelial masses, we prepared a cellular suspension by placing a couple of loopfuls of the bacterial culture in a glass test tube and then grinding it with a glass stick in order to suspend most of the bacterial mass. The ground colonies were suspended in 1 ml of saline solution and diluted with cation-adjusted Mueller-Hinton broth until turbidity matched that of the McFarland 0.5 standard. This suspension was diluted with Mueller-Hinton broth to obtain a final concentration of $1 \times 10^4$ to $5 \times 10^4$ CFU per well in 0.1 ml that was added to microplate wells (Microtest Primaria,
Becton Dickinson and Co., Franklin Lakes, N.J.) containing an equal volume of broth with serial dilutions of the drugs tested. As a growth control, we inoculated in the same way a well containing cation-adjusted Mueller-Hinton broth without drug. After 3 days of incubation at 35°C, the plates were read and the MIC was determined as the lowest concentration of drug totally inhibiting the nocardial growth. For the sulfonamides, we considered as the MIC the lowest concentration that inhibits 80% of the growth compared with the growth in the control well. As external controls, we utilized Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 29213. All the antimicrobials except SXT were tested at concentrations of 64 to 0.015 μg/ml according to the guidelines of the National Committee for Clinical Laboratory Standards (6). The trimethoprim-sulfamethoxazole combination (ratio, 1:20) was tested from 76/4 to 0.29/0.015 μg/ml.

The MICs of DA-7867 and the other antimicrobial agents for the 30 clinical isolates of N. brasiliensis are presented in Table 1. As published previously (1, 10), linezolid demonstrated in vitro activity against all isolates tested (MIC at which 90% of the isolates tested were inhibited, below 2 μg/ml). The activity of the quinolones was similar to that shown by amikacin. The most active quinolone was garenoxacin, with a MIC at which 90% of the isolates tested were inhibited of 0.5. When comparing the cumulative percent inhibited for every concentration evaluated, no significant difference was observed among the quinolones (Fig. 1A); however, the N. brasiliensis strains showed an exquisite sensitivity to DA-7867 compared to sensitivities to amikacin and linezolid (Fig. 1B).

Few efforts have been made to study the sensitivities of Nocardia spp. to antimicrobials, and most of them have been done using Nocardia asteroides. Although phylogenetically N. brasiliensis and N. asteroides are quite related, they have different biological and drug sensitivity patterns. N. asteroides is sensitive to drugs such as imipenem, ciprofloxacin, minocycline, etc. (4); on the other hand, N. brasiliensis is susceptible to a more limited amount of antimicrobials. The presence of chronic inflammatory tissue, pus, scarring tissue, abundant micro- abscesses, and a low pH make it difficult for drugs to reach the microorganisms in the mycetoma lesions. The presence of osteomyelitis, which is often seen in actinomycetoma patients, can worsen the prognosis of the infection. With the development of global bacterial resistance there have appeared many new antimicrobials, more potent and with a wider spectrum of activity. The drugs tested in these assays presented a high activity against N. brasiliensis, with MICs even lower than those presented by amikacin, a traditional bactericidal agent used to treat actinomycetoma by N. brasiliensis. The des-fluoro (6) quinolone garenoxacin showed similar results to gatifloxacin and moxifloxacin, corroborating the results observed before with a few N. brasiliensis strains (9). The sensitivity of N. brasiliensis strains to gatifloxacin and moxifloxacin has not been reported to our knowledge.

Oxazolidinones have shown high activity in vitro against gram-positive organisms, including Mycobacteria and Nocardia (1, 2, 3, 8, 10). The best-known drug of these kinds of compounds, linezolid, has been used recently with success in some cases of nocardial infection, producing remission of the infections (7). Its use in clinical cases of mycetoma is still unknown, although in vivo experimental assays have shown good results, decreasing the development of lesions in an experimental model of mycetoma in BALB/c mice (L. Vera-Cabrera, A. Gomez-Flores, et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. B-509, 2001). Given these promising results, the assay of new oxazolidinones is important in order to select those with stronger activity and lower toxicity. DA-7867 has been shown to be more active than linezolid against a group of diverse gram-positive organisms (K. Lee, J. H. Jum, et al., Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-1312, 2001); this high sensitivity was also observed in our assays when comparing the sensitivities of the N. brasiliensis isolates to both drugs.

The activities of DA-7867 and the quinolones assayed in the experiments are promising, although experiments with a murine model of actinomycetoma should be done before starting clinical trials with patients.

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