Pathogenic *Nocardia*, *Rhodococcus*, and Related Organisms Are Highly Susceptible to Imidazole Antifungals

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***Rhodococcus equi* and species of *Nocardia* and *Gordonia* may be human opportunistic pathogens. We find that these, as well as several isolates from closely related genera, are highly susceptible to the imidazoles bifonazole, clotrimazole, econazole, and miconazole, whose MICs are ≤ 1 $\mu\text{g/ml}$. In liquid cultures 1 μg of the drug/ml was bacteriostatic and 10 $\mu\text{g/ml}$ was bactericidal. On solid media at 10 μg of azole/ml no resistant mutants could be isolated. An MIC of 1 to 15 $\mu\text{g/ml}$ was observed with ketoconazole, whereas none of these organisms was inhibited by the triazoles fluconazole and voriconazole (100 $\mu\text{g/ml}$). Imidazoles may offer the prospect of treatment of nocardioform mycetomas and may provide the basis for the development of additional antimicrobial agents to combat these pathogens.**

There has been an increase in the number of individuals infected with opportunistic pathogens of the nocardioform group of bacteria as a result of the susceptibility of AIDS patients and some other categories of immunocompromised patients (1, 4, 9). The soilborne organism *Rhodococcus equi* may cause chronic and severe pyogranulomatous pneumonia in foals, and similar symptoms can arise in humans with weakened immune systems; subsequent dissemination from the lung to other body sites sometimes also occurs in either horses or humans (10). *Nocardia brasiliensis* is a common cause of localized chronic mycetoma in Africa and other tropical areas and may develop into a disseminated infection in immunocompromised individuals (16, 20, 21). *Nocardia otitidiscaviarum* (3), *Dietzia maris* (2, 17), *Gordonia bronchialis*, *Gordonia rubripertincta* (18), and *Mycobacterium vaccae* (8) may also infect humans. Increasing incidence of drug resistance and multiple resistance for all main classes of antibiotics in both *R. equi* (11, 15) and *N. brasiliensis* (25) makes it desirable to identify additional antibacterial agents, preferably targeting novel elements in the prokaryotic cell to minimize the likelihood of cross-resistance from familiar antibiotics. Antifungal azoles act on organisms such as *Candida albicans* by blocking a step in the synthesis of ergosterol, resulting in damage to membrane integrity. They are selective inhibitors of the cytochrome P450-dependent 14- α demethylation of lanosterol but have very little effect on mammalian cytochrome P450 (26). The specificity of their mode of action suggests that susceptibility in bacteria is unlikely; however, they inhibit *Helicobacter pylori* at a concentration of 2 to 64 $\mu\text{g/ml}$ (24). In earlier work miconazole and ketoconazole were shown to inhibit *Staphylococcus aureus* (22). Metronidazole and other nitroimidazoles are bactericidal against *H. pylori* through toxic metabolites that cause DNA strand breakage (14). Recently miconazole and clotrimazole have been shown to have in vitro activity against *Mycobacte-*

rium tuberculosis, the MIC being 2 to 5 $\mu\text{g/ml}$ (23). Here we show that nocardioform opportunistic pathogens and other related strains from seven genera are highly susceptible to bifonazole and econazole as well as to miconazole and clotrimazole. However, fluconazole and the newly developed voriconazole (19) showed no inhibitory effects on any of these organisms.

Strains used in this work are listed in Table 1. Cultures were grown on brain heart infusion (BHI; pH 7.4), Luria-Bertani (pH 7.0), or Sabouraud dextrose (SD; 4%, pH 5.6) media solidified where required with 1.5% agar. The nonionic detergent Tween 80 was added to a final concentration of 0.7% to reduce aggregation during optical density (OD) and CFU measurements of cultures. All incubations were at 30°C. Bifonazole, clotrimazole, econazole, and miconazole were purchased from Sigma Chemical Co. Fluconazole and voriconazole were kindly provided by Pfizer Inc. Solid medium MICs were measured by replicating $\sim 10^4$ CFU onto plates supplemented with 0.25, 0.5, 1.0, 2.0, 5.0, 10, 20, 50, or 100 μg of drug/ml, followed by further intermediate concentrations where necessary. MICs in liquid BHI medium (10 ml, with five 5-mm glass beads to promote dispersal) were measured by monitoring the effect of drug challenge on viable counts and on OD at 600 nm with a Milton Roy Spectronic 601 spectrophotometer.

On BHI solid medium the MIC of each of the four azoles for *R. equi* strain ATCC 14887 was ≤ 1 $\mu\text{g/ml}$; the MIC for *N. brasiliensis* was 0.5 to 1.0 $\mu\text{g/ml}$ (Table 1). The least susceptibility, an MIC of 2 $\mu\text{g/ml}$, was observed for *M. vaccae* with bifonazole. Ketoconazole, an orally administered imidazole, was less effective: most MICs were ~ 10 $\mu\text{g/ml}$. Fluconazole and voriconazole showed no inhibitory effects in these experiments. We included 4-nitroimidazole in our testing since the MIC for *M. tuberculosis* has been reported to be ~ 20 $\mu\text{g/ml}$, but no inhibitory effect was observed at 100 $\mu\text{g/ml}$. *H. pylori* is highly susceptible to metronidazole (14), but this compound did not affect the growth of the strains we investigated. Azole MICs were not medium dependent: MICs on BHI medium were similar to those on Luria-Bertani or SD medium. Comparison

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TABLE 1. Susceptibility of bacterial strains to antifungal drugs^a

Organism	Strain	MIC ($\mu\text{g/ml}$) on BHI agar				
		Bifonazole	Clotrimazole	Econazole	Ketoconazole	Voriconazole
<i>Dietzia maris</i>	JCM 6166	0.3	0.5	1.0	1.0	>100
<i>Gordonia bronchialis</i>	UNT N-654	1.0	0.5	1.0	1.5	>100
<i>Gordonia rubripertincta</i>	ATCC 25593	1.5	0.5	1.0	10	>100
<i>Mycobacterium parafortuitum</i>	IFM 0490	1.0	0.5	0.5	3	>100
<i>Mycobacterium vaccae</i>	IFM 0489	2.0	0.25	1.0	8	>100
<i>Nocardia brasiliensis</i>	IFM 0236	0.3	1.0	1.0	5	>100
<i>Nocardia otitidiscaviarum</i>	IFM 0239	1.0	1.5	0.5	6	>100
<i>Nocardioides albus</i>	JCM 3185	1.0	0.25	0.25	10	>100
<i>Rhodococcus equi</i>	ATCC 14887	1.0	0.5	0.25	15	>100
<i>Rhodococcus equi</i>	CS402	1.0	1.0	0.5	10	>100
<i>Tsukamurella paurometabola</i>	IFM 0811	1.0	1.5	1.0	3	>100

^a Plates were incubated at 30°C for 96 h. Organisms were provided by Y. Mikami, except strains ATCC 14887 and ATCC 25593 (N. Ferreira) and CS402 (N. Tomioka).

with fungi on SD plates showed that these bacterial strains were in every case inhibited at lower drug concentrations than those at which the fungal strains were inhibited; for example, the econazole MICs were 5 $\mu\text{g/ml}$ for *C. albicans*, 2 $\mu\text{g/ml}$ for *Fusarium* sp., and 3 $\mu\text{g/ml}$ for *Saccharomyces cerevisiae* and *Aspergillus niger*. Fluconazole and voriconazole had MICs similar to or lower than these values against the fungal strains. Nystatin also targets ergosterol and interferes with fungal membrane integrity. However, instead of blocking synthesis this polyene binds to the ergosterol, leading to formation of a pore, allowing leakage of intracellular fungal ions and macromolecules (5). We tested the effect of nystatin on these bacterial strains, but no inhibition was observed, suggesting limits to comparability between azole action on prokaryotic nocardioforms and eukaryotic fungi.

The efficacy of antimicrobial chemotherapy is greatly diminished in the face of selection of resistant mutants: the average mutation rate in *M. tuberculosis* for resistance to isoniazid is 1 in 10^{-5} to 1 in 10^{-6} ; the corresponding figures for rifampin and

ethambutol are 1 in 10^{-8} and 1 in 10^{-4} , respectively (12). We attempted to select mutants resistant to 10 μg of each of the four imidazoles/ml; approximately 1×10^9 CFU of *M. vaccae*, 1×10^9 CFU of *N. brasiliensis*, or 4×10^9 CFU of *R. equi* in BHI broth were challenged with each of the antifungals, but no clones resistant to this level of drug were obtained. Similar results were obtained from cultures spread on BHI plates. These experiments were repeated four or more times, indicating that resistance to these compounds arises extremely rarely. At concentrations about 10 times the MIC the imidazoles were bactericidal (Fig. 1).

Recently a sterol biosynthetic pathway has been identified in mycobacteria (13) and the putative target was identified (7). Our results suggest that this pathway is present in nocardioform bacteria too. The drugs we determined to be bactericidal at low concentrations are for topical rather than systemic use (6), so they might be tested against mycetomas, infections of the skin and subcutaneous tissue by *Nocardia* and related organisms *Actinomadura madurae* and *Streptomyces somaliensis*

FIG. 1. Response to clotrimazole (10 $\mu\text{g/ml}$) challenge for cultures of *R. equi* ATCC 14887 (A) and *D. maris* JCM 6166 (B) in the presence (+) or absence (–) of an imidazole. The drug was added at 12 h (third data point). The scales of the y axes are logarithmic.

(16). Since antifungal imidazoles are routinely used on humans, their pharmacology is well established. Thus there is a realistic potential for their use in treatment of infections by these opportunistic pathogens.

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