

## In Vitro Activities of Ketolide HMR 3647, Macrolides, and Clindamycin against Coryneform Bacteria

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**The in vitro activity of ketolide HMR 3647 against coryneform bacteria isolated from clinical samples was evaluated. Except against *Corynebacterium jeikeium* and *C. urealyticum*, HMR 3647 showed high activity against *Corynebacterium* spp., being more active than 14- and 16-membered macrolides, azithromycin, or clindamycin. HMR 3647 also had high in vitro activity against *Brevibacterium* spp. and *Listeria monocytogenes*.**

Coryneform bacteria are increasingly recognized as a cause of human infection, particularly in hospitalized patients (6, 9). Molecular and chemotaxonomic methods have successfully been used in the last decade to define new genera and species and to reclassify previously recognized coryneform bacteria, but very few studies have been performed relative to their medical and clinical importance. *Corynebacterium urealyticum*, *C. jeikeium*, *C. amycolatum*, *C. striatum*, and *C. minutissimum* and *Brevibacterium* spp. are some of the most frequently isolated coryneform bacteria in clinical microbiology laboratories (6, 9, 12). Coryneform Centers for Disease Control and Prevention (CDC) groups I2 and F2 are now included in the newly described species *C. amycolatum* (2, 4). Most of the organisms previously identified as *C. xerosis*, some *C. minutissimum* strains, and a few *C. striatum* strains are also currently known to be *C. amycolatum*. Another clinically important gram-positive rod to be considered is *Listeria monocytogenes*.

*C. jeikeium* and *C. urealyticum* are commonly multi-drug resistant, although strains of both species are known to be susceptible to a variety of antimicrobial agents (3, 11–14), such as glycopeptides, and variably resistant to erythromycin, tetracycline, and fluoroquinolones. Multi-drug resistance is not found only in these two organisms; other species and groups of coryneform bacteria (including, for example, *C. amycolatum* or coryneform CDC group G) are frequently resistant to many of the antimicrobial agents available for therapeutic use (5, 9).

Ketolide HMR 3647 is a new semisynthetic 14-membered ring macrolide with a 3-keto group instead of the L-cladinose sugar (1). Ketolides show the same antibacterial spectrum as reference macrolides but also have good activity against erythromycin-resistant isolates among gram-positive cocci (7). The purpose of this study was to evaluate the in vitro activity of the new ketolide HMR 3647 against coryneform bacteria and *L. monocytogenes* isolated from human samples compared with those of azithromycin, 14- and 16-membered macrolides, and clindamycin.

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The following test organisms (number of isolates) were isolated from clinical samples at the Department of Clinical Mi-

crobiology, University Hospital Virgen Macarena, Seville, Spain: *C. amycolatum* (35), *C. jeikeium* (20), *C. urealyticum* (20), *C. striatum* (20), *C. minutissimum* (20), *L. monocytogenes* (15), and *Brevibacterium* spp. (10). Organisms were identified by conventional phenotypic tests and the API Coryne System, according to Funke et al. (6). *C. amycolatum* strains included organisms identified as *C. xerosis*, *C. minutissimum*, and CDC coryneform group I2 by the CDC scheme for identification of coryneform bacteria (4). After isolation and identification, bacteria were maintained in 10% glycerol in tryptic soy broth at  $-70^{\circ}\text{C}$  until used. The following reference strains were included: *C. jeikeium* ATCC 43734, *C. urealyticum* ATCC 43042, and *C. striatum* ATCC 6940. *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as control strains.

HMR 3647 (Hoechst-Marion-Roussel, Romainville, France), erythromycin A (Sigma, St. Louis, Mo.), clarithromycin (Abbott Laboratories, Madrid, Spain), roxithromycin (Hoechst-Marion-Roussel), josamycin (Sigma), azithromycin (Pfizer S.A., Madrid, Spain), and clindamycin (Pharmacia-Upjohn, Kalamazoo, Mich.) were used as powders of known potency. Solutions of antimicrobial agents were prepared on the day of antimicrobial testing, according to the manufacturer's instructions.

A microdilution method was used for susceptibility testing. The guidelines provided by the National Committee for Clinical Laboratory Standards (NCCLS) for dilution susceptibility testing (10) were followed, with some exceptions, as coryneform bacteria are not discussed in this document. Cation-adjusted Mueller-Hinton broth (Difco, Detroit, Mich.) was supplemented with 0.5% Tween 80 in tests of the lyophilic species *C. jeikeium* and *C. urealyticum*. Twofold dilutions ranging from 0.03 to 64  $\mu\text{g/ml}$  for all antimicrobial agents were tested. The inoculum was prepared from bacterial cultures grown on Columbia agar with 5% sheep blood agar for 20 to 24 h. Bacteria were suspended in Mueller-Hinton broth with (*C. jeikeium* and *C. urealyticum*) or without 0.5% Tween 80 to a concentration of about  $10^8$  CFU/ml and diluted in the same medium to obtain a final concentration of  $5 \times 10^4$  CFU per well. The final volume in each well of the microtiter plate was 100  $\mu\text{l}$ . Plates were inoculated and then incubated in air at  $35^{\circ}\text{C}$  for 20 to 22 h or 48 h if no growth was observed at 20 to 22 h or if the organism was *C. striatum*.

The MICs, MICs at which 50% of the isolates are inhibited ( $\text{MIC}_{50\text{s}}$ ), and  $\text{MIC}_{90\text{s}}$  of the different antimicrobial agents evaluated are shown in Table 1. The macrolides and clindamycin had low activity against *Corynebacterium* spp. The

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TABLE 1. Ranges, MIC<sub>50</sub>s, and MIC<sub>90</sub>s of antimicrobial agents for coryneform bacteria

Species (n)	HMR 3647		Erythromycin		Clarithromycin		Roxithromycin		Josamycin		Azithromycin		Clindamycin	
	Range	MIC <sub>50</sub>	Range	MIC <sub>50</sub>	Range	MIC <sub>50</sub>	Range	MIC <sub>50</sub>	Range	MIC <sub>50</sub>	Range	MIC <sub>50</sub>	Range	MIC <sub>50</sub>
<i>C. amycolatum</i> (35)	≤0.03-1	0.25	0.25	0.25	0.06->64	4	0.06->64	16	0.125->64	16	0.125->64	>64	>64	>64
<i>C. jeikeium</i> (20)	0.06->64	1	>64	0.06->64	0.06->64	>64	>64	>64	0.25->64	>64	>64	0.06->64	>64	>64
<i>C. urealyticum</i> (20)	≤0.03->64	>64	>64	0.125->64	0.125->64	>64	>64	>64	0.5->64	>64	>64	0.5->64	>64	>64
<i>C. striatum</i> (20)	≤0.03-8	0.06	0.06	2->64	1->64	2	4	8	16->64	32	16->64	>64	>64	>64
<i>C. minutissimum</i> (20)	≤0.03-4	≤0.03	0.25	≤0.03->64	1	8	4	2	≤0.03->64	16	>64	>64	>64	32
<i>L. monocytogenes</i> (15)	0.06-1	0.125	0.25	0.06-1	0.125	0.25	0.125	0.125	0.25-0.5	0.5	0.25-1	1	1	2
<i>Brevibacterium</i> spp. (10)	0.06-0.25	0.125	0.25	≤0.03->64	0.125	0.125	2	0.5	≤0.03->64	0.5	≤0.03->64	0.25	0.25	1
All strains (140)	≤0.03->64	0.125	>64	≤0.03->64	8	>64	≤0.03->64	2	>64	≤0.03->64	16	>64	≤0.03->64	>64

ketolide HMR 3647 showed high activity against *C. amycolatum*, *C. striatum*, and *C. minutissimum* but low activity against the more-resistant species *C. urealyticum* and *C. jeikeium*. Similar activities of macrolides and clindamycin against *Corynebacterium* spp. have been reported in other studies (5, 8, 14), although Soriano et al. (14) reported the MIC<sub>90</sub>s of erythromycin for *C. minutissimum* strains to be several times higher than those obtained here. It is possible that some of the *C. minutissimum* strains included in the study of Soriano et al. belong to the newly-recognized *C. amycolatum* species.

It is important that remarkable differences in the MICs of ketolides and macrolides can be observed for *C. striatum* if readings are made at 48 h instead of 20 h. At 48 h, all *C. striatum* strains showed a hazy growth, lower than that observed in control strains or in other coryneforms, in wells containing concentrations of 32 to 64 µg/ml. Since the meaning of this residual growth remains unclear, and *C. striatum* is a nonfastidious bacterium, we chose to establish MICs at 20 h following NCCLS guidelines for nonfastidious microorganisms. This phenomenon could partially explain the higher MICs of HMR 3647 and erythromycin for *C. striatum* isolates that were obtained by other authors (15).

At a concentration of 0.5 µg/ml, which is the breakpoint of the NCCLS for erythromycin and clindamycin, more than 90% of *C. amycolatum*, *C. striatum*, and *C. minutissimum* strains were inhibited by HMR 3647. These values decreased to 20 and 45% for *C. urealyticum* and *C. jeikeium*, respectively. At this concentration, none of the macrolides inhibited more than 20% of *Corynebacterium* spp., with the exception of *C. minutissimum*. Forty to 70% of strains of the latter species were inhibited by macrolides at 0.5 µg/ml.

The lowest MIC<sub>90</sub>s for *L. monocytogenes* were those of clarithromycin (0.125 µg/ml) and those of HMR 3647 and erythromycin (0.25 µg/ml for both antimicrobial agents). At a concentration of 0.5 µg/ml, more than 90% of the strains were inhibited by HMR 3647, erythromycin, clarithromycin, and roxithromycin. Josamycin, azithromycin, and clindamycin showed very low activity against this microorganism.

HMR 3647 was the most-active agent against *Brevibacterium* spp. The MIC<sub>90</sub> of clarithromycin (2 µg/ml) was much lower than those of the other macrolides (≥16 µg/ml).

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REFERENCES

- Bryskier, A., C. Agouridas, and J. F. Chantot. 1997. Ketolide: new semisynthetic 14-membered ring macrolide, p. 39-50. In S. H. Zinner, L. S. Young, J. P. Acar, and H. C. Neu (ed.), Expanding indications for the new macrolides, azalides and streptogramins. Marcel Dekker Inc., New York, N.Y.
- Collins, M. D., R. A. Burton, and D. Jones. 1988. *Corynebacterium amycolatum* sp. nov., a new mycolic acid-less *Corynebacterium* species from human skin. FEMS Microbiol. Lett. 49:349-352.
- García-Rodríguez, J. A., J. E. García Sánchez, J. L. Muñoz Bellido, T. Nebreda Mayoral, E. García Sánchez, and I. García García. 1991. In vitro activity of 79 antimicrobial agents against *Corynebacterium* group D2. Antimicrob. Agents Chemother. 35:2140-2143.
- Funke, G., P. A. Lawson, K. A. Bernard, and M. D. Collins. 1996. Most *Corynebacterium xerosis* strains identified in the routine clinical laboratory correspond to *Corynebacterium amycolatum*. J. Clin. Microbiol. 34:1124-1128.
- Funke, G., V. Pünter, and A. Von Graevenitz. 1996. Antimicrobial susceptibility patterns of some recently established coryneform bacteria. Antimicrob. Agents Chemother. 40:2874-2878.
- Funke, G., A. von Graevenitz, J. E. Clarridge, and K. A. Bernard. 1997. Clinical microbiology of coryneform bacteria. Clin. Microbiol. Rev. 10:125-159.
- Jones, R. N., and D. J. Biedenbach. 1997. Antimicrobial activity of RU-66647, a new ketolide. Diagn. Microbiol. Infect. Dis. 27:7-12.
- Martínez-Martínez, L., A. Pascual, K. Bernard, and A. I. Suárez. 1996. Antimicrobial susceptibility pattern of *Corynebacterium striatum*. Antimi-

- crob. Agents Chemother. **40**:2671–2672.
9. **Martínez-Martínez, L.** 1998. Clinical significance of newly recognized coryneform bacteria. *Rev. Med. Microbiol.* **9**:55–68.
  10. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard. NCCLS document M7-A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
  11. **Philippon, P., and E. Bimet.** 1990. In vitro susceptibility of *Corynebacterium* group D2 and *Corynebacterium jeikeium* to twelve antibiotics. *Eur. J. Clin. Microbiol. Infect. Dis.* **9**:892–895.
  12. **Riegel, P., R. Ruimy, R. Christen, and H. Monteil.** 1996. Species identities and antimicrobial susceptibilities of corynebacteria isolated from various clinical sources. *Eur. J. Clin. Microbiol. Infect. Dis.* **15**:657–662.
  13. **Soriano, F., C. Ponte, M. Santamaría, A. Torres, and R. Fernández-Roblas.** 1987. Susceptibility of urinary isolates of *Corynebacterium* group D2 to fifteen antimicrobials and acetohydroxamic acid. *J. Antimicrob. Chemother.* **20**:349–355.
  14. **Soriano, F., J. Zapardiel, and E. Nieto.** 1995. Antimicrobial susceptibilities of *Corynebacterium* species and other non-spore-forming gram-positive bacilli to 18 antimicrobial agents. *Antimicrob. Agents Chemother.* **39**:208–214.
  15. **Soriano, F., R. Calvo, and R. Fernández-Roblas.** 1997. In vitro activity of ketolide HMR 3647 (RU 66647) against gram-positive bacilli, abstr. F-118, p. 166. *In* Program and abstracts of the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.