JOURNAL OF CLINICAL MICROBIOLOGY, July 1997, p. 1904–1908 Vol. 35, No. 7
0095-1137/97/$04.00+0

Emergence of Rifampin-Resistant *Rhodococcus equi* in an Infected Foal

SHINJI TAKAI,[1]* KAZUYO TAKEDA,[1] YOSHIMI NAKANO,[1] TETSUYA KARASAWA,[1] JUICHI FURUGOORI,[1] YUKAKO SASAKI,[1] SHIRO TSUBAKI,[1] TOHRU HIGUCHI,[2] TOHRU ANZAI,[3] RYUICHI WADA,[3] AND MASANOBU KAMADA[3]

Department of Animal Hygiene, School of Veterinary Medicine and Animal Sciences, Kitasato University, Towada, Aomori 034,[1] Hidaka Agriculture Mutual Aid Association, Shizunai, Hokkaido 056,[2] and Epizootic Research Station, Equine Research Institute, Japan Racing Association, Kokubunji, Tochigi 329-04,[3] Japan

Received 18 July 1996/Returned for modification 21 January 1997/Accepted 18 March 1997

To investigate the emergence of rifampin resistance in *Rhodococcus equi* strains isolated from foals and their environment in Japan, we compared the in vitro antimicrobial susceptibilities to rifampin of 640 isolates from 64 infected foals and 98 soil isolates from their horse-breeding farms. As a control, 39 human isolates from patients with and without AIDS were also tested for susceptibility to rifampin. All of the isolates showed rifampin sensitivity, except isolates from one infected foal and two patients with AIDS that showed rifampin resistance. To investigate the emergence of rifampin-resistant *R. equi* in the infected foal, which had received rifampin monotherapy for a month before euthanasia, 99 isolates of *R. equi* from the lesions and 20 isolates from the intestinal contents of the one foal with rifampin-resistant organisms were analyzed for rifampin susceptibilities, pathogenicities, and ribotypes. Of the 99 isolates from the lesions, all of which were virulent *R. equi* strains containing a virulence plasmid with a size of 85 or 90 kb, 90 (91%) isolates were rifampin resistant (MIC, ≧12.5 μg/ml). On the other hand, of the 20 isolates from the intestinal contents, 11 (55%) isolates showed rifampin resistance (MIC, ≧25 μg/ml), and 5 of them were avirulent *R. equi* strains. Among these 101 rifampin-resistant *R. equi* isolates with and without virulence plasmids characterized by ribotyping, 58 were type I, 20 were type II, 11 were type III, and 12 were type IV. These results demonstrated that at least eight different rifampin-resistant *R. equi* strains emerged concurrently and respectively from the different lesions and intestinal contents of the infected foal.

Rhodococcus equi is a well-known pathogen in domestic animals, especially horses, which causes suppurative bronchopneumonia with high mortality in foals aged 1 to 3 months (2, 15, 28). Though rarely seen in immunocompromised humans before the rise in cases of human immunodeficiency virus infection, *R. equi* is now recognized as an emerging opportunistic pathogen in patients with AIDS (5–7, 19, 32). In foals, the disease has worldwide distribution, and in general, *R. equi* causes sporadic disease, although it has become endemic on some farms (2, 15, 28). The disease progresses insidiously in many cases, and delay in diagnosis and treatment tends to increase the morbidity rate. Morbidity rates of 5 to 17% worldwide and mortality rates of up to 80% have been reported (2, 15).

The introduction of a combination of erythromycin and rifampin for the treatment of *R. equi* pneumonia of foals has improved survival rates considerably (8, 9, 17, 20). The results of several studies revealed that the combination provided the most successful antimicrobial treatment, because of the low MIC of these drugs, as well as good tissue and macrophage penetration (17). At present, these two antibiotics are the drugs of choice for the treatment of this disease in foals and infected human patients (3, 5–7, 12, 13).

The majority of *R. equi* strains are highly susceptible to rifampin, usually at 0.06 μg/ml or less (15). However, mutants arise spontaneously in strains not exposed previously to the antibiotic at a rate of 1 mutation per 10^{-7} to 10^{-8} organisms (1), indicating that the drug should be used only in combination with other antibiotics. In human patients, resistance to rifampin has developed during treatment (3, 13), and although the problem has been anticipated (9), it has not been described previously for foals, except for a 10-month-old filly which had a chronic respiratory tract disease (9).

We have recently reported that the 15- to 17-kDa antigens of *R. equi* are associated with virulence in foals and mice (25) and that the presence of large plasmids (85- and 90-kb virulence plasmids) is essential for virulence and expression of 15- to 17-kDa antigens (23, 29, 30). Recent studies have revealed that the virulence-associated 15- to 17-kDa antigens and virulence plasmids are useful epidemiological markers for virulent *R. equi* (24–30, 32).

In this paper, we report the first case of rifampin resistance in an infected foal in Japan and describe the process of emergence as determined by using plasmid profiles and ribotyping.

Clinical isolates were obtained from the lung lesions of 64 foals infected with *R. equi* during a 4-year surveillance study in Aomori and Hokkaido in Japan. A total of 847 isolates were examined and included 630 isolates recovered from 63 infected foals, 119 isolates from lesions and the intestinal contents of 1 foal with rifampin-resistant organisms, and 98 isolates recovered from soil on horse-breeding farms. For the selective isolation of *R. equi*, nalidixic acid-novobiocin-actidione (cycloheximide)-potassium tellurite (NANAT) medium was used (34). The 39 human isolates used in the investigation were obtained from immunocompromised patients with and without AIDS and are described elsewhere (24, 27).

The disk diffusion method of susceptibility testing was used

* Corresponding author. Mailing address: Department of Animal Hygiene, School of Veterinary Medicine and Animal Sciences, Kitasato University, Towada, Aomori 034, Japan. Phone: 81-176-23-4371, ext. 459. Fax: 81-0176-23-8703. E-mail: takai@vmas.kitasato-u.ac.jp.

for screening the large numbers of *R. equi* isolates. Mueller-Hinton II agar (Becton Dickinson and Company, Cockeysville, Md.) was employed for disk diffusion testing, which was performed according to the manufacturer's directions.

MICs were determined by the broth dilution method with Mueller-Hinton II broth. The antimicrobial agent was supplied as tablets of known potency. The inoculum was prepared with *R. equi* suspensions with turbidity adjusted to that of a 0.5 McFarland standard. All of the cultures were incubated for 18 h at 37°C. The MIC was taken as the lowest concentration of antibiotic which prevents visible growth. Control strains *R. equi* ATCC 6939 and *Staphylococcus aureus* ATCC 29213 were included in each test. The MICs for these control strains were within the acceptable range throughout the experiments.

Whole-cell antigens, which were prepared by harvesting bacteria grown at 38°C for 48 h in brain heart infusion broth and solubilized in sodium dodecyl sulfate (SDS) reducing buffer, were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, electrotransfer of proteins to nitrocellulose sheets, and Western blot (immunoblot) analysis, as described previously (25, 29). A monoclonal antibody specific for the 15- to 17-kDa antigens was used for the immunoblotting procedures (22).

Plasmid DNA was isolated from *R. equi* by the alkaline lysis method with the following modifications, as described previously (29). Samples of plasmid preparations were separated along with the plasmids of *R. equi* ATCC 33701 (85-kb plasmid) and L1 (90-kb plasmid) in 0.7% agarose gels at approximately 5 V/cm for 2 h.

Ribotyping was performed essentially as described previously by Lasker et al. (10) with the enzyme *Pvu*II (Takara, Tokyo Japan). *R. equi* isolates were grown in brain heart infusion broth for 2 days at 30°C before being harvested by centrifugation. DNA was extracted from *R. equi* by the method described by Lasker et al. (10). The 16S rRNA gene of *Corynebacterium kutscheri* ATCC 15677 was used as a probe for ribotyping (21). An *Escherichia coli* strain harboring the plasmid pBR322CK containing the 16S rRNA gene of *C. kutscheri* was donated by T. Takahashi, Rakuno Gakuen University. The digoxigenin-11-dUTP-labeled 16S rRNA gene probe was amplified with the primers by PCR with the DIG-DNA Labeling and Detection Kit (Boehringer Mannheim-Yamanouchi, Tokyo, Japan) as described previously (23). The selection of two primers was based on the gene sequence published by Takahashi et al. (21). Two oligonucleotide primers were custom synthesized (Nisseiken Co., Ltd., Tokyo, Japan): 5′-GAGTG GCGAACGGTGAGTAA-3′ (primer 1) and 5′-CAGCGTTG CATTCTGCGATT-3′ (primer 2). Primer 1 corresponds to the sense strand at positions 52 to 71 of the gene; primer 2 corresponds to the antisense strand at positions 1,297 to 1,278.

One microgram of chromosomal DNA was digested with 10 U of *Pvu*II (Takara) for 4 to 6 h at 37°C in buffer as recommended by the manufacturer. Restriction endonuclease fragments were then separated by electrophoresis in a 0.7% (wt/vol) agarose gel (Pharmacia) in Tris-borate buffer (0.089 M Tris-borate, 0.089 M boric acid, 0.008 M disodium EDTA [pH 8.2]). DNA fragments were then transferred to a sheet of Nytran (Schleicher & Schuell, Germany) by the vacuum transfer method with VacuGene (Pharmacia-LKB Biotechnology AB, Uppsala, Sweden) and without depurination, as described in the instructions of the manufacturer. Hybridization was carried out at 68°C for at least 6 h in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate [pH 7.0]) containing 0.1% sodium *N*-lauroylsarcosine, 0.02% SDS, and 1% blocking agent. After hybridization, the sheets were washed twice at room temperature for 5 min in 2× SSC containing 0.1% SDS and then twice for 15 min at 68°C in 0.1× SSC containing 0.1% SDS. Immunological detection of hybridized digoxigenin-labeled probe was performed with the commercial kit.

TABLE 1. Isolation of virulent *R. equi* from an infected foal

Source of isolate[a]	No. of bacteria/g ($\log_{10}$)	No. of isolates	No. of isolates containing virulence plasmid		No. of avirulent isolates
			85 kb	90 kb	
Lung Ab1	7.9	10	0	10	0
Lung Ab2	4.7	10	0	10	0
Lung Ab3	7.9	10	10	0	0
Lung Ab4	4.0	10	10	0	0
Kidney	3.3	9	0	9	0
Bronchial L	3.7	10	10	0	0
Mesenteric L1	8.0	10	10	0	0
Mesenteric L2	8.1	10	0	10	0
Mesenteric L3	7.9	10	0	10	0
Synovial fluid	7.7	10	10	0	0
Stomach C	3.2	3	1	2	0
Duodenum C	2.7	1	0	1	0
Jejunum C	2.7	2	0	0	0
Ileum C	3.5	2	2	0	0
Cecum C	4.4	4	0	1	3
Colon C	4.3	5	2	2	1
Rectum C	4.3	5	2	0	3

[a] Ab, abscess; L, lymph node; C, contents.

To survey the emergence of rifampin resistance in *R. equi* isolates from foals and their environment in Japan, the in vitro antimicrobial susceptibilities to rifampin of 640 isolates from lung lesions of 64 infected foals (10 isolates each), 98 soil isolates from their horse-breeding farms, and 39 isolates from patients with and without AIDS were tested. Almost all of the isolates showed rifampin sensitivity; however, isolates from one infected foal and two patients with AIDS showed rifampin resistance. The susceptibilities of 201 isolates (64 independent isolates from the 64 foals, 98 soil isolates, and 39 human isolates) to erythromycin and gentamicin were also tested, and all of the isolates revealed sensitivity to these agents.

The infected foal, from which rifampin-resistant *R. equi* strains were isolated, showed initial clinical signs of respiratory disease at 40 days of age and then was treated with changing combinations of penicillin, ampicillin, cephalothin, streptomycin, and gentamicin for several weeks. However, since no clinical improvement was observed, the combination of rifampin and erythromycin was used for the treatment. Because of the onset of self-limiting diarrhea in the foal during the first week of therapy, erythromycin was discontinued and rifampin alone was used for 1 month. When the foal showed no signs of improvement, it was euthanized at 111 days of age. Necropsy revealed that the foal had *R. equi* infection and that the lesions involved both lungs and lymphadenitis of gut-associated lymph nodes with abdominal abscesses.

Bacteriological examinations of isolates from the foal revealed the presence of *R. equi* in pure cultures from the abscesses of the lungs, bronchial and mesenteric lymph nodes, and kidneys. *R. equi* was also isolated from the contents of the intestinal tract with NANAT medium (Table 1). One or more isolates per specimen were subcultured, tested for the presence of 15- to 17-kDa antigens by Western immunoblotting with a monoclonal antibody, and then analyzed for the presence of virulence plasmid DNA by agarose gel electrophoresis (Table 1). All of the isolates from lesions expressed the 15- to 17-kDa antigens and possessed a virulence plasmid. Both virulent and

TABLE 2. Distribution of MICs of rifampin for 119 *R. equi* isolates from an infected foal

Source of isolate (*n*)	No. of isolates for which MIC (μg/ml) was:									
	≦0.195	0.39	0.78	1.56	3.12	6.25	12.5	25	50	≧100
Lung Ab1 (10)	1								5	4
Lung Ab2 (10)	8						2			
Lung Ab3 (10)									9	1
Lung Ab4 (10)								2	8	
Bronchial L (10)								1	9	
Kidney (9)									9	
Mesenteric L1 (10)										10
Mesenteric L2 (10)								8	1	
Mesenteric L3 (10)								5	4	1
Synovial fluid (10)							8	2		
Stomach C (3)	1		1							1
Duodenum C (1)	1									
Ileum C (2)		1	1							
Cecum C (4)	1							1		2
Colon C (5)		1						1		3
Rectum C (5)	2									3

[a] Ab, abscess; L, lymph node; C, contents.

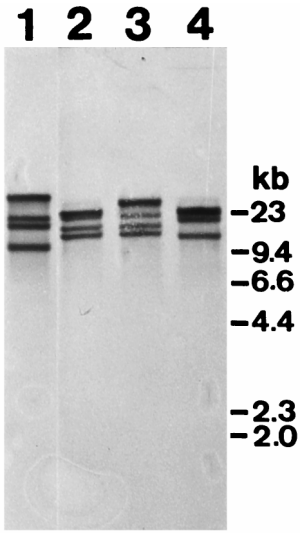

FIG. 1. rRNA gene restriction patterns of *R. equi* DNA after cleavage with *Pvu*II and hybridization with a 16S ribosomal DNA digoxigenin-labeled probe obtained by PCR. Lanes: 1, isolate 94L6 (type I); 2, isolate 94K3 (type II); 3, isolate 94I10 (type III); 4, isolate 94L4 (type IV). Molecular mass markers (λ DNA cleaved by *Hin*dIII) are shown in the right margin.

avirulent *R. equi* strains were isolated from the intestinal contents.

To investigate how rifampin-resistant *R. equi* strains emerged in the infected foal, 99 isolates of *R. equi* from the lesions and 20 isolates from the intestinal contents of the foal were analyzed for their rifampin susceptibilities, plasmid profiles, and ribotypes. As shown in Table 2, 90 (91%) of the 99 isolates from the 10 lesions showed rifampin resistance (MIC, ≧12.5 μg/ml). Nine isolates from two lung abscesses showed sensitivity. On the other hand, of the 20 isolates from the intestinal contents, 11 (55%) isolates showed rifampin resistance (MIC, ≧25 μg/ml).

The 99 isolates from the lesions contained an 85-kb (59 isolates) or a 90-kb (40 isolates) plasmid, and 13 of the 20 isolates from the intestinal contents harbored an 85-kb (7 isolates) or a 90-kb (6 isolates) plasmid (Table 1). The remaining seven isolates revealed avirulent *R. equi* strains.

The 119 isolates were characterized by ribotyping and divided into four types (Fig. 1); 62 were type I, 23 were type II, 14 were type III, and 20 were type IV. Table 3 summarizes the results for the virulence plasmids and ribotypes of the rifampin-resistant isolates. All of the isolates containing an 85-kb plasmid were type I, and those containing a 90-kb plasmid were divided into four types. Avirulent isolates which had no plasmids were divided into three types. Therefore, there were at least eight different *R. equi* isolates discriminated among these rifampin-resistant isolates.

The present study demonstrated the presence of rifampin-resistant *R. equi* strains in an infected foal which had been treated with rifampin alone for a month. At least eight different rifampin-resistant isolates, which were discriminated by plasmid profiles and ribotyping, emerged from different sites of the lesions and from the intestinal contents of the foal concurrently and respectively. These results revealed that rifampin-resistant mutants are easily selected by monotherapy and that a combination of antibiotics is crucial for treatment of *R. equi* infection in foals.

The combination of erythromycin and rifampin was introduced for the treatment of *R. equi* infection in foals after the synergistic activity of the combination was reported (16, 17, 20), because these drugs can be administered orally and have the ability to penetrate cells, including macrophages and neutrophils, which *R. equi* infects (11). Another important reason for the combined use of rifampin and erythromycin is that it reduces the likelihood of the emergence of mutants during treatment, since the rapid mutation rate of bacteria allowing development of rifampin resistance and bacterial development of chromosomal resistance with long-term use of erythromycin have been reported (1, 4). However, a recent report by Kenney et al. (9) demonstrated that resistance of rifampin and erythromycin developed during the treatment of *R. equi* pneumonia in a 10-month-old foal with reactive arthritis. The MICs of rifampin for Kenney's isolates were >4 μg/ml; on the other hand, those for our isolates ranged from 12.5 to >100 μg/ml. The molecular basis of rifampin resistance has been studied extensively with *E. coli*, in which the drug target has been

TABLE 3. Virulence plasmids and ribotypes of rifampin-resistant *R. equi* isolates from an infected foal

Source of rifampin-resistant isolate (*n*)[a]	No. of isolates with ribotype							
	Virulence plasmid					Avirulent		
	85 kb I	90 kb						
		I	II	III	IV	I	II	IV
Lung Ab1 (9)					9			
Lung Ab2 (2)		2						
Lung Ab3 (10)	10							
Lung Ab4 (10)	10							
Bronchial L (10)	10							
Kidney (9)			9					
Mesenteric L1 (10)	10							
Mesenteric L2 (10)				10				
Mesenteric L3 (10)			10					
Synovial fluid (10)	10							
Stomach C (1)					1			
Cecum C (3)						2		1
Colon C (4)	1			1	1		1	
Rectum C (3)	2					1		

[a] Ab, abscess; L, lymph node; C, contents.

shown to be the beta subunit of DNA-dependent RNA polymerase, which is encoded by the *rpoB* gene (1). Mutants resistant to high levels of the antibiotic have shown an alteration in a subunit of RNA polymerase (1). Recently, a novel mechanism of resistance to rifampin has been identified in two nocardioform species, *Rhodococcus erythropolis* and *Mycobacterium smegmatis*, in which this antibiotic is inactivated (4, 31). The molecular mechanisms of resistance to rifampin have not been established in *R. equi* (31). Therefore, further studies are needed to elucidate these mechanisms in isolates of *R. equi*.

Diarrhea is reportedly a side effect of erythromycin treatment of horses (18, 20). In the present study, a self-limiting diarrhea was noted in the foal during the first week of combined rifampin and erythromycin therapy, and the erythromycin was discontinued. The combination of rifampin and erythromycin was introduced as the preferred therapy for *R. equi* infection at actual clinical sites in the early 1990s in Japan, but little information was offered to farm managers. This might be the reason for the emergence of rifampin-resistant mutants of *R. equi* in Japanese foals.

In the present study, two human isolates showing rifampin resistance were isolated from patients with AIDS (3, 13). These strains were kindly provided by D. Clave (3), and their in vitro susceptibilities to rifampin and other antibiotics have already been tested. Our results completely agreed with those from their report, so these strains were used as a control in this study. The remaining 37 human isolates were sensitive to rifampin and erythromycin. Clave et al. (3) warned about the emergence of rifampin resistance and a marked increase in the MIC of imipenem in the human isolates, and Nordmann et al. (13) also reported rifampin- and fluoroquinolone-resistant isolates from a patient with AIDS. Early diagnosis of *R. equi* infection in humans is still difficult, since the presence of mycolic acids in the cell wall of *R. equi* may be misidentified as mycobacteria or may be regarded as a component of normal flora or a contaminant (6, 7, 33). Therefore, *R. equi* infections in humans may be mistaken for tuberculosis or other infections and treated accordingly with appropriate antibiotics, which could account for the association of human isolates of *R. equi* with resistance to antibiotics (3, 13).

Epidemiological studies of *R. equi* infection in foals have been advanced since the discovery of virulence-associated antigens and plasmids (14, 24–30, 32). A variety of typing procedures have been applied to different bacterial species to differentiate beyond the species and subspecies levels (10). In the present study, ribotyping was performed to discriminate between rifampin-resistant isolates which had been typed by plasmid profiles. This typing method is based on *Pvu*II digestion of chromosomal DNA, followed by Southern hybridization probing with the *E. coli* 16S rRNA operon, and has been shown to differentiate several different types (10). The present results demonstrated that ribotyping is a suitable tool for molecular characterization of virulent *R. equi*, and it may also provide a useful technique for studying the molecular epidemiology of *R. equi* infection in foals.

In the present study, avirulent isolates from the intestinal contents showed a high frequency of rifampin resistance (five of seven isolates). Since rifampin was administered orally, intestinal habitants were also influenced by the treatment. Rifampin-resistant isolates containing either of these virulence-associated plasmids were also isolated from the intestinal contents. At present, we are unable to differentiate the origins of these isolates containing either of these virulence-associated plasmids, whether from intestinal contents or lung lesions. The emergence of rifampin-resistant virulent *R. equi* strains due to the wrong therapy should be prevented, since infected foals are the major source of virulent *R. equi* strains and since they contaminate farm environments by shedding large quantities of *R. equi* cells in their feces (28).

We thank T. Takahashi, Faculty of Veterinary Medicine, Rakuno Gakuen University, for kindly providing the 16S rRNA gene of *C. kutscheri*. The help of a veterinarian (Y. Ichiyama) and farmers in providing samples is gratefully acknowledged.

This study was supported by Grants-in-Aid for Scientific Research B06454119 and C0687028 from the Ministry of Education, Science, Sports and Culture, Japan.

REFERENCES

1. **Arora, S. K., and P. Arjunan.** 1992. Molecular structure and conformation of rifamycins, a potent inhibitor of DNA-dependent RNA polymerase. J. Antibiot. **45:**428–431.
2. **Barton, M. D., and K. L. Hughes.** 1980. *Corynebacterium equi*: a review. Vet. Bull. **50:**65–80.
3. **Clave, D., M. Archambaud, R. M. Rouquet, P. Massip, and N. Moatti.** 1991. In vitro activity of twenty antibiotics to *Rhodococcus equi*. Pathol. Biol. **39:**424–428.
4. **Dabbs, E. R.** 1987. Rifampicin inactivation by *Rhodococcus* and *Mycobacterium*. FEMS Microbiol. Lett. **44:**395–399.
5. **Drancourt, M., E. Bonnet, H. Gallais, Y. Peloux, and D. Raoult.** 1992. *Rhodococcus equi* infection in patients with AIDS. J. Infect. **24:**123–131.
6. **Gradon, J. D., J. G. Timpone, and S. M. Schnittman.** 1992. Emergence of unusual opportunistic pathogens in AIDS: a review. Clin. Infect. Dis. **15:** 134–157.
7. **Harvey, R. L., and J. C. Sunstrum.** 1991. *Rhodococcus equi* infection in patients with and without human immunodeficiency virus infection. Rev. Infect. Dis. **13:**139–145.
8. **Hillidge, C. J.** 1987. Use of erythromycin-rifampin combination in treatment of *Rhodococcus equi* pneumonia. Vet. Microbiol. **14:**337–342.
9. **Kenney, D. G., S. C. Robbins, J. F. Prescott, A. Kaushik, and J. D. Baird.** 1994. Development of reactive arthritis and resistance to erythromycin and rifampin in a foal during treatment for *Rhodococcus equi* pneumonia. Equine Vet. J. **25:**246–248.
10. **Lasker, B. A., J. M. Brown, and M. M. McNeil.** 1992. Identification and epidemiological typing of clinical and environmental isolates of the genus *Rhodococcus* with use of a digoxygenin-labeled rDNA gene probe. Clin. Infect. Dis. **15:**223–233.
11. **Maggi, N., C. R. Pasqualucci, R. Ballotta, and P. Sensi.** 1966. Rifampicin: a new orally active rifamycin. Chemotherapia **11:**285–292.
12. **McNeil, M. M., and J. M. Brown.** 1992. Distribution and antimicrobial susceptibility of *Rhodococcus equi* from clinical specimens. Eur. J. Epidemiol. **8:**437–443.
13. **Nordmann, P., R. Rouveix, M. Guenounou, and M. H. Nicolas.** 1992. Pulmonary abscess due to a rifampin and fluoroquinolone resistant *Rhodococcus equi* strain in a HIV infected patient. Eur. J. Clin. Microbiol. Infect. Dis. **11:**557–558.
14. **Nordmann, P., M. Keller, F. Espinasse, and E. Ronco.** 1994. Correlation between antibiotic resistance, phage-like particle presence, and virulence in *Rhodococcus equi* human isolates. J. Clin. Microbiol. **32:**377–383.
15. **Prescott, J. F.** 1991. *Rhodococcus equi*: an animal and human pathogen. Clin. Microbiol. Rev. **4:**20–34.
16. **Prescott, J. F., and V. M. Nicolson.** 1984. The effects of combination of selected antibiotics on the growth of *Corynebacterium equi*. J. Vet. Parmacol. Ther. **7:**61–64.
17. **Prescott, J. F., and C. R. Sweeney.** 1985. Treatment of *Corynebacterium equi* pneumonia of foals: a review. J. Am. Vet. Med. Assoc. **187:**725–728.
18. **Prescott, J. F., D. J. Hoover, and I. R. Dohoo.** 1983. Pharmacokinetics of erythromycin in foals and in adult horses. J. Vet. Pharmacol. Ther. **6:**67–74.
19. **Samies, J. H., B. N. Hatrhaway, R. M. Echols, J. M. Veazey, and V. A. Pilon.** 1986. Lung abscess due to *Corynebacterium equi*: report of the first case in a patient with acquired immune deficiency syndrome. Am. J. Med. **80:**685–688.
20. **Sweeney, C. R., R. W. Sweeney, and T. J. Divers.** 1987. *Rhodococcus equi* pneumonia in 48 foals: response to antimicrobial therapy. Vet. Microbiol. **14:**329–336.
21. **Takahashi, T., M. Tsuji, N. Kikuchi, C. Ishihara, T. Osanai, N. Kasai, R. Yanagawa, and T. Hiramune.** 1995. Assignment of the bacterial agent of urinary calculus in young rats by the comparative sequence analysis of the 16S rRNA genes of corynebacteria. J. Vet. Med. Sci. **57:**515–517.
22. **Takai, S., M. Iie, C. Kobayashi, T. Morishita, T. Nishio, T. Ishida, T. Fujimura, Y. Sasaki, and S. Tsubaki.** 1993. Monoclonal antibody specific to virulence-associated 15- to 17-kilodalton antigens of *Rhodococcus equi*. J. Clin. Microbiol. **31:**2780–2782.
23. **Takai, S., T. Ikeda, Y. Sasaki, Y. Watanabe, T. Ozawa, S. Tsubaki, and T. Sekizaki.** 1995. Identification of virulent *Rhodococcus equi* by amplification of gene coding for 15- to 17-kilodalton antigens. J. Clin. Microbiol. **33:**1624–1627.

24. **Takai, S., Y. Imai, N. Fukumaga, Y. Uchida, K. Kamisawa, Y. Sasaki, S. Tsubaki, and T. Sekizaki.** 1995. Identification of virulence-associated antigens and plasmids in *Rhodococcus equi* from patients with AIDS. J. Infect. Dis. **172:**1306–1311.
25. **Takai, S., K. Koike, S. Ohbushi, C. Izumi, and S. Tsubaki.** 1991. Identification of 15- to 17-kilodalton antigens associated with virulent *Rhodococcus equi*. J. Clin. Microbiol. **29:**439–443.
26. **Takai, S., S. Ohbushi, K. Koike, S. Tsubaki, H. Oishi, and M. Kamada.** 1991. Prevalence of virulent *Rhodococcus equi* in isolates from soil and feces of horses from horse-breeding farms with and without endemic infections. J. Clin. Microbiol. **29:**2887–2889.
27. **Takai, S., Y. Sasaki, T. Ikeda, T. Uchida, S. Tsubaki, and T. Sekizaki.** 1994. Virulence of *Rhodococcus equi* isolates from patients with and without AIDS. J. Clin. Microbiol. **32:**457–460.
28. **Takai, S., Y. Sasaki, and S. Tsubaki.** 1995. *Rhodococcus equi* infection in foals: current concepts and implication for future research. J. Equine Sci. **6:**105–119.
29. **Takai, S., T. Sekizaki, T. Ozawa, T. Sugawara, Y. Watanabe, and S. Tsubaki.** 1991. Association between a large plasmid and 15- to 17-kilodalton antigens in virulent *Rhodococcus equi*. Infect. Immun. **59:**4056–4060.
30. **Takai, S., Y. Watanabe, T. Ikeda, T. Ozawa, S. Matsukura, Y. Tamada, S. Tsubaki, and T. Sekizaki.** 1993. Virulence-associated plasmids in *Rhodococcus equi*. J. Clin. Microbiol. **31:**1726–1729.
31. **Tanaka, Y., K. Yazawa, E. R. Dabbs, K. Nishikawa, H. Komaki, Y. Mikami, M. Miyaji, N. Morisaki, and S. Iwasaki.** 1996. Different rifampicin inactivation mechanisms in *Nocardia* and related taxa. Microbiol. Immunol. **40:**1–4.
32. **Tkachuk-Saad, O., and J. Prescott.** 1991. *Rhodococcus equi* plasmids: isolation and partial characterization. J. Clin. Microbiol. **29:**2696–2700.
33. **Van Etta, L. L., G. A. Filice, R. M. Ferguson, and D. N. Gerding.** 1983. *Corynebacterium equi*: a review of 12 cases of human infection. Rev. Infect. Dis. **5:**1012–1015.
34. **Woolcock, J. B., A.-M. T. Farmer, and M. D. Mutimer.** 1979. Selective medium for *Cornybacterium equi* isolation. J. Clin. Microbiol. **9:**640–642.