JOURNAL OF CLINICAL MICROBIOLOGY, June 2003, p. 2337–2340 Vol. 41, No. 6
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.6.2337–2340.2003

Emergence of Rifampin-Resistant *Rhodococcus equi* with Several Types of Mutations in the *rpoB* Gene among AIDS Patients in Northern Thailand

Norichika Asoh,[1]* Hiroshi Watanabe,[1] Marguerite Fines-Guyon,[2] Kiwao Watanabe,[1] Kazunori Oishi,[1] Weerayut Kositsakulchai,[3] Tippaya Sanchai,[3] Khemrassamee Kunsuikmengrai,[3] Sumpun Kahintapong,[3] Banyong Khantawa,[4] Prasit Tharavichitkul,[4] Thira Sirisanthana,[5] and Tsuyoshi Nagatake[1]

Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan[1]; Service de Microbiologie, CHU Côte de Nacre, 14033 Caen Cedex, France[2]; and Nakornping Hospital,[3] and Departments of Microbiology[4] and Medicine,[5] Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

Received 6 December 2002/Returned for modification 12 February 2003/Accepted 11 March 2003

The antimicrobial susceptibilities of 30 *Rhodococcus equi* isolates obtained from 30 patients between 1993 and 2001 in northern Thailand were investigated. The MICs showed a tendency toward resistance to various antibiotics but sensitivity to imipenem, minocycline, vancomycin, and teicoplanin (MICs, ≤0.5 μg/ml) and relative sensitivity to meropenem, clarithromycin, and ciprofloxacin (MICs, ≤2 μg/ml). Of the 30 isolates, 26 were susceptible (MICs, ≤1 μg/ml), 1 showed low-level resistance (MIC, 8 μg/ml), and 3 showed high-level resistance (MICs, ≥64 μg/ml) to rifampin. PCR amplification and DNA sequencing of the *rpoB* gene and molecular typing by pulsed-field gel electrophoresis (PFGE) were performed for eight *R. equi* isolates from eight AIDS patients with pneumonia or lung abscess caused by *R. equi* between 1998 and 2001, including one low- and three high-level rifampin-resistant isolates. As a result, two high-level rifampin-resistant strains with PFGE pattern A had a Ser531Trp (*Escherichia coli* numbering) mutation, and one high-level rifampin-resistant strain with PFGE pattern B had a His526Tyr mutation, whereas one low-level rifampin-resistant strain with PFGE pattern C had a Ser509Pro mutation. Four rifampin-susceptible strains with PFGE patterns D and E showed an absence of mutation in the *rpoB* region. Our results indicate the presence of several types of rifampin-resistant *R. equi* strains among AIDS patients in northern Thailand.

Rhodococcus equi, an aerobic, intracellular, gram-positive, acid-fast coccobacillus initially isolated from foals in 1923 as *Corynebacterium equi* (8), is a well-known pathogen in domestic animals, especially horses, which causes suppurative bronchopneumonia with a high mortality rate in young foals (15). It has been well known that this organism is an important pathogen in immunosuppressed human hosts since the first case of a human infection was reported in 1967 (6). A marked increase in the incidence of infection caused by *R. equi* has been reported since the human immunodeficiency virus (HIV) disease epidemic began in 1981 (4, 16, 21).

A combination of erythromycin and rifampin is considered to be effective for treating *R. equi* infections (9, 13, 19). However, the emergence of rifampin resistance in *R. equi* has been reported (10, 17), although it is still rare. Recently, Fines et al. reported that several mutations in the *rpoB* gene are associated with rifampin resistance in *R. equi* isolated from foals, as well as in *Mycobacterium tuberculosis*, *Escherichia coli*, and *Staphylococcus aureus* (5).

However, the issue of whether rifampin-resistant *R. equi* isolated from humans has such mutations is unclear. To address this issue, we conducted the study described here.

* Corresponding author. Mailing address: Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan. Phone: 81 (95) 849-7842. Fax: 81 (95) 849-7843. E-mail: nori-a@net.nagasaki-u.ac.jp.

MATERIALS AND METHODS

Bacterial strains. Thirty *R. equi* strains were isolated from 22 patients, whose clinical histories, including HIV, were not known in detail, at Chiang Mai University Hospital in northern Thailand between 1993 and 1996 and from 8 AIDS patients who had been admitted to Nakornping Hospital in northern Thailand due to pneumonia or lung abscess caused by *R. equi* from 1998 to 2001. The strains were detected in sputum (n = 22), blood (n = 5), pleural effusions (n = 2), and gastric juice (n = 1). Cultures were performed using TSA II medium (Becton Dickinson Microbiology Systems, Cockeysville, Md.) supplemented with 7% rabbit blood agar for 24 h at 37°C. The morphology of the colonies was similar to that of *Corynebacterium* species. Subsequently, *R. equi* was distinguished from *Corynebacterium* spp. by its biochemical characteristics, including the fact that it is nitrate positive, phosphatase alkaline positive, and alpha-glucosidase positive.

Antimicrobial susceptibility test. The MIC was determined by the agar dilution method according to the guidelines of the National Committee for Clinical Laboratory Standards (12). The susceptibilities of 30 *R. equi* isolates to the following 20 antibiotics were tested: amikacin (Meiji Seika Kaisha, Tokyo, Japan), ampicillin (Meiji Seika Kaisha), cefazolin (Fujisawa Pharmaceutical Co., Osaka, Japan), cefotiam (Takeda Chemical Industries, Osaka, Japan), ceftriaxone (Nippon Roche Co., Tokyo, Japan), chloramphenicol (Sankyo Co., Tokyo, Japan), ciprofloxacin (Bayer Yakuhin, Osaka, Japan), clarithromycin (Taisho Pharmaceutical Co., Tokyo, Japan), clindamycin (Pharmacia K.K., Tokyo, Japan), erythromycin (Dainippon Pharmaceutical Co., Osaka, Japan), fosfomycin (Meiji Seika Kaisha), gentamicin (Schering-Plough K.K., Osaka, Japan), imipenem (Banyu Pharmaceutical Co., Tokyo, Japan), meropenem (Sumitomo Chemical Co., Tokyo, Japan), minocycline [Lederle (Japan), Tokyo, Japan], penicillin G (Meiji Seika Kaisha), rifampin (Daiichi Pharmaceutical Co., Tokyo, Japan), teicoplanin (Aventis Pharma, Tokyo, Japan), tetracycline (Lederle), and vancomycin (Shionogi Co., Osaka, Japan).

PCR and DNA sequencing. PCR and DNA sequencing in the *rpoB* gene were performed for eight *R. equi* isolates from eight AIDS patients with pneumonia or lung abscess caused by *R. equi* between 1998 and 2001 as described previously (5). A set of primers, MF (5′-CGACCACTTCGGCAACCG-3′) and MR (5′-T

TABLE 1. Distribution of MICs of various antibiotics against 30 strains of *R. equi* in northern Thailand

Antibiotic	No. of strains for which MIC (μg/ml) is:													
	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16	32	64	128	>128
Penicillin G							2	3	11	9	5			
Ampicillin									2	4	15	9		
Cefazolin								1	2		3	7	7	5	5
Cefotiam						2	2	4	5	3	5		2		7
Ceftriazone						5	5	18	1			1			
Imipenem				11	16	3									
Meropenem						4	17	9							
Fosfomycin															30
Gentamicin							1	1	15	13					
Amikacin								7	13	10					
Minocycline				4	13	13									
Tetracycline								3	4	9	6	6	2		
Erythromycin				8	9	11				2					
Clarithromycin	3		16	9				2							
Clindamycin						4	13	11							2
Chloramphenicol								10	16	4					
Vancomycin					15	15									
Teicoplanin					1	29									
Rifampin			1	2	6	15	2			1			1		2
Ciprofloxacin						2	19	9							

CGATCGGGCACATCCGG-3′), was chosen to amplify a portion of the *rpoB* region of *R. equi* that includes the rifampin resistance-determining region.

PFGE. Pulsed-field gel electrophoresis (PFGE) was also performed for eight *R. equi* isolates as mentioned above in order to determine genetic relatedness, as described previously (11). The DNA was digested with *Psh*BI (Takara Bio Co., Shiga, Japan) at 37°C overnight. A CHEF Mapper pulsed-field electrophoresis system (Bio-Rad Life Science Group, Hercules, Calif.) was used for electrophoresis, with a potential of 6 V/cm, switch times of 0.47 and 63 s, and a run time of 20 h and 18 min. After the gels were stained with ethidium bromide, the interpretation of PFGE patterns was based on criteria described by Tenover et al. (18).

RESULTS

Antimicrobial susceptibility test. The MICs of 20 antibiotics for 30 *R. equi* isolates showed that the isolates had a tendency to be resistant to various antibiotics but that they were sensitive to imipenem, minocycline, vancomycin, and teicoplanin (MICs, ≤0.5 μg/ml) and relatively sensitive to meropenem and ciprofloxacin (MICs, ≤2 μg/ml). Of the 30 isolates, 28 showed sensitivity, but 2 isolates detected from 1993 to 1996 showed low-level resistance (MICs, 8 and 2 μg/ml, respectively) to erythromycin and clarithromycin. Twenty-six isolates were susceptible (MICs, ≤1 μg/ml), one showed low-level resistance (MIC, 8 μg/ml), and three showed high-level resistance (MICs, ≥64 μg/ml) to rifampin (Table 1). The one low- and three high-level rifampin-resistant isolates were detected in four AIDS patients between 1998 and 2001.

AIDS patients. Eight AIDS patients (seven males and one female; mean age, 32.6 years), who presented with fever, a productive cough, and sputum, were admitted to Nakornping Hospital in northern Thailand due to pneumonia or lung abscess caused by *R. equi* from 1998 to 2001. The mean CD4 lymphocyte count was 9.4/mm^3 (CD4/CD8 ratio, 0.04). Five patients were treated with a combination of erythromycin and rifampin; two patients were treated with antituberculosis drugs, including rifampin; and one patient was treated with a combination of penicillin G and gentamicin. Four patients improved after treatment, three patients died of respiratory failure, and the prognosis of one patient was unknown because of transfer. Two of the three patients who died were infected by high-level rifampin-resistant strains (Table 2).

Amino acid sequence analysis and susceptibility to rifampin. Two high-level rifampin-resistant strains had a Ser531Trp mutation and one high-level rifampin-resistant strain had a His526Tyr mutation in the *rpoB* gene, whereas one low-level rifampin-resistant strain had a Ser509Pro mutation. Four rifampin-susceptible strains showed an absence of mutation in the *rpoB* region (Table 2).

Interpretation of PFGE. The PFGE patterns in two high-level rifampin-resistant strains with the Ser531Trp mutation were closely related to patterns A1 and A2, but one other high-level rifampin-resistant strain with a His526Tyr mutation showed a different pattern, B, while one low-level rifampin-resistant strain with a Ser509Pro mutation showed pattern C. The PFGE patterns among the four rifampin-susceptible strains with no mutation were different from those of rifampin-resistant strains, but two of four strains revealed identical patterns (D), and the two remaining cases were possibly related to patterns E1 and E2 (Table 2 and Fig. 1).

DISCUSSION

R. equi is now recognized as an important pathogen in animals and immunocompromised humans, especially AIDS patients (3, 4, 15). In humans, mortality rates from 25 to 60% in *R. equi* pneumonia have been reported (3, 19, 20). The introduction of a combination of erythromycin and rifampin for the treatment of *R. equi* pneumonia in foals has improved survival rates considerably (7) and is also considered to be effective in *R. equi* infections in humans due to the low MICs, intracellular

TABLE 2. Clinical characteristics of *R. equi* isolates detected in AIDS patients, rifampin MICs, RpoB amino acid substitutions, and PFGE patterns

Strain no.	Patient age (yr); sex	Source	Diagnosis	No. of CD4$^+$ cells/mm^3 (CD4/CD8)	Treatment[a]	Prognosis	RFP MIC (μg/ml)	Amino acid substitution (*E. coli* numbering)	PFGE pattern
1	21; male	Sputum	Pneumonia	10 (0.01)	RFP, INH, EB, PZA	Death	>128	Ser531Trp	A1
2	32; male	Sputum	Pneumonia	0	RFP, EM	Improved	>128	His526Tyr	B
3	42; male	Sputum	Pneumonia	10 (0.01)	RFP, EM	Death	64	Ser531Trp	A2
4	38; male	Blood	Lung abscess	21 (0.03)	RFP, EM	Improved	8	Ser509Pro	C
5	42; female	Sputum	Lung abscess	10 (0.15)	RFP, INH, EB, PZA	Unknown	0.5	None	D
6	32; male	Sputum	Lung abscess	Not determined	RFP, EM	Death	0.5	None	D
7	24; male	Sputum	Pneumonia	10 (0.09)	PCG, GM	Improved	0.5	None	E1
8	30; male	Blood	Lung abscess	5 (0.02)	RFP, EM	Improved	0.5	None	E2

[a] RFP, rifampin; EM, erythromycin; INH, isoniazid; EB, ethambutol; PZA, pyrazinamide; PCG, penicillin G; GM, gentamicin.

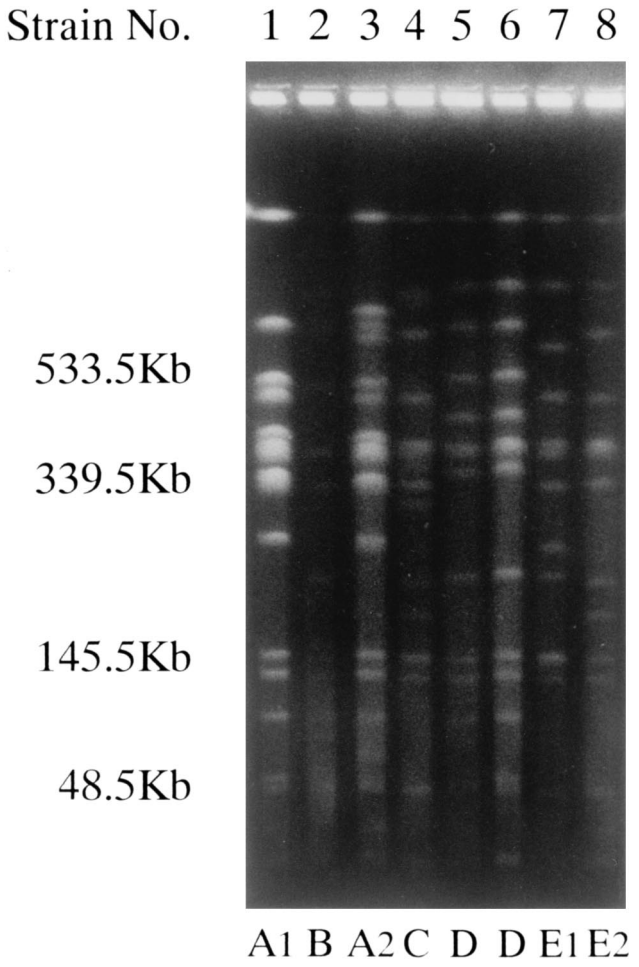

FIG. 1. PFGE patterns of *Psh*BI-digested DNAs from *R. equi* isolates from eight AIDS patients with pneumonia or lung abscess caused by *R. equi* between 1998 and 2001. The PFGE patterns in two high-level rifampin-resistant strains were the closely related patterns A1 and A2 (lanes 1 and 3), but another high-level rifampin-resistant strain showed a different pattern, B (lane 2). One low-level rifampin-resistant strain showed pattern C (lane 4). The PFGE patterns of two rifampin-susceptible strains were identical (pattern D; lanes 5 and 6), and another two rifampin-susceptible strains showed the possibly related patterns E1 and E2 (lanes 7 and 8).

penetration, and synergistic action (9, 13, 19). The emergence of erythromycin (3, 10) and rifampin (10, 17) resistance in *R. equi* has been reported, although it is still rare. In our study, antimicrobial susceptibilities (*R. equi* was found to be resistant to various antibiotics but sensitive to imipenem, minocycline, vancomycin, and teicoplanin) were similar to those in previous studies, except that the susceptibility to aminoglycosides was lower (9, 13). Of 30 *R. equi* isolates, 2 isolates detected from 1993 to 1996 showed low-level resistance to erythromycin (MIC, 8 μg/ml), while among those detected from 1998 to 2001, one showed low-level resistance (MIC, 8 μg/ml) and three showed high-level resistance (MICs, ≥64 μg/ml) to rifampin. In fact, two of three AIDS patients with pneumonia caused by high-level rifampin-resistant *R. equi* had fatal outcomes despite treatment that included rifampin. *R. equi* is largely a soil organism but is widespread in the feces of herbivores. Most human patients who have developed *R. equi* infections are known to have been in contact with herbivores, their manure, or soil (15). It is likely that the eight AIDS patients in our study had such contact, because they lived close to a farm in northern Thailand. On the other hand, AIDS is still prevalent in Thailand, and tuberculosis is the most commonly reported opportunistic infection (2). Therefore, AIDS patients in Thailand have many opportunities for treatment with antituberculosis drugs, including rifampin, and this may explain the selection for rifampin resistance in *R. equi* as well. In our study, two types of high-level rifampin-resistant strains and one low-level rifampin-resistant strain with mutations in the *rpoB* gene were confirmed despite their small numbers, and no rifampin-suscepible strains had such mutations. Moreover, molecular analysis by PFGE was consistent with this type of mutation. In a previous study of *R. equi* isolated from foals, the His526Asp mutation was found to be predominantly associated with high-level rifampin resistance (5). One high-level rifampin-resistant strain in our study had a mutation at position 526, but the amino acid substitution was not the same. However, two high-level rifampin-resistant strains had a Ser531Trp mutation. Although it has been reported that one low-level rifampin-resistant *R. equi* strain had a mutation at position 531 with a different amino acid substitution (5), a mutation at this position was associated with high-level rifampin resistance in *M. tuberculosis* (1, 14). Meanwhile, one low-level rifampin-resistant strain in our study showed a mutation at position 509 which had never been reported in *R. equi*.

In conclusion, our results demonstrated the presence of several types of rifampin-resistant *R. equi* among AIDS patients in northern Thailand. Therefore, treatment should be considered based on antimicrobial susceptibility and intracellular penetration.

ACKNOWLEDGMENTS

We thank all the staff of the Department of Microbiology, Faculty of Medicine, Chiang Mai University, and of Nakornping Hospital for their help in the completion of this study. We also thank Akihiro Wada (Department of Bacteriology, Institute of Tropical Medicine, Nagasaki University), Chieko Shimauchi (Miyazaki Prefectural Nursing University), and Matsuhisa Inoue (Kitasato University School of Medicine) for their help in the completion of the PFGE studies.

This study was supported by a Monbukagakusho Grant-in-Aid for Scientific Research (09045083) from the Japanese government.

REFERENCES

1. **Billington, O. J., T. D. McHugh, and S. H. Gillespie.** 1999. Physiological cost of rifampin resistance induced in vitro in *Mycobacterium tuberculosis*. Antimicrob. Agents Chemother. **43:**1866–1869.
2. **Chariyalertsak, S., T. Sirisanthana, O. Saengwonloey, and K. E. Nelson.** 2001. Clinical presentation and risk behaviors of patients with acquired immunodeficiency syndrome in Thailand, 1994–1998: regional variation and temporal trends. Clin. Infect. Dis. **32:**955–962.
3. **Donisi, A., M. G. Suardi, S. Casari, M. Longo, G. P. Cadeo, and G. Carosi.** 1996. *Rhodococcus equi* infection in HIV-infected patients. AIDS **10:**359–362.
4. **Drancourt, M., E. Bonnet, H. Gallais, Y. Peloux, and D. Raoult.** 1992. *Rhodococcus equi* infection in patients with AIDS. J. Infect. **24:**123–131.
5. **Fines, M., S. Pronost, K. Maillard, S. Taouji, and R. Leclercq.** 2001. Characterization of mutations in the *rpoB* gene associated with rifampin resistance in *Rhodococcus equi* isolated from foals. J. Clin. Microbiol. **39:**2784–2787.
6. **Golub, B., G. Falk, and W. W. Spink.** 1967. Lung abscess due to *Corynebacterium equi*. Report of first human infection. Ann. Intern. Med **66:**1174–1177.
7. **Hillidge, C. J.** 1987. Use of erythromycin-rifampin combination in treatment of *Rhodococcus equi* pneumonia. Vet. Microbiol. **14:**337–342.
8. **Magnusson, H.** 1938. Pyaemia in foals caused by *Corynebacterium equi*. Vet. Res. **50:**1459–1468.

9. **Mascellino, M. T., E. Iona, R. Ponzo, C. M. Mastroianni, and S. Delia.** 1994. Infections due to *Rhodococcus equi* in three HIV-infected patients: microbiological findings and antibiotic susceptibility. Int. J. Clin. Pharmacol. Res. **14:**157–163.
10. **McNeil, M. M., and J. M. Brown.** 1992. Distribution and antimicrobial susceptibility of *Rhodococcus equi* from clinical specimens. Eur. J. Epidemiol. **8:**437–443.
11. **Morton, A. C., A. P. Begg, G. A. Anderson, S. Takai, C. Lammler, and G. F. Browning.** 2001. Epidemiology of *Rhodococcus equi* strains on thoroughbred horse farms. Appl. Environ. Microbiol. **67:**2167–2175.
12. **National Committee for Clinical Laboratory Standards.** 1998. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A4. National Committee for Clinical Laboratory Standards, Wayne, Pa.
13. **Nordmann, P., and E. Ronco.** 1992. In-vitro antimicrobial susceptibility of *Rhodococcus equi*. J. Antimicrob. Chemother. **29:**383–393.
14. **Ohno, H., H. Koga, S. Kohno, T. Tashiro, and K. Hara.** 1996. Relationship between rifampin MICs for and *rpoB* mutations of *Mycobacterium tuberculosis* strains isolated in Japan. Antimicrob. Agents Chemother. **40:**1053–1056.
15. **Prescott, J. F.** 1991. *Rhodococcus equi*: an animal and human pathogen. Clin. Microbiol. Rev. **4:**20–34.
16. **Sirisanthana, T., and K. Supparatpinyo.** 1996. Pulmonary infection caused by *Rhodococcus equi* in patients with human immunodeficiency virus infection. J. Infect. Dis. Antimicrob. Agents **13:**95–99.
17. **Takai, S., K. Takeda, Y. Nakano, T. Karasawa, J. Furugoori, Y. Sasaki, S. Tsubaki, T. Higuchi, T. Anzai, R. Wada, and M. Kamada.** 1997. Emergence of rifampin-resistant *Rhodococcus equi* in an infected foal. J. Clin. Microbiol. **35:**1904–1908.
18. **Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33:**2233–2239.
19. **Vestbo, J., J. D. Lundgren, J. Gaub, B. Roder, and E. Gutschik.** 1991. Severe *Rhodococcus equi* pneumonia: case report and literature review. Eur. J. Clin. Microbiol. Infect. Dis. **10:**762–768.
20. **Votava, M., B. Skalka, H. Hrstkova, R. Tejkalova, and L. Dvorska.** 1997. Review of 105 cases of isolation of *Rhodococcus equi* in humans. Cas. Lek. Cesk. **136:**51–53.
21. **Weingarten, J. S., D. Y. Huang, and J. D. Jackman, Jr.** 1988. *Rhodococcus equi* pneumonia. An unusual early manifestation of the acquired immunodeficiency syndrome (AIDS). Chest **94:**195–196.