ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1984, p. 385–386
0066-4804/84/030385-02$02.00/0

Vol. 25, No. 3

Antibiotic Resistance of *Erysipelothrix rhusiopathiae* Isolated from Pigs with Chronic Swine Erysipelas

TOSHIO TAKAHASHI,[1]* TAKUO SAWADA,[1] KENICHI OHMAE,[1] NOBUYUKI TERAKADO,[2] MASATAKE MURAMATSU,[1] KENJI SETO,[3] TSUTOMU MARUYAMA,[4] AND MASAKO KANZAKI[4]

National Veterinary Assay Laboratory, Kokubunji, Tokyo 185,[1] National Institute of Animal Health, Tsukuba, Ibaraki 305,[2] Nippon Institute for Biological Science, Ohme, Tokyo 198,[3] and Tokyo Metropolitan Research Laboratory of Public Health, Tokyo 160,[4] Japan

Received 27 July 1983/Accepted 28 November 1983

The susceptibility of 258 isolates of *Erysipelothrix rhusiopathiae* from slaughtered pigs affected with chronic erysipelas in Japan to antimicrobial agents was determined. A total of 111 (43.0%) strains showed resistance to erythromycin, oleandomycin, oxytetracycline, or dihydrostreptomycin. Plasmids were not detected. This is the first report of resistance of *E. rhusiopathiae* to these antibiotics.

Erysipelothrix rhusiopathiae (*insidiosa*) is a causative agent of swine erysipelas, which causes great economic loss in pig production. Human *E. rhusiopathiae* infection has also been reported (2, 4, 12, 14). Antibiotics, especially penicillins, have been widely used for the treatment of this disease. However, in Japan, pigs are usually fed food containing various antibiotics, mainly tetracyclines and macrolides, for the purpose of growth stimulation. It seems, therefore, that long-term administration of antibiotics will give a selective advantage to antibiotic-resistant strains of *E. rhusiopathiae*. This paper deals with the antibiotic resistance in *E. rhusiopathiae* strains isolated from pigs in Japan.

A total of 258 strains of *E. rhusiopathiae* were submitted for assay of antibiotic susceptibility. They were isolated from cases of chronic swine erysipelas in slaughterhouses from October 1980 to December 1982. Infections associated with the isolates included arthritis (148 cases), lymphadenitis (65 cases), endocarditis (30 cases), and urticaria (15 cases). Antimicrobial agents studied were penicillin G (PC-G), ampicillin (APC), erythromycin (EM), oleandomycin (OM), oxytetracycline (OTC), chloramphenicol (CP), dihydrostreptomycin (DSM), kanamycin (KM), and sulfadimethoxine (SDM). MICs were determined by an agar dilution method (5). A 10^{-2} dilution of an overnight tryptic soy broth culture was inoculated by microplanter (Ebara Works, Tokyo, Japan) onto Mueller-Hinton agar containing serial twofold dilutions of the test antibiotic. The plates were incubated at 37°C for 48 h. The MIC was defined as the lowest concentration of antimicrobial agent that prevented macroscopic growth. Isolates were serotyped by using a modification of the agar gel diffusion method of Kucsera (11).

MICs are shown in Table 1. All of the strains were highly susceptible to PC-G and APC (MIC, 0.025 to 0.1 U or μg/ml) and moderately susceptible to CP (MIC, 1.56 to 25 μg/ml). KM and SDM showed no activity against the strains (MICs, >100 and >400 μg/ml, respectively). MICs of EM, OM, OTC, and DSM presented two distribution peaks. The MIC breakpoints of strains resistant to EM, OM, OTC, and DSM were assumed to be 0.78, 3.13, 3.13, and 100 μg/ml, respectively. The frequencies of isolation of *E. rhusiopathiae* strains resistant to each drug were as follows: OTC (42.6%), DSM (17.4%), EM (5.8%), and OM (5.0%).

The relationship between antimicrobial resistance patterns, sources, and serotypes of isolates is shown in Table 2. A total of 111 (43.0%) strains were resistant to EM, OM, OTC, or DSM. Six different resistance patterns were found. Strains resistant only to OTC (25.6%) were most frequent, followed by those resistant to OTC and DSM (10.8%), EM, OM, OTC, and DSM (4.6%), EM, OTC, and DSM (1.2%), OM, OTC, and DSM (0.4%), and DSM (0.4%). In the *E. rhusiopathiae* strains isolated from cases of endocarditis, the frequency of resistance was significantly ($P < 0.05$, by Fisher's exact test) lower than in the isolates from other infections. Triple resistance and quadruple resistance were found only in isolates from cases of arthritis and lymphadenitis. Of the 111 resistant strains, 104 (93.7%) belonged to serotype 2. Isolation of plasmid DNA was attempted by the method of Womble et al. (15) and Kado and Liu (6), except for the use of sodium laurolyl sarcosinate (6%) in the procedure of bacteriolysis. However, we could not detect any plasmid in strains showing the various resistance patterns (data not shown).

The present results on susceptibility of *E. rhusiopathiae* strains to PC-G, APC, CP, KM, and SDM are in general agreement with those reported previously by others (1, 3, 7–10, 13), indicating that penicillins remain the antibiotics of choice for the treatment of swine erysipelas. It should be noted, however, that 43% of *E. rhusiopathiae* strains examined showed resistance to EM, OM, OTC, or DSM. This is the first report on resistant strains of *E. rhusiopathiae* of porcine origin. Although the detailed mechanisms of antibiotic resistance were not established, plasmid DNA in resistant strains was not found. In any case, frequent use of tetracyclines and macrolides for pig production will undoubtedly give a selective advantage to antibiotic-resistant strains of *E. rhusiopathiae*.

Our results also showed that most of the resistant *E. rhusiopathiae* strains belonged to serotype 2. Further epidemiological studies are necessary to clarify this correlation.

* Corresponding author.

TABLE 1. Susceptibility of 258 isolates of *E. rhusiopathiae* to antimicrobial agents

Drug	No. of isolates with MIC (μg/ml) of:															MIC (μg/ml) breakpoint of resistance	No. of resistant strains (%)
	0.025	0.05	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100	>400		
PC-G[a]	23	213	22														
APC	23	162	73														
EM	2	54	177	10					3	6	4	2				0.78	15 (5.8)
OM				6	10	174	55		5	8						3.13	13 (5.0)
OTC				2	3	87	56		5	15	15	72	3			3.13	110 (42.6)
CP							11	16	205	23	3						
DSM									173	24	7	4	5	45		100	45 (17.4)
KM														258			
SDM															258		

[a] Units per milliliter.

TABLE 2. Relationship between resistance patterns, sources, and serotypes of 258 *E. rhusiopathiae* isolates

Resistance pattern[a]	No. of isolates								
	Source[b]				Serotype				Total (%)
	A	L	E	U	1a	1b	2	Other[c]	
EM, OM, OTC, DSM	5	7	0	0	0	0	12	0	12 (4.6)
EM, OTC, DSM	2	1	0	0	0	0	3	0	3 (1.2)
OM, OTC, DSM	1	0	0	0	0	0	1	0	1 (0.4)
OTC, DSM	20	3	1	4	0	0	28	0	28 (10.8)
OTC	44	11	4	7	0	3	59	4	66 (25.6)
DSM	0	1	0	0	0	0	1	0	1 (0.4)
Total									
Resistant	72	23	5	11	0	3	104	4	111 (43.0)
Susceptible	76	42	25	4	29	15	87	16	147 (57.0)

[a] EM, Resistant to erythromycin; OM, resistant to oleandomycin; OTC, resistant to oxytetracycline; DSM, resistant to dihydrostreptomycin.

[b] A, Arthritis; L, lymphadenitis; E, endocarditis; U, urticaria.

[c] Includes serotypes 3, 5, 6, 8, 11, 21, and N.

LITERATURE CITED

1. **Azechi, H., H. Nakamura, S. Yonezawa, I. Takahashi, and K. Suzuki.** 1971. Sensitivity of freshly isolated strains of *Erysipelothrix insidiosa* to antibiotics. J. Jpn. Vet. Med. Assoc. **24**:92–97.
2. **Cabot, R. C.** 1978. Case records of the Massachusetts General Hospital. N. Engl. J. Med. **298**:957–962.
3. **Cooper, M. S., and G. A. Allen.** 1959. The effect of chlortetracycline on immune response. II. Influence on development of protective antigen after vaccination with a live avirulent strain of *Erysipelothrix rhusiopathiae*. J. Immunol. **83**:232–236.
4. **Ewald, F. W.** 1965. Erysipeloid und septishe Rotlauf-komplikation. Muench. Med. Wochenschr. **107**:365–369.
5. **Ishiyama, S., Y. Ueda, S. Kuwabara, N. Kosakai, G. Koya, M. Konno, and R. Fujii.** 1968. On the standardization of method for determination of minimum inhibitory concentrations. Chemotherapy (Tokyo) **16**:98–99.
6. **Kado, C. I., and S.-T. Liu.** 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. **145**:1365–1373.
7. **Kalich, J., and D. Neubrand.** 1957. Tierexperimentelle Studien über die Rotlaufpathogese unter Berücksichtigung der Beifutterung von Antibiotica. Mh. Tierheilk. **9**:298–312.
8. **Kalich, J., and K. Neubrand.** 1959. Zur Frageder Epidemiologie und Pathogenese des Schweinerotlaufs. Mh. Tierheilk. **11**:85–89.
9. **Kielstein, P.** 1961. Zur bakteriellen Chemoresistenz des Rotlauferregers. Arch. Exp. Vet. Med. **15**:1161–1171.
10. **Kinjo, T., and S. Machida.** 1973. Sensitivity of *Erysipelothrix insidiosa* to antibiotics and trials of artificial induction of penicillin resistant strains in vitro. Sci. Bull. Coll. Agric. Univ. Ryukyus Okinawa **20**:335–341.
11. **Kucsera, G.** 1973. Detection of new serotypes among *Erysipelothrix rhusiopathiae* strains of different origin. Acta Vet. Acad. Sci. Hung. **21**:211–219.
12. **McCracken, A. W., C. U. Mauney, T. W. Huber, and R. V. McCloskey.** 1973. Endocarditis caused by *Erysipelothrix insidiosa*. Am. J. Clin. Pathol. **59**:219–222.
13. **Polityńskabans, E.** 1965. Susceptibility of various phage and serotypes of *Erysipelothrix insidiosa* to antibiotics. Bull. Vet. Inst. Pulawy **9**:78–83.
14. **Simerkoff, M. S., and J. J. Rahal.** 1973. Acute and subacute endocarditis due to *Erysipelothrix rhusiopathiae*. Am. J. Med. Sci. **266**:53–57.
15. **Womble, D. D., D. P. Taylor, and R. H. Rownd.** 1977. Method for obtaining more-accurate covalently closed circular plasmid-to-chromosome ratios from bacterial lysate by dye-buoyant density centrifugation. J. Bacteriol. **130**:148–153.