Molecular Epidemiology of *Rhodococcus equi* of Intermediate Virulence Isolated from Patients With and Without Acquired Immune Deficiency Syndrome in Chiang Mai, Thailand

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We investigated the prevalence of virulent *Rhodococcus equi* in clinical isolates from 69 sporadic cases (60 men, 8 women, and 1 patient of unknown sex) in Chiang Mai, Thailand, between 1993 and 2001. Fifty were human immunodeficiency virus (HIV) positive, 3 were HIV negative, and HIV status was unknown for 16. Fifty-two (75%) of 69 isolates were strains of intermediate virulence that contained the virulence-associated 20-kDa antigen, and 17 isolates (25%) were avirulent. No virulent strains with the virulence-associated 15–17-kDa antigens were identified. *R. equi* was isolated from HIV-positive patients' houses and those of their neighbors: avirulent strains were widespread, but only 1 strain of intermediate virulence was isolated. *R. equi* strains of intermediate virulence were isolated from 4 (0.8%) of 500 submaxillary lymph nodes from apparently healthy pigs in Chiang Mai. The routes of *R. equi* acquisition should be investigated from the viewpoint of zoonosis and public health.

Rhodococcus equi is a facultative, intracellular, gram-positive coccobacillus that causes suppurative pneumonia and ulcerative enteritis in foals aged 1–3 months [1–3]. This organism is present in soil and sporadically causes pulmonary abscesses and lymphadenitis in other domesticated animals, such as pigs, cattle, and goats [1, 2, 4]. *R. equi* was an uncommon cause of infection in humans before the onset of AIDS [1, 2]. More than 100 cases of *R. equi* infection have been reported since its initial isolation as a human pathogen [5–10]. Most patients infected with *R. equi* are immunocompromised, and two-thirds have human immunodeficiency virus (HIV) infection [10]. The clinical manifestations of *R. equi* infection are diverse, although 80% of patients present with pulmonary involvement [2, 10].

The discovery of virulence-associated antigens and virulence plasmids has allowed the classification of *R. equi* strains and molecular epidemiological studies of virulent *R. equi* strains [3, 11–13]. At least 3 virulence levels of *R. equi* have been identified: virulent, intermediate virulence, and avirulent [3, 12, 14–16]. Virulent *R. equi* is characterized by the presence of virulence-associated 15–17-kDa antigens (VapA), a virulence plasmid of 85–90 kb, and it causes suppurative pneumonia in foals (murine LD_{50}, 10^6) [14, 16, 17]. VapA-positive *R. equi* is widespread on horse-breeding farms but not on farms of other domesticated animals [18]. *R. equi* strains of intermediate virulence are identified by a vir-

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ulence-associated 20-kDa antigen (VapB) and a virulence plasmid of 79–100 kb and are found in the submaxillary lymph nodes of pigs (murine LD_{50}, 10^7). They have not been found in horses or other domesticated animals [15, 19–22]. Avirulent *R. equi* shows no evidence of virulence-associated antigens or plasmid DNA (murine LD_{50}, $>10^8$) and is widespread in soil [3, 22].

The pathogenicity of human isolates has been studied previously [15, 23–25]. Caterino-de-Araujo et al. [23] reported that 1 in 4 Brazilian isolates contained VapA and that the others contained VapB. Two of 9 Italian isolates contained VapA, 1 had VapB, and the other 6 were avirulent. Our previous study also demonstrated that most *R. equi* isolates from humans, with or without AIDS, are virulent (8 VapA-positive and 15 VapB-positive of 39 isolates) [15, 24]. The route of infection in humans remains unknown, although contact with farm animals and manure has been reported in approximately one-third of cases [2, 5–7, 10].

Thailand has one of the highest rates of HIV/AIDS in the world [26]. It is estimated that 1 million Thai citizens have been infected with HIV. Pulmonary infection is a major cause of morbidity and mortality in HIV-infected patients. In Thailand, *Mycobacterium tuberculosis, Pneumocystis carinii*, and *Penicillium marneffei* are major etiological agents of such pulmonary infection [26]. Infection by *R. equi* has also been reported [15, 33], and, in a preliminary surveillance study, we found that 4 of 6 human isolates from Chiang Mai were VapB positive [25]. In the present study, *R. equi* was isolated from 69 sporadic cases in patients with and without HIV infection in Chiang Mai between 1993 and 2001. The pathogenicity of those isolates was investigated.

MATERIALS AND METHODS

Bacterial strains. Strain ATCC 33701 (a virulent strain of equine origin) and 12 representative strains of intermediate virulence (of human and pig origin) were used as reference strains, because some of the protein profiles, plasmid characteristics, and virulence levels of these strains have been described elsewhere [15, 17, 19, 24]. We reviewed microbiology records at Chiang Mai University Hospital from June 1993 through May 2001. *R. equi* was isolated from 69 patients. The information sought included presenting signs and symptoms, subsequent course of infection, findings from chest X-ray, laboratory data (including the presence of anti-HIV antibody), treatment regimen, and outcome. Unfortunately, details of the clinical manifestations were not available in most cases, because the medical record system in the hospitals in Chiang Mai does not allow for this kind of retrospective study. The bacteria were harvested from various lesions and identified by the Department of Microbiology, Faculty of Medicine, Chiang Mai University. Plasmid profiles of 4 isolates (32, 62, 63, and 64) from the 69 patients in the preliminary study have been reported elsewhere [25].

Isolation of* R. equi *from the patients' environment. Seventy-nine samples were collected from 5–17 sites from around the houses of 4 patients and their neighbors. These patients were from a rural area in Chiang Mai (patients 3 and 4), and the neighbors of patients 1 and 2 bred pigs and chickens in their back yards. As controls, soil and fecal samples from domestic animals were collected randomly from farms in 6 distinct areas (Hang Dong, Mae Rim, Muang, San Kamphaeng, San Patong, and San Sai) in Chiang Mai. Soil was scraped from the ground surface around houses (including pig pens) with a small spoon and poured into sterile tubes.

One gram of soil was diluted serially with a 10-fold volume of sterile saline. Each dilution was inoculated onto 2 plates of nalidixic acid–novobiocin–actidione (cycloheximide)–potassium tellurite (NANAT) medium, as described elsewhere [27]; the plates were incubated at 30°C for 2 or 3 days. *R. equi* colonies were counted, and the number of viable organisms per gram of soil was calculated. Three to 10 colonies of *R. equi* per specimen were subcultured and examined for VapA and VapB by colony-blot ELISA with monoclonal antibodies [15, 28]. For colony-blot analysis, bacterial strains were injected onto brain-heart–infusion agar plates with an inoculation needle and incubated at 38°C for 24 h. A nitrocellulose filter (pore size 0.45 μm, BAS 85; Schleicher & Schuell) was then placed over the cultures for a few minutes until it was completely wet. The membrane was removed, air dried, and treated by autoclaving at 105°C for 1 min. All buffers, antibodies, and conjugate dilutions for the immunoblot were as described elsewhere [15, 29].

Isolation of* R. equi *from the submaxillary lymph nodes of pigs. Submaxillary lymph nodes were removed from freshly slaughtered pigs at the slaughterhouses in Chiang Mai and placed in sterile dishes for transport to the laboratory. The lymph nodes were immersed in boiling water for 3 s prior to being minced finely with sterile scissors, and the pieces were placed onto a modified NANAT agar plate [21] and incubated for 2–3 days at 30°C. All suspected colonies were counted, and 1–10 colonies or *R. equi* per specimen were subcultured and examined for VapA and VapB by colony blot analysis.

Isolation of plasmid DNA. Plasmid DNA was isolated from *R. equi* by the alkaline lysis method [30], with modifications as described elsewhere [17]. Plasmid DNAs were analyzed by digestion with restriction endonucleases *Eco*RI and *Eco*T22I [15, 21]. Samples of the plasmid preparations were separated in 0.7% or 1.0% agarose gels at ~5 V/cm for 2 h.

Virulence-associated gene. The target DNAs for polymerase chain reaction (PCR) amplification were the published sequences of the VapA and VapB genes (GenBank database accession nos. D212361 and D44469) from *R. equi* strains ATCC 33701

and 5, respectively. Primer 1 (5′-GACTCTTCACAAGACGGT-3′) corresponded to the sense strand at position 6–23, and primer 2 (5′-TAGGCGTTGTGCCAGCTA-3′) corresponded to the antisense strand at position 569–552 in the sequence of the VapB gene [14, 31]. Primer 3 (5′-AACGTAGTCGCGGTGAGAA-3′) corresponded to the sense strand at position 240–258, and primer 4 (5′-ACCGAGACTTGAGCGACTA-3′) corresponded to the antisense strand at position 1066–1048 in the sequence of a cloned fragment containing the VapA gene [32].

PCR amplification was done with 10 μL of the DNA preparation in a 50-μL reaction that contained 10 mmol/L Tris-HCl (pH 8.3 at 25°C), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L each dNTP, 1 μmol/L each primer, and 2.5 U of *Taq* DNA polymerase (Takara), as described elsewhere [14]. The samples were subjected to 30 cycles of amplification on a GeneAmp PCR system 2400 (Perkin-Elmer). The cycling conditions were as follows: denaturation for 90 s at 94°C, primer annealing for 1 min at 55°C, and an extension step for 2 min at 72°C [14].

Southern hybridization. The VapB gene on fragments of plasmid DNAs was detected by Southern hybridization [15]. Digested plasmid DNAs were transferred from gels to a sheet of nylon membrane (Hybond N; Amersham Japan) by the vacuum-transfer method, using a VacuGene (Pharmacia-LKB Biotechnology) and without depurination, according to the manufacturer's instructions. Hybridization was done at 68°C for at least 6 h in 5× standard saline citrate (SSC; 1× SSC is 0.15 mol/L NaCl plus 0.015 mol/L sodium citrate [pH 7.0]) that contained 0.1% sodium *N*-lauryl sarcosine, 0.02% SDS, and 1% blocking reagent. After hybridization, the sheets were washed twice at room temperature for 5 min in 2× SSC that contained 0.1% SDS and then washed twice more for 15 min at 68°C in 0.1× SSC that contained 0.1% SDS. The immunological detection of hybridized digoxigenin-labeled probe was done with a commercial kit (Boehringer Mannheim-Yamanouchi). For Southern-blot analysis, a digoxigenin-11–dUTP–labeled probe was amplified using the primers and PCR conditions described above [14].

RESULTS

Isolation of* R. equi *from patients. Patients infected with *R. equi* were identified over the period 1993–2001 with a frequency of 5–14 cases/year in Chiang Mai, Thailand. The 69 patients were 60 men and 8 women, and the sex of 1 patient was unknown. Most patients lived in a rural area in Chiang Mai. The age of the patients was 19–69 years, with two-thirds being aged 20–40 years. Of the 69 patients, 50 were also infected with HIV, as defined by the presence of anti-HIV antibody in the serum samples on ⩾2 occasions. Three patients were not infected with HIV, and the HIV infection status of the other 16 patients was unknown. The majority of the 50 HIV-infected patients were in the later stages of infection, as evidenced by the presence of opportunistic infection, including pneumocystic pneumonia, *P. marneffei* infection, salmonella septicemia, or oral candidiasis prior to or at the time of presentation. The primary site of infection was the lung, and *R. equi* was isolated from sputum (47 patients), blood (15 patients), pus from the chest (3 patients), bronchoalveolar lavage (4 patients), and pleural fluids (1 patient). The 69 isolates were then investigated for the presence of virulence-associated protein and plasmids.

Protein and plasmid profiles of the 69 clinical isolates. Of 69 clinical isolates from the patients, 52 (75%) were positive for VapB and virulence plasmid DNA (table 1). The remaining 17 isolates were negative for both virulence-associated antigens and plasmid. To confirm the above results, PCR was used to detect VapA and VapB genes in the isolates. Fifty-two strains expressing VapB and a positive control (strain 5) gave positive results, showing a product of 827 bp in the PCR amplification. The 17 isolates were negative for both *vapA* and *vapB*.

Plasmid DNA preparations of the 52 isolates were analyzed further by restriction-enzyme digestion with endonucleases *Eco*RI and *Eco*T22I. The restriction cleavage patterns of the plasmid DNA divided the 52 isolates into 8 different patterns. Four of these 8 patterns have been previously reported in isolates from pigs and from patients with AIDS (of the 52 isolates, 34 were plasmid type 1, 4 were plasmid type 4, 7 were plasmid type 7, and 1 was plasmid type 12). The remaining 4 had unique restriction cleavage patterns and did not match any of the 12 previously reported *Eco*RI and *Eco*T22I digestion patterns for *R. equi*. Lanes 1–12 in figure 1*A* and 1*B* show the 12 previously reported *Eco*RI and *Eco*T22I digestion patterns, and lanes 13–16 show the newly identified plasmid types found in the present study. Plasmid DNAs of the 12 representative and 4 new types digested with *Eco*T22I were examined by Southern-blot analysis with PCR probes. As shown in figure 1*C*, the PCR products labeled with digoxigenin-11–dUTP hybridized with 1 of the fragments of each plasmid DNA. From these results, we tentatively designated these new plasmid types as 13 (1 isolate), 14 (2 isolates), 15 (2 isolates), and 16 (1 isolate).

Isolation of virulent* R. equi *from soil samples and feces. The quantitative culture of *R. equi* was achieved for 78 soil samples from 4 patients' houses and their neighbors. Sixty-four (82%) of 78 soil samples were culture-positive for *R. equi*. The number of *R. equi* organisms from these soil samples ranged from 5.0×10^2 to 4.4×10^5 cfu/g of soil. Three to 10 colonies per positive sample were subcultured, yielding 577 colonies for analysis. Colony blotting revealed VapB in 1 (0.2%) of 577 isolates, which was taken from the house of patient 4. The other isolates did not express any virulence-associated antigens. The VapB-positive isolate contained a virulence plasmid that was identified as type 12; however, the plasmid type of VapB-

Table 1. Distribution of virulence-associated 20-kDa antigen (VapB)–positive *Rhodococcus equi* in isolates from human patients.

Characteristic	No. of isolates	No. of VapB-positive *R. equi*	No. of avirulent *R. equi*	VapB-positive *R. equi*, %
Total	69	52[a]	17	75
Sex				
Male	60	45	15	75
Female	8	6	2	75
Unknown	1	1	0	100
Age, years				
10–19	1	0	1	0
20–29	29	22	7	76
30–39	17	13	4	76
40–49	8	8	0	100
50–59	2	2	0	100
60–69	2	0	2	0
Unknown	10	7	3	70
HIV infection				
Yes	50	37	13	74
No	3	2	1	67
Unknown	16	13	3	81
Clinical presentation at admission				
Pneumonia	10	6	4	60
Lung abscess	6	4	2	67
Others	6	2	4	33
Unknown	47	40	7	85
Type of clinical specimen collected, total				
Sputum	47	35	12	74
Blood	15	9	6	60
BAL	4	3	1	75
Pus from chest	3	3	0	100
Pleural fluid	1	1	0	100
Unknown	5	5	0	100

NOTE. BAL, bronchoalveolar lavage; HIV, human immunodeficiency virus.

[a] Restriction cleavage patterns of plasmid DNA divided the 52 VapB-positive isolates into 8 different patterns: type 1, 34 isolates; type 4, 4 isolates; type 7, 7 isolates; type 12, 1 isolate; type 13, 1 isolate; type 14, 2 isolates; type 15, 2 isolates; and type 16, 1 isolate.

positive isolate from patient 4 did not match the plasmid type of VapB-positive isolate from the environment of his house. Of the 629 isolates from the control samples in 6 distinct areas (Hang Dong, Mae Rim, Muang, San Kamphaeng, San Patong, and San Sai) in Chiang Mai, none were positive for the VapA and VapB according to the results of colony blotting.

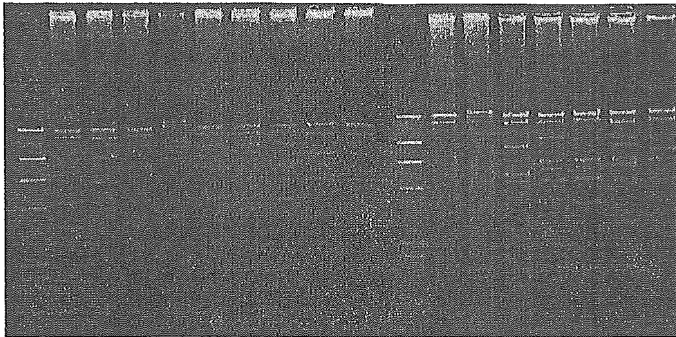
Isolation of virulent* R. equi *from the submaxillary lymph nodes of pigs. *R. equi* was isolated from 4 (0.8%) of 500 submaxillary lymph nodes from apparently healthy pigs in Chiang Mai. One to 10 colonies per positive specimens were subcultured, and all of the isolates ($n = 20$) were identified by biochemical characteristics. Fifteen of 20 isolates showed VapB, and the remaining 5 isolates were avirulent *R. equi* without any virulence-associated antigens. The 15 VapB-positive isolates contained virulence plasmids that were identified as types 1 (8 isolates), 7 (1 isolate), 13 (5 isolates), and 15 (1 isolate), respectively.

DISCUSSION

More than 100 cases of *R. equi* infection have been reported since the first description of human disease caused by this organism [5–7, 9, 10, 33]. We examined 69 human cases in Chiang Mai over the course of 9 years (1993–2001) and found that most patients were infected with VapB-positive *R. equi.* Furthermore, the results of a bacteriological examination of soil and animal feces samples from the houses of patients and their neighbors showed that only 1 of 125 isolates was VapB positive. The prevalence of intermediately virulent *R. equi* in

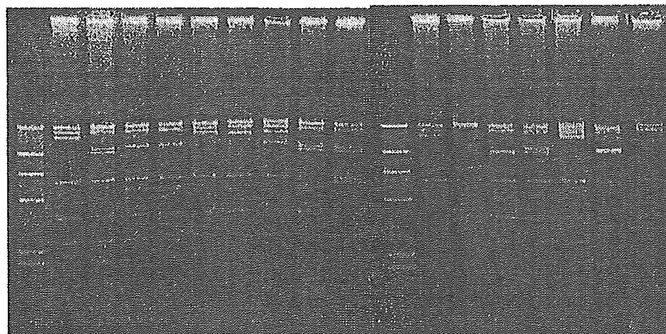
A

M 1 2 3 4 5 6 7 8 9 M 10 11 12 13 14 15 16



B

M 1 2 3 4 5 6 7 8 9 M 10 11 12 13 14 15 16



C

M 1 2 3 4 5 6 7 8 9 M 10 11 12 13 14 15 16

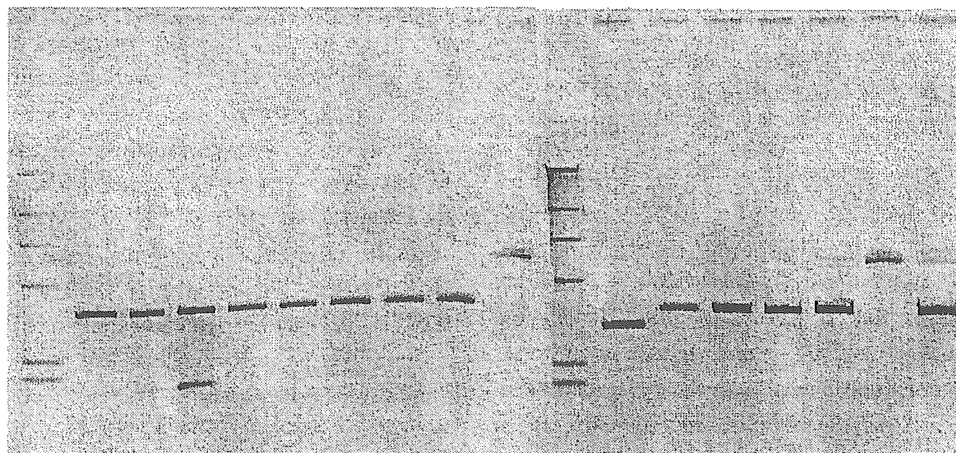


Figure 1. *Eco*RI *(A)* and *Eco*T22I *(B)* restriction fragments of the 16 plasmid types of *Rhodococcus equi* isolates of intermediate virulence and Southern hybridization (*Eco*T22I restriction fragments) of digoxigenin-labeled *vapB* probe *(C)*. *Lane 1,* strain A2 (plasmid type 1); *lane 2,* strain S2 (plasmid type 2); *lane 3,* strain S3 (plasmid type 3); *lane 4,* strain S4 (plasmid type 4); *lane 5,* strain A5 (plasmid type 5); *lane 6,* strain S6 (plasmid type 6); *lane 7,* strain S7 (plasmid type 7); *lane 8,* strain S8 (plasmid type 8); *lane 9,* strain S9 (plasmid type 8); *lane 10,* strain A11 (plasmid type 10); *lane 11,* strain A43 (plasmid type 11); *lane 12,* strain 70 (plasmid type 12); *lane 13,* strain H3 (new plasmid type 13); *lane 14,* strain H25 (new plasmid type 14); *lane 15,* strain H43 (new plasmid type 15); and *lane 16,* strain H66 (new plasmid type 16). The markers *(lane M)* are *Hin*dIII digestion products of bacteriophage λ DNA.

soil was extremely low, as we had expected. In the case of *R. equi* infection in foals, susceptible foals are exposed to virulent *R. equi* that contains VapA on horse-breeding farms. VapA-positive *R. equi* is found in 20%–30% of isolates from the soil of farms with infected foals [3, 11, 12]. For humans, contact with horse-breeding farms or manure, and pigs and their environment, have been suggested as routes of human transmission by virulent and intermediately virulent strains [10, 15, 24]. In the rural areas of Chiang Mai, most houses are on farms with pig pens in the back yard. Therefore, we focused on pigs. An *R. equi* strain of intermediate virulence was isolated from the submaxillary lymph nodes of apparently healthy pigs, and the same virulence plasmid types were found in both human and pig isolates in Chiang Mai. This circumstantial evidence suggests that exposure to pigs may play an important role in most cases of *R. equi* infection in Chiang Mai.

Of interest, VapB-positive but not VapA-positive *R. equi* were found in human isolates in Chiang Mai. In previous studies, we demonstrated that most (21/29 [72%]) isolates from patients with AIDS in 5 countries (Australia, France, Italy, Denmark, and the United States) were of virulent and intermediately virulent strains: 7 isolates (from France, Spain, Italy, New Zealand, and the United States) expressed VapA, and 14 (from France, Australia, Italy, Switzerland, and the United States) expressed VapB [15, 24]. Caterino-de-Araujo et al. [23] also found both VapA- and VapB-positive *R. equi* in Italian and Brazilian isolates. Those authors tried to discover the routes of infection, but only 1 Italian patient reported contact with horses, and his *R. equi* isolate was avirulent [23]. In addition, although isolates from 3 of 4 Brazilian patients infected with *R. equi* showed VapB, the researchers were unable to detect *R. equi* infection in pigs slaughtered in Brazil. They could not confirm these routes of *R. equi* transmission for their patients and speculated that cattle and their environment might be the source of *R. equi* transmission in Brazil, because eating and social habits there include the consumption of beef and contact with large cattle-breeding farms [23]. However, a study on the distribution of *R. equi* lesions in cattle lymph nodes and the pathogenicity of isolates in Ireland found a prevalence of *R. equi* in the lymph nodes of 0.008% of cattle, and all isolates were VapA and VapB negative [34]. In Chiang Mai, people eat more pork and chicken than beef. This was evidenced by several reports of outbreak of trichinellosis in northern Thailand, the sources of which were traced to the consumption of undercooked pork, usually mixed with spices and chilies [35]. We do not know whether these eating habits and epidemiological data contribute to the differences in the routes of transmission and the diversity of *R. equi* strains isolated from Brazilian, Italian, and Thai patients, but it is interesting to note that, in Chiang Mai, patients were infected mostly with VapB-positive strains, and this may reveal a correlation between human infection and this route of transmission in the region.

There are at least 12 distinct plasmids of 79–100 kb in human and pig isolates that are associated with the expression of VapB, as shown by restriction-enzyme digestion patterns of virulence plasmids using *Eco*RI and *Eco*T22I [15, 19, 21, 25]. In the present study, 44 of 52 VapB-positive isolates contained virulence plasmids that have been isolated from humans or pigs: 34 isolates contained a 79-kb plasmid (designated as type 1), 4 isolates contained an 88-kb plasmid (designated as type 4), 7 isolates contained an 88.5-kb plasmid (designated as type 7), and 1 isolate contained a type 12 plasmid (78.6-kb). However, the remaining 6 isolates contained 4 different large plasmids, which were different from the 12 representative patterns. Four new plasmid types were found in human isolates in Chiang Mai. Of the 12 representative plasmid types, 9 have been found in Japanese pig isolates [15, 19, 21, 25]. Type 1 plasmid has been found in ⩾50% of pig isolates in Japan [19, 21], and 34 (65.4%) of 52 human isolates from Chiang Mai contained this plasmid. The prevalence of *R. equi* strains of intermediate virulence in the 500 submaxillary lymph nodes of pigs in Chiang Mai was investigated in the present study, and it was lower than the prevalence that we found in our previous study, which showed a 3.1% isolation rate in Japanese pigs (56/1832 lymph nodes) [21]. However, the same virulence plasmid types were found in both human and pig isolates, which provides insight into the source and route of infection in humans in Chiang Mai and other parts of Thailand. The sites of collection (slaughterhouse) and number of pig isolates was too small for comparison, so a further surveillance study will reveal the geographical differences or similarities in the distribution of virulence plasmids in pig isolates from Chiang Mai and Japan.

The plasmid type of the soil isolate was type 12, which was also found in 1 of 69 human isolates in the present study and in a pig isolate from a pig-breeding farm in Chiang Mai that was reported elsewhere [25]. The prevalence of virulent *R. equi* was too low (1/577 soil isolates) in the environment of the patients to establish the infection, compared with cases in foals. As a control, we collected soil and fecal samples from farms randomly selected in 6 regions of Chiang Mai, and no virulent *R. equi* was identified from 629 isolates. These data coincide with our previous surveillance study, which was conducted in different regions in Chiang Mai [25]. These results suggest that VapA- and VapB-positive *R. equi* might not be widespread in the soil environment of rural Chiang Mai. However, the number of cases with *R. equi* is unusual in Chiang Mai. This evidence is contradictory, so further surveillance studies to search the source of infection are needed to identify VapB-positive *R. equi* in the environment in Chiang Mai.

The manifestations of *R. equi* infection are diverse, although pulmonary infection is present in ~80% of patients with HIV

infection [10]. In the present study, specimens for *R. equi* isolation were from the respiratory tract and included sputum (47 cases), bronchoalveolar lavage (4 cases), and pus from the chest (1 case), and most of these patients had pulmonary infection with *R. equi*. The number of cases with *R. equi* infection during the first 5 years of the study was 47, but, during the 4 years after 1998, this decreased to 22 cases. Weinstock and Brown [10] mentioned that, by inducing immune reconstitution, highly active antiretroviral therapy has probably reduced the incidence of *R. equi* infection among HIV-infected patients in developed countries. None of 69 patients received any antiretroviral therapy in the hospitals in Chiang Mai. Unfortunately, the majority of the patients did not come back to our hospitals for follow-up, so we could not provide clinical information about the outcome of the patients and information about the relationship between outcome and level of virulence. At present, we do not know the reason for the decrease in cases, but surveillance studies on the *R. equi* infection in patients should be continued in Chiang Mai and other parts of Thailand.

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Laboratory and Epidemiology Communications

Spread of Erythromycin-, Tetracycline-, and Aminoglycoside-Resistant Genes in Methicillin-Resistant *Staphylococcus aureus* Clinical Isolates in a Kumamoto Hospital

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Various drug-resistance genes with different mechanisms have been identified in methicillin-resistant *Staphylococcus aureus* (MRSA). Knowing the prevalence of these drug-resistance genes is important for controlling of MRSA spread in hospitals.

In our previous paper (1), 24 MRSA clinical isolates obtained in October 2002 in a hospital with 550 beds in Kumamoto Prefecture were assessed by restriction fragment length polymorphisms (RFLP) of genomic DNA using pulsed-field gel electrophoresis (PFGE), plasmid DNA typing by

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Table 1. PFGE patterns of MRSA isolates; MICs of GM, EM, and TC from these isolates; and distribution of GM-, EM-, and TC-resistance genes among these isolates

No.	Lane No.[1]	PFGE pattern[2]	MIC (μg/ml) of GM	MIC of EM	MIC of TC	PCR products A[3]	B	C	D	E	F	G	H	Southern blot *acc6'-aph2"* plasmid/chromosome (kb)	*ermA*	*tetM*
1245	1	A1	≧16 (R)	≧8 (R)	≧16 (R)	+	–	–	+	–	–	–	+	40, 200/110	40/220, 580	40/290
1247	2	A1	≧16 (R)	≧8 (R)	2 (S)	+	–	–	+	–	–	–	+	40, 200/110	40/220, 580	40/290
1250	3	A1	≧16 (R)	≧8 (R)	≧16 (R)	+	–	–	+	–	–	–	+	40, 200/110	40/220, 580	40/290
1251	4	A1	≧16 (R)	≧8 (R)	≧16 (R)	+	–	–	+	–	–	–	+	40, 200/110	40/220, 580	40/290
1252	5	A1	≧16 (R)	≧8 (R)	≧16 (R)	+	–	–	+	–	–	–	+	40, 200/110	40/220, 580	40/290
1255	6	A1	≧16 (R)	≧8 (R)	≧16 (R)	+	–	–	+	–	–	–	+	40, 200/110	40/220, 580	40/290
1256	7	A1	≧16 (R)	≧8 (R)	≧16 (R)	+	–	–	+	–	–	–	+	40, 200/110	40/220, 580	40/290
1258	8	A1	≧16 (R)	≧8 (R)	≧16 (R)	+	–	–	+	–	–	–	+	40, 200/110	40/220, 580	40/290
1259	9	A1	≧16 (R)	≧8 (R)	≧16 (R)	+	–	–	+	–	–	–	+	40, 200/110	40/220, 580	40/290
1261	10	A1	≦2 (S)	≧8 (R)	≧16 (R)	–	–	–	+	–	–	–	+	—/—	40/220, 580	40/290
1264	11	A1	≧16 (R)	≧8 (R)	≧16 (R)	+	–	–	+	–	–	–	+	40, 200/110	40/220, 580	40/290
1246	12	A16	≧16 (R)	≧8 (R)	≧16 (R)	+	–	–	+	–	–	–	+	40, 200/110	40/220, 580	40/290
1263	13	A25(M5)	≧16 (R)	≧8 (R)	≧16 (R)	+	–	–	+	–	–	–	+	40, 200/110	40/220, 580	40/290
1257	14	A25(M5)	≧16 (R)	≧8 (R)	≧16 (R)	+	–	–	+	–	–	–	+	40, 200/110	40/220, 580	40/290
1248	15	A6	≧16 (R)	≧8 (R)	≧16 (R)	+	–	–	+	–	–	–	+	40, 200/110	40/220, 580	40/290
1268	16	AE	≧16 (R)	≦0.5 (S)	≦1 (S)	+	–	–	–	–	–	–	–	—/280	—/—	—/—
1249	17	AH2	≧16 (R)	≧8 (R)	≧16 (R)	+	–	–	+	–	–	–	+	40, 200/110	40/220, 580	40/290
1253	18	A12(O5)	≧16 (R)	≧8 (R)	≧16 (R)	+	–	–	+	–	–	–	+	—/120	40/220, 580	40/340
1267	19	AJ	≧16 (R)	≧8 (R)	≧16 (R)	+	–	–	+	–	–	–	+	50, 280/—	40/220, 580	40/320
1262	20	AK	≦2 (S)	≧8 (R)	≧16 (R)	–	–	–	+	–	–	–	+	—/—	40/220, 580	40/290
1254	21	AL	≧16 (R)	≧8 (R)	≦1 (S)	+	–	–	+	–	–	–	–	50, 280/240	40/240, 530	—/—
1265	22	AM1	≧16 (R)	≧8 (R)	≧16 (R)	+	–	–	+	–	–	–	+	40/130	40/260, 580	40/530
1260	23	AM2	≧16 (R)	≧8 (R)	≧16 (R)	+	–	–	+	–	–	–	+	40/180	40/260, 580	40/530
1266	24	AN	≧16 (R)	≧8 (R)	≦1 (S)	–	–	–	–	–	–	–	–	—/—	—/—	—/—

[1]: Lane No. in electrophoresis shown in Fig. 1 and Fig. 2.
[2]: The PFGE patterns was reported in reference 1.
[3]: A: *aac6'-aph2"*, B: *aph(3')-III*, C: *ant(4')-I*, D: *ermA*, E: *ermB*, F: *ermC*, G: *tetK*, H: *tetM*.

using agarose gel electrophoresis, and antibiotic resistance. The same isolates were analyzed here by PCR and Southern blot to detect drug-resistance genes including gentamicin (GM)-resistant genes *aac6'-aph2"* and *aph(3')-III*; kanamicin (KM)-resistant gene *ant(4')-I*(2); erythromycin (EM)-resistant genes *ermA*, *ermB*, and *ermC*(3); and tetracycline (TC)-resistant genes *tetK* and *tetM*(4). The PCR results were evaluated based on the expected sizes of PCR products or confirmed by DNA sequencing.

Among the 24 MRSA isolates, 22 isolates, 23 isolates, and 20 isolates were resistant to GM, EM, and TC, respectively (Table 1). The majority of the isolates (18 of 24) were resistant to all of three antibiotics; five of the remaining isolates were resistant to two of the three, i.e., to EM and GM (Nos. 1247, 1254, and 1266), or to EM and TC (Nos. 1261 and 1262); and the last isolate (No. 1268) was resistant to GM. No isolate was sensitive to all three of the antibiotics. Isolate No. 1247 was sensitive to TC but showed an increase in MIC (2 μg/ml) compared with other TC-sensitive isolates (Nos. 1268, 1254, and 1266).

The results of PCR are shown in Table 1. Among the 24 MRSA isolates, 21 were PCR-positive for *aac6'-aph2"*, 22 were positive for *ermA*, and 21 were positive for *tetM*. None of the isolates was positive for *aph(3')-III*, *ant(4')-I*, *ermB*, *ermC* or *tetK*. The majority of the isolates (19 of 24) were positive for three genes: *aac6'-aph2"*, *ermA*, and *tetM*. Two isolates (Nos. 1261 and 1262) were positive for the two genes *ermA* and *tetM*. Isolate No. 1254 was positive for the two genes *ermA* and *aac6'-aph2"*. Isolate No.1268 was positive for the gene *aac6'-aph2"*. Isolate No.1266 was negative for all genes tested. The existence of *aac6'-aph2"*, *ermA*, and *tetM* was consistent with the susceptibility to GM, EM, and TC, respectively, in all MRSA isolates excepting two (Nos. 1247 and 1266). That is, all the isolates resistant to GM, EM, and TC, had *aac6'-aph2"*, *ermA*, and *tetM*, respectively. Isolates Nos. 1261 and 1262, resistant to EM and TC but sensitive to GM, had *ermA* and *tetM* but not *aac6'-aph2"*. Isolate No. 1254, resistant to GM and EM but sensitive to TC, had *ermA* and *aac6'-aph2"* but not *tetM*. Isolate No.1268, resistant to GM but sensitive to EM and TC, had *aac6'-aph2"* but not *ermA* or *tetM*.

There were exceptional isolates in which the existence of the drug-resistance genes was not consistent with the phenotype. Isolate No. 1247, resistant to GM and EM but sensitive to TC (with relatively higher MIC, as above described), had all these three genes. Other TC genes might be affecting the susceptibility to TC, or the detected TC resistance gene was non-functional due to mutation. Isolate No.1266, resistant GM and EM but sensitive to TC, did not have any of the genes tested, indicating that there are other GM- and EM-resistant genes.

To determine whether the drug-resistant genes *aac6'-aph2"*, *ermA*, and *tetM* existed on the plasmid DNA or genomic DNA of these MRSA isolates, Southern blotting was carried out (Fig. 1). The GM-resistance gene *aac6'-aph2"* was detected on four different-sized plasmids (40 kb, 50 kb, 200 kb, and 280 kb), as well as in a 15 kb *Sma*I-digest chromosome fragment derived from (Fig. 1B and Table 1). The majority of the

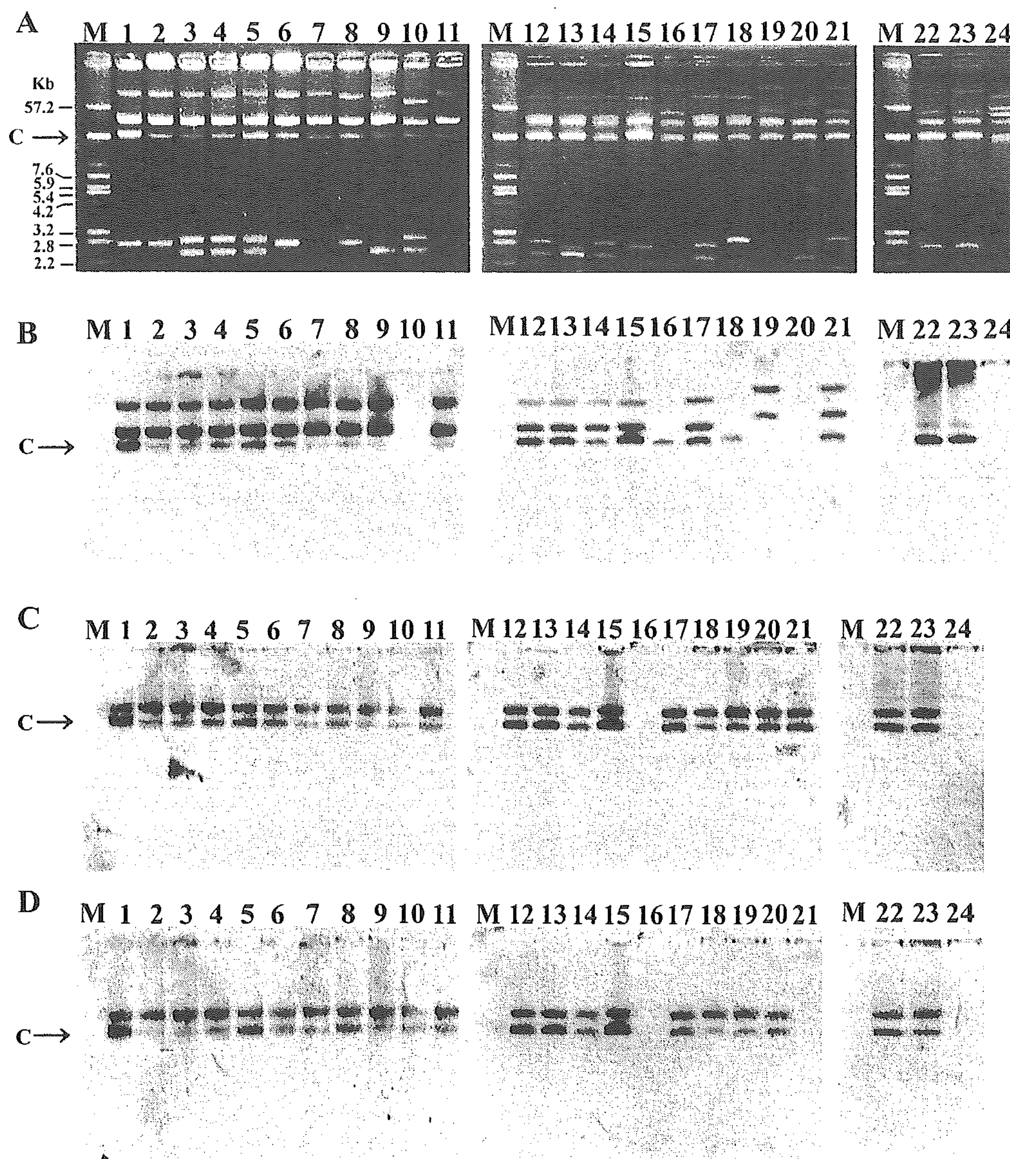

Fig. 1. Agarose gel electrophoresis of plasmid DNA from MRSA isolates (A) and Sourthern blotting hyblidized with *aac6'-aph2"*(B), *ermA* (C), and *tetM* (D).
M: marker plasmids derived from *E. coli* V517. C: DNA fragments derived from genomes. Lanes 1 to 24: MRSA isolates Nos. were listed in Table 1.

isolates had *aac6'-aph2"* on the 40 kb and 200 kb plasmids and on the chromosome. Isolates No. 1254 (lane 21) had *aac6'-aph2"* on the 50 kb and 280 kb plasmids and also on the chromosome. Isolate No.1267 (lane 19) had *aac6'-aph2"* on the 50 kb and 280 kb plasmids. Isolates Nos. 1265 and 1260 (lanes 22 and 23) had *aac6'-aph2"* on the 40 kb plasmid. Isolates Nos.1268 and 1253 (lanes 16 and 18, respectively) had *aac6'-aph2"* on the chromosome but not on the plasmid. The *aac6'-aph2"* gene was not detected in isolates Nos. 1261, 1262, and 1266 (lanes 10, 20, and 24, respectively). The majority of the isolates had *ermA* and *tetM* on the 40 kb plasmid and on the chromosome (Fig. 1C and D). Isolate No. 1254 (lane 21) had *ermA* on both the plasmid and the chromosome but had no *tetM*. Isolates Nos. 1268 and 1266 had neither *ermA* nor *tetM*.

To locate the drug-resistant genes on the chromosome, Southern blotting was done after separation of *Sma*I digests of the genomic DNA by PFGE (Fig. 2). The *aac6'-aph2"* was detected on 110 Kb of the *Sma*I digest in the majority of the isolates with the PFGE pattern A (A1, A16, A25[M5], and A6). Notably, *aac6'-aph2"* was not detected in isolate No. 1261 (lane 10) even though it was detected in other isolates with the same PFGE pattern. In isolates with other PFGE patterns, *aac6'-aph2"* was detected on variously sized *Sma*I digests (100 Kb, 120 Kb, 130 Kb, 180 Kb, 240 Kb, and 280 Kb). The *ermA* was detected on both the 220 Kb and 580 Kb *Sma*I digests in all isolates with the PFGE pattern A (lanes 1-15) and in isolates Nos. 1249 (lane 17), 1267 (lane 19), and 1262 (lane 20), respectively, with PFGE patterns AH2, AJ, and AK (Fig. 2C and Table 1). The *ermA* was also detected on the 230 Kb and 580 Kb *Sma*I fragments in isolates No. 1253 (lane 18), on the 240 Kb and 530 Kb fragments in No. 1254 (lane 21), and on the 260 Kb and 580 Kb fragments in Nos. 1265 (lane 22) and 1260 (lane 23). The *tetM* was detected on the 290 Kb *Sma*I fragment in all isolates with the PFGE pattern A (lanes 1 - 15), in isolates No. 1249 with the PFGE pattern AH2 (lane 17), and in isolate No. 1262 with PFGE pattern AK (lane 20). In isolates with other PFGE

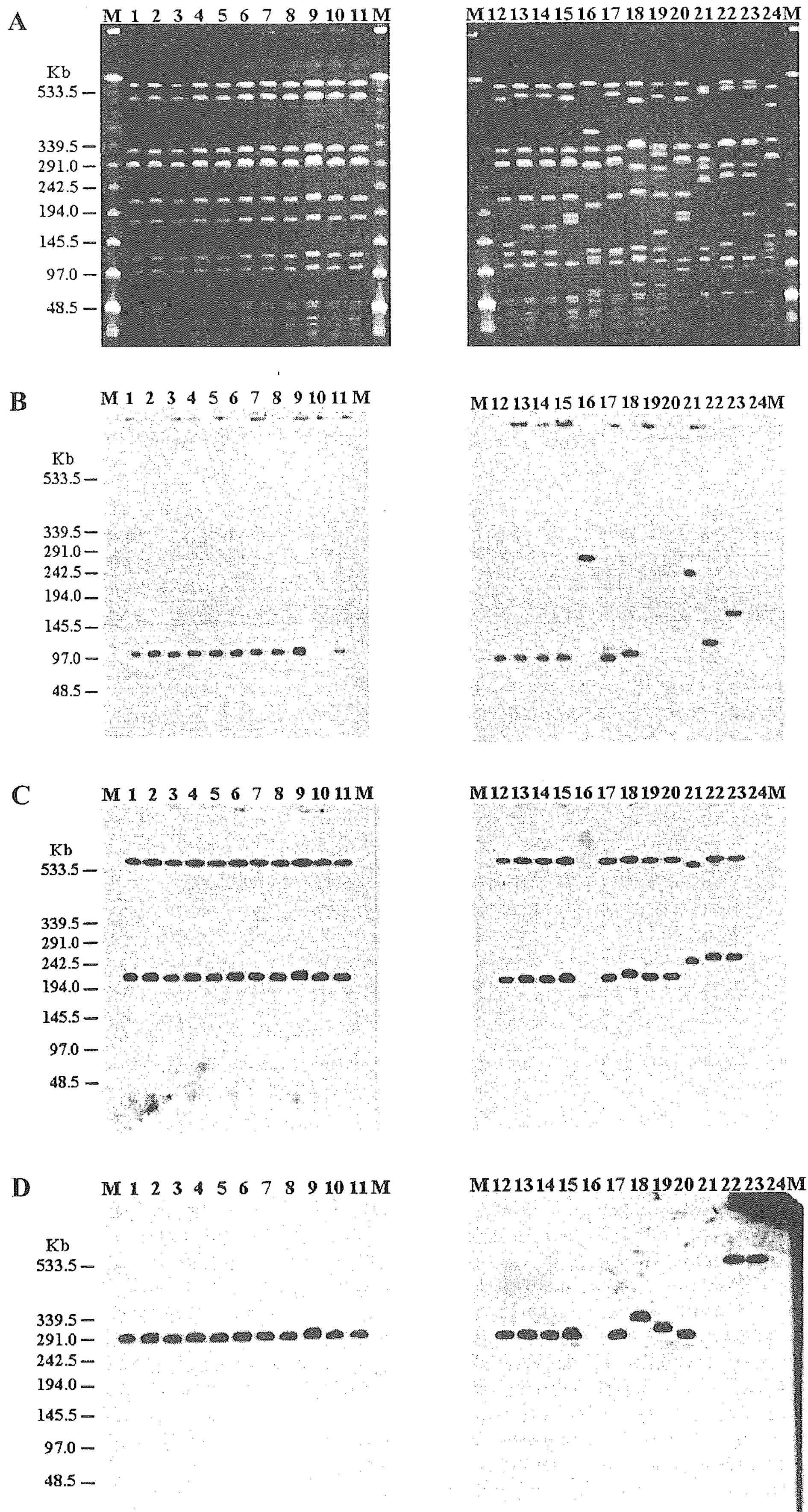

Fig. 2. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates (A) and Sourthern blotting hyblidized with *aac6'-aph2"*(B), *ermA* (C), and *tetM* (D).
M: low range PFG Marker. Lanes 1 to 24: MRSA isolates Nos. were listed in Table 1.

patterns, the *tetM* was detected on different-sized *Sma*I fragments of 320 Kb, 340 Kb, and 530 Kb.

Based on the PFGE patterns, 14 MRSA isolates among the 24 isolates belonged to one group. Among these isolates, 13 were resistant to GM, EM, and TC. All of them had a multidrug resistant 40 kb plasmid harboring *aac6′-aph2″*, *ermA*, and *tetM*, and a large plasmid of 200 kb with *aac6′-aph2″*. They had *aac6′-aph2″* and *tetM* each on at least one chromosome site, and *ermA* on at least two chromosomal sites.

The above molecular analysis of the drug-resistance genes clearly indicates the clonal expansion of MRSA and confirms the data obtained with RFLP, although our previous antibiogram data appears to have given results less convincing than those of RFLP.

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Laboratory and Epidemiology Communications

Molecular Epidemiology of Intra-Familial Tuberculosis Transmission

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The risk of tuberculosis transmission has been estimated to be relatively high among household contacts of index tuberculosis patients (1). We report here two cases of tuberculosis transmission from index patients to family members, based on restriction fragment length polymorphism (RFLP)-analysis of the pathogens.

Case 1: A 43-year-old male (patient A1) visited his home doctor complaining of fever and a left chest pain. He had smoked 15 cigarettes per day for 27 years. At the age of 28, he had experienced right side pneumothorax. He worked in a factory. He was single and lived with his father and sister. A chest radiograph revealed a cavitary lesion in the left apical region and left pleural effusion. A CT scan confirmed the cavitary lesion. He was referred to a city hospital in Yamanashi Prefecture. His sputum was negative for acid-fast bacilli (AFB). His bronchial lavage was positive for both AFB and *Mycobacterium tuberculosis* cultures. His gastric juice was positive for *M. tuberculosis* in polymerase chain reaction (PCR) (COBAS AMPLICOR™ MTB, Roche Diagnostics, Branchburg, N.J., USA). He was referred to a national hospital for treatment of pulmonary tuberculosis. The household contact investigation then performed revealed that his father (patient A2), but not his sister, had contracted tuberculosis. His father had no symptoms. He was a 73-year-old retired man, who had smoked 30 cigarettes per day for 50 years. He had hypertension. His sputum was positive for AFB and *M. tuberculosis* culture. A chest radiograph showed multiple small cavitary lesions in the bilateral upper lung fields. His chest CT scan revealed centrilobular infiltrations in the right upper lung field.

Case 2: A 54-year-old male (patient B1) visited his home doctor in October 2001 with complaints of fatigue, fever, shortness of breath, and a 20 kg loss of body weight over the previous 3 months. He had smoked 20 cigarettes per day for 37 years and was a carpenter. His father had died of tuberculosis after being ill for 10 years. His sputum was positive for AFB and *M. tuberculosis*-positive in culture and PCR. His chest radiograph revealed multiple cavitary lesions in the bilateral upper and middle lung fields. He was referred to the national hospital for treatment of pulmonary tuberculosis in October 2001. His son (patient B2), a 25-year-old office worker who lived with him until 2000, was referred to the same hospital in February 2002 when a routine radiograph check revealed an abnormal shadow. He had smoked 20 cigarettes per day for 6 years. His sputum was positive for AFB and *M. tuberculosis*-positive in culture and PCR. A chest radiograph revealed a cavitary lesion in the left upper field and infiltration shadows in the left middle and right upper lung fields. His chest CT scan showed a cavitary lesion in the left apical region. He was admitted for treatment of pulmonary tuberculosis.

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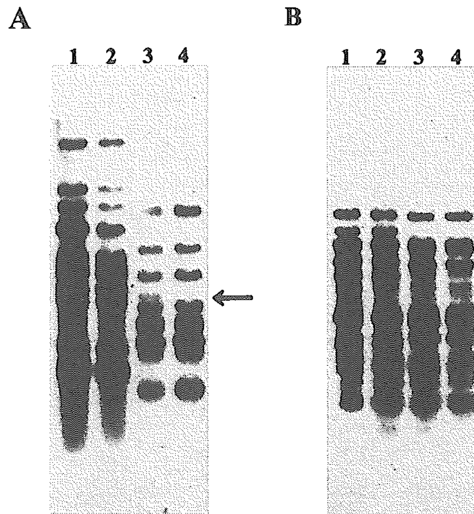

Fig. 1. IS*6110* and $(CGG)_5$ restriction fragment length polymorphism (RFLP) typing.
*Pvu*II-digested genomic DNA was analyzed by Southern blot hybridization with a peroxydase-labeled DNA probe for IS*6110* (A) and $(CGG)_5$ (B). Lane 1: *M. tuberculosis* isolate from patient A1 (isolate A1); lane 2: isolate A2; lane 3: isolate B1; lane 4: isolate B2.

To determine whether the strains of *M. tuberculosis* infecting the respective patients and their contacts were of the same origin, chromosomal DNAs from these isolates were analyzed by RFLP using a IS*6110* probe (2) (Fig. 1A) and a trinucleotide repeat sequence $(CGG)_5$ probe (Y. Otsuka et al., in preparation for a paper) (Fig. 1B). The IS*6110* patterns were identical for isolates from patients A1 and A2 (lanes 1 and 2). The IS*6110* patterns of isolates from patients B1 and B2 were identical except in one band (indicated by an arrow in Fig. 1A), and differed from the pattern observed in isolates A1 and A2. The $(CGG)_5$ pattern was identical between isolates A1 and A2, and between isolates B1 and B2, respectively. All these isolates were sensitive to isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin. No mutations were found in the drug resistance-related genes, including *kat*G and *inh*A responsive for isoniazid-resistance, *rpo*B for rifampin-resistance, *pnc*A for pyrazinamide, *emb*B for ethambutol-resistance, *rrs*L and *rrs* for streptomycin, and *gyr*A for flouroquinolones. The data indicated that *M. tuberculosis* isolates A1 and A2, and isolates B1 and B2, respectively, were of the same origin. The data clearly supported transmission of *M. tuberculosis* within a family.

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Laboratory and Epidemiology Communications

Molecular Epidemiology of Methicillin-Resistant *Staphylococcus aureus* in a Kumamoto Hospital in 2002

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a prevalent nosocomial pathogen in healthcare facilities. Epidemiological analysis of MRSA isolates present in a hospital, based, for example, on the restriction fragment length polymorphisms of genomic DNA determined using pulsed-field gel electrophoresis (PFGE), is essential for assessment of hospital infection control (1,2).

Fifty-six MRSA isolates were obtained from 24 inpatients during October 2002 in a hospital with 11 wards and 550 beds in Kumamoto Prefecture. Of these isolates, 24 isolates, each derived from a single patient, were analyzed for chromosomal DNA typing by using a contour-clamped homogeneous electric field system (CHEF Mapper™: Bio-Rad Laboratories, Hercules, Calif., USA), plasmid DNA typing by use of agarose gel electrophoresis, antibiotic resistance (VITEK™: bioMerieux, Marcy-l'Etoile, France), enterotoxin serotyping (SET-RPLA: Denka Seiken Co., Tokyo), toxic shock syndrome toxin-1 (TSST-1) production (TST-RPLA: Denka Seiken), and coagulase serotyping (Denka Seiken).

Thirteen different PFGE patterns of *Sma*I DNA digests were detected (Fig. 1). A band-based cluster analysis of these patterns (Molecular Analyst™: Bio-Rad) revealed nine clusters (clusters A, AH, AI, AE, AM, AL, AN, AJ, and AK) (a cluster was defined as a group of patterns with more than 70% similarity (Fig. 2A). The frequency distribution of MRSA isolates based on PFGE patterns is shown in Fig. 2B. The most frequent pattern (A1) represented 45.8% of the total

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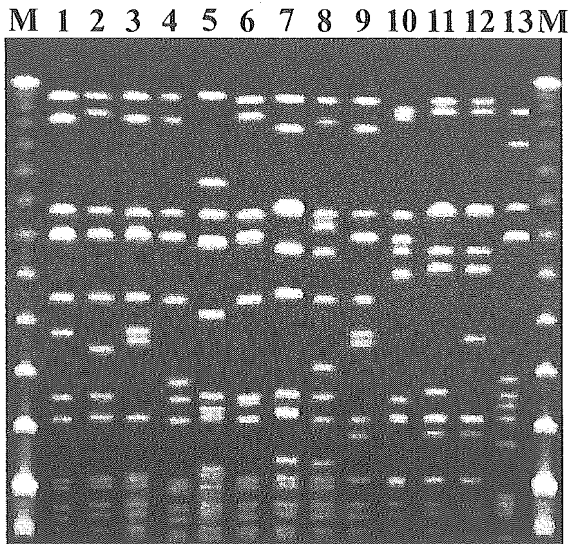

Fig. 1. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA from MRSA isolates. M: low range PFG Marker. Lanes 1 to 30: MRSA isolates with different PFGE patterns A1 to AK, respectively, shown in Fig. 2.

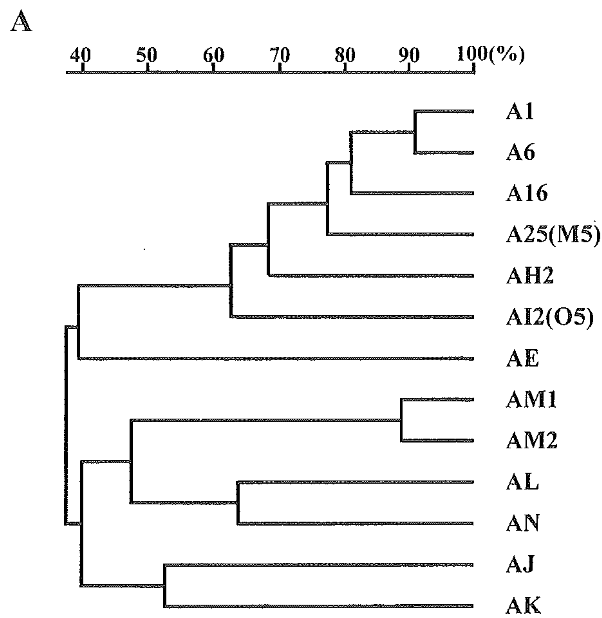

Fig. 2. Cluster analysis of MRSA isolates based on PFGE patterns of *Sma*I-digested genomic DNA.

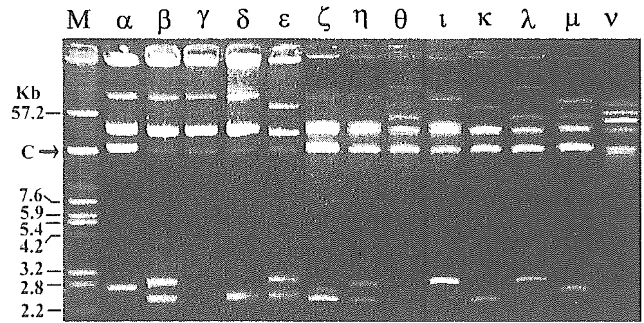

Fig. 3. Agarose gel electrophoresis of plasmid DNA from MRSA isolates. M: marker plasmids derived from *E. coli* V517. C: DNA fragments derived from genomes.

isolates. PFGE pattern A25 was detected in two isolates. The other 11 PFGE patterns were detected in only one isolate.

The profiles of plasmid typing are shown in Fig. 3. All MRSA isolates had plasmids. Twelve different sizes of plasmids, ranging from 2.4 kb to 280 kb, were detected. Each isolate had two to five different sized plasmids. Thirteen plasmids different in size were detected (Table 1). The frequency distribution of MRSA isolates based on plasmid patterns is shown in Table 1. All isolates had the 40 kb plasmid. Isolates with patterns α, β, and γ accounted for 50% of the total, and these isolates had both 200 kb and 40 kb plasmids.

The geographic distribution of MRSA isolates in the hospital is shown in Table 2. The two, two, two, one, three, and one isolates from intensive care unit and wards e2, w3, a2, a5, and a6, respectively, belonged to the same cluster, A1, suggesting a clonal spread of MRSA in the hospital. Among these isolates, one from ward a2 and two from ward a5 belonged to the same plasmid pattern α; and one from ward e2, one from ward a5 and one from ward a6 belonged to the pattern β, indicating that transmission of a clone of MRSA with PFGE pattern A1 and plasmid pattern α occurred in the ward a5. Two isolates belonging to PFGE pattern A25, but having different respective plasmid patterns (ζ and η) were isolated from wards a3 and a5, respectively. Isolates with other PFGE and plasmid patterns appeared to be limited to wards w3, e2, w2, a3, a5, e1, a3, w1, e1, a3, and w2.

Sensitivity to antibiotics is shown in Table 3. The MRSA

Table 1. Plasmid typing pattern classified by plasmid size and its frequency

Plasmid pattern	Frequency	Plasmid size (kb)											
		280	200	150	57	55	50	45	40	3.0	2.6	2.5	2.4
α	5		○						○		○		
β	4		○						○	○			○
γ	3		○						○				
σ	1		○						○				○
ε	1			○					○	○			○
ζ	1		○						○			○	○
η	1		○					○	○	○			○
θ	2	○					○		○				
ι	1		○					○	○	○			
κ	1				○				○				○
λ	1	○					○		○	○			
μ	2	○					○		○				○
ν	1			○		○	○		○				

Table 2. Distribution of MRSA in a hospital

PFGE pattern	Plasmid pattern	Ward e 1	e 2	e 3	w 1	w 2	w 3	a 1	a 2	a 3	a 5	a 6	ICU
A1	α								1		2		1
	β		1#								1	1	
	γ						1						1
	δ						1						
	ε		1										
(A1##	α - ε		2				2		2		3	1	2)
A6	α						1						
A16	β		1										
A25	ζ									1			
	η										1		
AE	θ					1							
AH2	η									1			
AI2	ι										1		
AJ	θ	1											
AK	κ									1			
AL	λ				1								
AM1	μ	1											
AM2	μ									1			
AN	ν					1							

Number of patient with MRSA.
Total numbers of patients with MRSA having PFGE pattern A1.

Table 3. Antibiotic pattern classfied by antibiotic pattern of antibiotics against MRSA

Antibiotic pattern	Antibiotics EM	GM	TC	MINO	ABK	VCM	TEIC
a	R	R	R	R	S	S	S
b	R	R	R	S	R	S	S
c	R	R	R	S	S	S	S
d	R	R	R	I	S	S	S
e	R	S	R	R	S	S	S
f	R	R	S	S	S	S	S
g	R	S	R	S	S	S	S
h	S	R	S	S	S	S	S

All the isolates were resistant to MPIPC, PCG, ABPC, PIPC, CEZ, CMZ, IPM, SBT/ABPC.
MPIPC: oxacillin, PCG: benzyl-penicillin, ABPC: ampicillin, PIPC: piperacllin, CEZ: cefazolin, CMZ: cefmetazole, IPM: imipenem/cilastatin, SBT/ABPC: sulbactam/ampicillin, EM: erythromycin, GM: gentamicin, TC: tetracycline, MINO: minocyclin, ABK: arbekacin, VCM: vancomycin, TEIC: teicoplanin.
R: resistant, S: susceptible, I: intermediate.

isolates were resistant to 9-12 of 15 tested drugs. Those isolates had a spectrum of drug-resistance showing eight different patterns. The spectra of drug-resistance were similar to each other; e.g., a difference in susceptibility was found against only one drug when isolates with pattern c were compared with those with pattern a, b, d, e, f, or g. One isolate was resistant to arbekacin (pattern b). Those isolates were resistant to 9-12 of 15 tested drugs. (One isolate with PFGE pattern AH2.) All the isolates were sensitive to vancomycin or teicoplanin. No correlation was found between the antibiotic resistance patterns and PFGE patterns (Table 4).

Among 24 MRSA isolates, 23 isolates produced coagulase type II, and the remaining isolate produced coagulase type III (Table 4). Twenty-two isolates produced enterotoxin type C; the twenty-third produced isolate enterotoxin types B and C; and the last isolated produced neither enterotoxin types A, B, C, or D. Twenty-three isolates produced TSST-1; the twenty-fourth did not. Collectively, among 24 MRSA

Table 4. Genotypic and phenotypic characterization of MRSA

No.	PFGE pattern	Plasmid pattern	Antibiotic pattern	Enterotoxin	TSST-1	Coagulase
1245	A1	α	c	C	+	II
1247	A1	α	f	C	+	II
1250	A1	β	c	C	+	II
1251	A1	β	d	C	+	II
1252	A1	β	c	C	+	II
1255	A1	α	c	C	+	II
1256	A1	γ	c	C	+	II
1258	A1	α	c	C	+	II
1259	A1	δ	c	C	+	II
1261	A1	ε	g	C	+	II
1264	A1	γ	d	C	+	II
1246	A16	β	c	C	+	II
1263	A25(M5)	ζ	c	C	+	II
1257	A25(M5)	η	c	C	+	II
1248	A6	α	c	C	+	II
1268	AE	θ	h	C	+	III
1249	AH2	η	b	C	+	II
1253	AI2(O5)	ι	c	B,C	+	II
1267	AJ	θ	c	C	+	II
1262	AK	κ	e	C	+	II
1254	AL	λ	f	C	+	II
1265	AM1	μ	a	C	+	II
1260	AM2	μ	a	C	+	II
1266	AN	ν	f	–	–	II

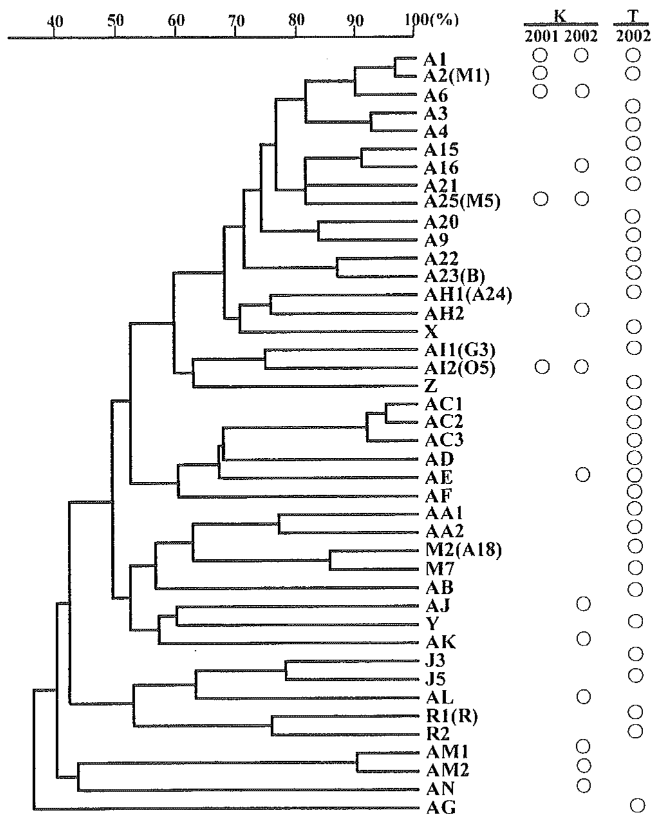

Fig. 4. Cluster analysis of MRSA isolates based on PFGE patterns. K: Kumamoto hospital, T: Tokyo hospital.

isolates, 21 isolates produced coagulase type II, enterotoxin type C, and TSST-1; i.e., most of the isolates shared common characteristics regarding these parameters.

PFGE-based MRSA surveillance was conducted in the same hospital in October 2001 (1) and in a hospital in Tokyo in October 2002 (2). In these surveillance studies, a total of 42 PFGE patterns were detected (Fig. 4). PFGE patterns A1, A6, A25(M5), and AI2(O5) were common in Kumamoto in 2001 and 2002, whereas the other patterns were unique to each year (Fig. 4)(1), indicating the co-existence of persistence and rapid turnover of MRSA in a hospital. Patterns A1, A2(M1), A16, AE, detected in the hospital in Kumamoto in either 2001 or 2002, were also detected in a hospital in Tokyo in 2002 (Fig. 4)(2). Among these patterns, pattern A1 was most frequently detected in both hospitals (Fig. 2B)(2). The data indicate the clonal expansion of MRSA not only within hospitals but also nationwide.

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広範囲 血液・尿化学検査 免疫学的検査

—その数値をどう読むか—

［第6版］

(3)

VIII. 免疫学的検査

F. ウイルス感染症関連検査(抗原および抗体を含む)

SARSコロナウイルス

倉辻忠俊　　切替照雄

SARSコロナウイルス

SARS-Corona virus

倉辻忠俊 切替照雄

Key words: SARS, 呼吸器感染症, 血清抗体, 診断, バイオセーフティー

1. 概 説

重症急性呼吸器症候群(severe acute respiratory syndrome: SARS)は, 2003年2月末から(発端は2002年11月)東アジアを中心に, 短期間に全世界へと流行拡散した, SARSコロナウイルスによる急性呼吸器感染症である. 感染者の20%前後が発症後数日以内に乾性咳嗽, 呼吸困難, 低酸素血症へと急速に重症化する進行性肺炎(呼吸窮迫症候群)で, 死亡率は10%前後である. 飛沫感染, 接触感染によりヒト-ヒト感染が主であるが, まれに糞口感染, 空気感染の可能性を示唆する例もある. 20-50%が病院感染で, 早期診断, 院内感染対策が重要である.

診断はウイルス分離, RT-PCR, LAMP法などによる遺伝子診断, 血清抗体検査などによる.

ウイルスのレザボアはハクビシンなど野生の動物が考えられているが, まだ確定していない.

2. 検査の目的

ウイルス分離および遺伝子診断は治療および疫学的対策, 鑑別診断のために行う. ペア血清の抗体検査は確認のために行う.

3. 試料の採取方法

飛沫感染するので, 試料採取の際はN95マスク, ゴーグル, 手袋などの個人防護具を必ず着用する.

ウイルス分離, 遺伝子診断のための試料は, 鼻咽頭拭い液, 喀痰などの気道分泌液, 便, 尿などが用いられる. ウイルス分泌は発症後10日頃が最大となるので, 発症初期の診断が陰性でも, 10日余り後にもう一度採取することが望ましい.

血清抗体価検査は急性期(10病日以内)および20病日以降のペアとして採取する. 20病日前後の血清が陰性の場合, 30病日以降の血清を採取して確認することが望ましい.

4. 測 定 法

日本におけるSARSコロナウイルスの標準的確定診断は確立されていない. また, 施設内感染が多いため, 患者(疑いを含む)の診療, 試料の取り扱いの際は原則としてN95マスク, ゴーグル, 手袋など個人防護具の着用ばかりでなく, バイオセーフティレベル2以上の部屋で, 安全キャビネット内で, 十分な準備と細心の注意をもって実施する.

a. ウイルス分離

BSL3対応の施設が必要で, 通常の病院検査室では行ってはならない. したがって, 試料は保健所に連絡のうえ, 指定の地方衛生研究所あるいは国立感染症研究所へ, 万国郵便条約に則って送付する. すなわち, 試料の入ったスクリューキャップで密閉できる第1次容器を吸収性の良い布あるいは紙タオルで包み, WHOの基準を満たす耐圧の第2次容器に入れる. 第3次容器に試料の内容, 患者情報, 依頼元を記した封筒, 第2次容器, 保冷剤を入れて封をし, 外側に宛先, 依頼元を記し, 更にバイオハザードのシールを貼る. 発送の前に受け入れ先(地方衛生研究所あるいは国立感染症研究所)に連絡をしておく.

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