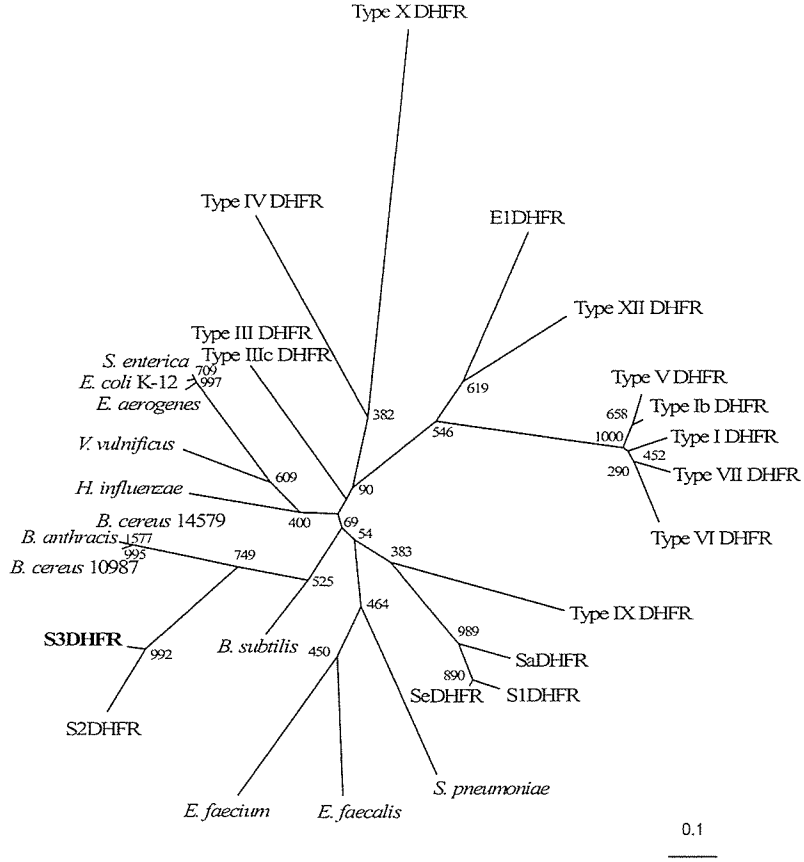

FIG. 2. Dendrogram of S3DHFR and DHFR from a variety of organisms. The dendrogram was created by the ClustalW program. Branch lengths correspond to the number of amino acid exchanges of the DHFR proteins (accession number and species given in parentheses) of types I (X00926, from *E. coli*), Ib (I40985, from *E. coli*), IV (A60935, from *E. coli*), V (X12868, from enterobacterial plasmid pLMO150), VI (Z86002, from *Proteus mirabilis*), VII (X58425, from *E. coli*), VIII (U10186, from *E. coli*), IX (A49788, from *E. coli*), X (AY123253, from *Klebsiella pneumoniae*), XII (I41043, from *E. coli*), E1 (AF028812, from *E. faecalis*), S1 (X13290, from *S. aureus*), and S2 (Z50141, from *S. haemolyticus* MUR313) and the chromosomal DHFRs of *B. anthracis* Ames (AE017031), *B. cereus* ATCC 14579 (AE017005), *B. cereus* ATCC 10987 (AE017271), *B. subtilis* Marburg (L77246), *Enterobacter aerogenes* (M26022), *E. coli* K-12 (P00379), *E. faecalis* V583 (AE016951), *E. faecium* mutant strain A (741860A), *Haemophilus influenzae* R1047 (X84205), *Salmonella enterica* serovar Paratyphi ATCC 9150 (CP000026), *S. aureus* ATCC 25923 (SaDHFR; Z16422), *S. epidermidis* ATCC 14900 (SeDHFR; Z48233), *Streptococcus pneumoniae* ATCC 49619 (Z74778), and *Vibrio vulnificus* YJ016 (BA000037).

indicating that the region is in an insertion sequence (IS). The DNA sequence, ranging from 275 bp upstream of *dfrG* to the 363 bp of the 5′-terminal region of *dfrG*, was identical to that previously reported for plasmid pMG1 in *Enterococcus faecium* (18). The *dfrG* gene may have been acquired from *E. faecium* via IS-mediated recombination. The ancestral origin of S3DHFR, however, remains unknown; S3DHFR showed little similarity to and considerable phylogenetic distance from intrinsic DHFR of *E. faecium* (Fig. 2).

The MICs of TMP in *E. coli* transformants harboring pSA1 or pT7dfrG carrying *dfrG* were significantly increased than those in control strains (Table 1), indicating that *dfrG* is responsible for TMP resistance. An *E. coli* transformant harboring pT7dfrB carrying *dfrB* also showed increased MIC, but it was not as high as those of *E. coli* strains expressing *dfrG*. *dfrB* is believed to encode a TMP-sensitive DHFR of *S. aureus* because it was found in all *S. aureus* strains, regardless of TMP susceptibility. Similar results were reported for *dfrE* encoding *Enterococcus faecalis* DHFR (4). The increased MIC for TMP in *E. coli* carrying *dfrB* may be explained by the multicopy effects of high expression of the housekeeping protein DHFR.

For functional analysis of S3DHFR and DHFR from *S. aureus* CM.S2 ($SaDHFR_{CM.S2}$), overexpression and purification of these recombinant DHFRs were achieved. Overexpression of S3DHFR or $SaDHFR_{CM.S2}$ was accomplished by integration of the respective coding regions downstream of the His-tagged coding region of the pCR/T7NT expression vector and transformation into the *E. coli* strain BL21-AI. Recombinant protein in soluble extracts was purified by affinity chromatography to determine enzymatic activities. The K_m values of recombinant S3DHFR for DHF and NADPH were 2.68 ± 1.09 μM and 2.38 ± 1.97 μM, respectively (Table 2). The K_m values of DHF and NADPH for S3DHFR did not differ from those of $SaDHFR_{CM.S2}$, but the IC_{50} values of TMP for these DHFRs differed significantly. The IC_{50} of TMP for S3DHFR was more than 8,000-fold greater than IC_{50} values for TMP-

sensitive SaDHFR and $SaDHFR_{CM.S2}$, indicating that S3DHFR and $SaDHFR_{CM.S2}$ are indeed DHFRs but that only S3DHFR plays a critical role in TMP resistance. The K_m values of crude extracts for NADPH were sixfold greater than those of recombinant S3DHFR (Table 2). Crude extracts may contain other factor(s) that bind to NADPH.

Detection of *dfrG* was performed by PCR on isolates from Chiang Mai, Thailand, and Tokyo, Japan. All Chiang Mai isolates were resistant to TMP and contained *dfrG*, whereas all Tokyo isolates but one were sensitive to TMP and did not contain *dfrG* (data not shown). The single Tokyo isolate IMCJ934 was resistant to TMP and contained *dfrG* (Table 1).

Pulsed-field gel electrophoresis (PFGE) analysis revealed 13 patterns of SmaI digestion in the 43 MRSA isolates from Chiang Mai, Thailand (data not shown). Cluster analysis showed that 12 of the 13 PFGE patterns formed a cluster (>75% similarity). The PFGE pattern of *S. aureus* CM.S2 genomic DNA was identical to that of 18 MRSA isolates. These results suggest that clonal expansion of MRSA carrying *dfrG* occurred at the hospital in Chiang Mai. The TMP-resistant isolate from Tokyo, Japan, IMCJ934, showed the same PFGE pattern as that of one of the Chiang Mai isolates, *S. aureus* CM.S2 (data not shown).

dfrG was detected by Southern blotting on fragments of SmaI-digested genomic DNA, but it was not detected on plasmids (data not shown). Conjugal transfer of TMP resistance from *S. aureus* CM.S2 to recipient strains *S. aureus* IMCJ565RFP^r or IMCJ644RFP^r was unsuccessful, suggesting that *dfrG* is located on the chromosome and not on a plasmid of these clinical isolates. It remains to be determined whether *dfrG* can be transferred by phages or mobile elements.

A single amino acid substitution (Phe to Tyr) at codon 98 of SaDHFR was reported to be associated with TMP resistance in *S. aureus* (5). Therefore, approximately 390 bp of internal DNA sequence of *dfrB* encoding SaDHFR was determined. When *S. aureus* ATCC 29213 was used as a control (5), all isolates from Chiang Mai, Thailand, exhibited three silent mutations: CAT to CAC in codon 77 and TTT to TTC in codons 91 and 118. All isolates from Tokyo, Japan, contained four silent mutations: AAA to AAG in codon 30, CAT to CAC in codon 77, and TTT to TTC in codons 91 and 118. These results indicate that these mutational changes are not associated with TMP resistance in the isolates from Chiang Mai or Tokyo. Other possible mechanisms of TMP resistance, such as overexpression of intrinsic DHFR, efflux, or impermeability, may be involved.

The CM.S2 strain was the dominant clone from Chiang Mai, Thailand. MRSA surveillance is being carried out in the hospital from which these isolates were obtained. *S. aureus* CM.S2 is resistant to clindamycin, erythromycin, gentamicin, and tetracycline and is less sensitive to arbekacin. Fosfomycin, linezolid, and vancomycin are effective in vitro; quinupristin-dalfopristin and daptomycin were not available for testing. Results of this surveillance will be reported in the future.

Our data strongly suggest that the TMP resistance-associated gene *dfrG* is prevalent in Thailand, and an isolate harboring this gene was found in Japan. This gene may spread worldwide, and measures against this, such as gene monitoring and adequate use of TMP, should be established.

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Survey of human immunodeficiency virus (HIV)-seropositive patients with mycobacterial infection in Japan

Yayoi Otsuka[a,1], Tomoko Fujino[a], Namiko Mori[a], Jun-ichiro Sekiguchi[a], Emiko Toyota[a], Katsutoshi Saruta[a], Yoshihiro Kikuchi[b], Yuka Sasaki[c], Atsushi Ajisawa[d], Yoshito Otsuka[e], Hideaki Nagai[f], Makoto Takahara[g], Hideo Saka[h], Takuma Shirasaka[i], Yasuki Yamashita[i], Makiko Kiyosuke[j], Hideyuki Koga[j], Shinichi Oka[a], Satoshi Kimura[a], Toru Mori[k], Tadatoshi Kuratsuji[a], Teruo Kirikae[a,*]

[a]*International Medical Center of Japan, Toyama 1-21-1, Shinjuku-ku, Tokyo 162-8655, Japan*
[b]*National Medical Organization, Sendai Medical Center, Miyagino 2-8-8, Miyagino-ku, Sendai 983-8520, Japan*
[c]*National Hospital Organization, Chiba-Higashi Hospital, Nitona-cho 673, Chuo-ku, Chiba 260-8712, Japan*
[d]*Tokyo Metropolitan Komagome Hospital, Honkomagome 3-18-22, Bunkyo-ku, Tokyo 113-8677, Japan*
[e]*Social Insurance Central General Hospital, Hyakuninchou 3-22-1, Shinjuku-ku, Tokyo 169-0073, Japan*
[f]*National Hospital Organization, Tokyo Hospital, Takeoka 3-1-1, Kiyose, Tokyo 204-8585, Japan*
[g]*National Hospital Organization, West Kofu Hospital, Yamamiya 3368, Kofu 400-0075, Japan*
[h]*National Hospital Organization, Nagoya Medical Center, Sannomaru 4-1-1, Naka-ku, Nagoya 406-0001, Japan*
[i]*National Hospital Organization, Osaka National Hospital, Hoenzaka 2-1-14, Chuo-ku, Osaka 540-0006, Japan*
[j]*National Hospital Organization, Kyushu Medical Center, Jigyohama 1-8-1, Chuo-ku, Fukuoka 810-8563, Japan*
[k]*Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Matsuyama 3-1-24, Kiyose, Tokyo 204-8533, Japan*

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KEYWORDS
Mycobacterial infection;
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Abstract *Objective*. To assess DNA polymorphisms in mycobacterial isolates obtained from human immunodeficiency virus (HIV)-seropositive patients with tuberculosis in Japan from 1996 to 2003.

Methods. Restriction fragment length polymorphisms (RFLP) from *Mycobacterium tuberculosis* and *Mycobacterium avium* isolates obtained from individual seropositive patients with tuberculosis ($n=78$) were analysed with the use of IS*6110* and $(CGG)_5$ or IS*1245* and IS*1311*, respectively, as markers. As a control, the same procedures were applied to isolates from HIV-seronegative tuberculosis patients ($n=87$).

* Corresponding author. Tel.: +81 3 3202 7181x2838; fax: +81 3 3202 7364.
E-mail address: tkirikae@ri.imcj.go.jp (T. Kirikae).
[1] Present address affiliation: Laboratory of Molecular Medicine, Department of Veterinary Clinical Sciences, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan.

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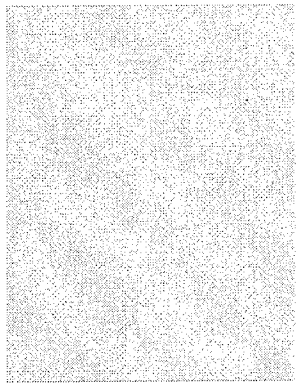

Results. Of 86 mycobacterial strains, *M. tuberculosis*, *M. avium* and *Mycobacterium chelonae* were identified in 48 (55.8%), 36 (41.9%) and 2 (2.3%) isolates, respectively. The obtained RFLP patterns of *M. tuberculosis* isolates from both the HIV-seropositive and -seronegative groups were variable, suggesting no obvious clustering among the isolates. Similar results were obtained in isolates of *M. avium*.

Conclusions. This is the first report on the molecular epidemiology of *Mycobacterium* spp. isolated from HIV-seropositive patients in Japan. The results indicate that no particular clones of *M. tuberculosis* or *M. avium* prevail in HIV-seropositive patients in Japan. Further monitoring of mycobacterial infection associated with HIV infection in Japan should be continued.

Introduction

In recent years, a decline in the number of new patients with acquired immunodeficiency syndrome (AIDS) has been observed in several industrialized countries, including the United States, Western European countries, Australia and New Zealand.[1-6] However, no decline in patients with human immunodeficiency virus (HIV) has been observed in Japan.[7] Mycobacterial infections, such as those of *Mycobacterium tuberculosis* and *Mycobacterium avium*, are important opportunistic infections in HIV-seropositive patients. With respect to tuberculosis (TB), several studies based on clinical observations[8-12] and on epidemiologic surveys[13-17] have provided evidence that HIV infection is a risk factor for the development of active and often lethal TB. Outbreaks of TB among communities of HIV patients have been reported in the United States, but multidrug resistant (MDR) *M. tuberculosis* strains were rarely isolated from these patients.[12,18] In sub-Saharan Africa, TB associated with HIV has played an important role in increasing TB transmission throughout the population.[17,19]

Non-tuberculous mycobacterial infection can be difficult to treat because of primary resistance against most of the commonly used anti-tubercular drugs, such as isoniazid, rifampin, streptomycin, ethambutol, pyrazinamide and kanamycin.[20] A relatively high prevalence of non-tuberculous mycobacterial infections has been observed in HIV/AIDS patients, and 25-50% of patients with AIDS in the United States and Europe are infected with this group of bacteria, primarily with *M. avium*, which mainly causes disseminated mycobacteraemia in AIDS patients.[21]

Japan is considered to have a low prevalence of HIV/AIDS, with a cumulative number of 2556 AIDS cases and 5140 HIV cases reported by the end of 2002.[7] However, the recent trend of HIV cases shows a substantial increase, particularly among men who have sex with men and youth/young adults. A considerable number of HIV patients in Japan have experienced discrimination or breach of confidentiality and they feel insufficiency of social and economical supports.[22] Patients with mycobacterial infection used to be discriminated, but the prejudice toward the patients declines. The medical, social and economic backgrounds of HIV patients in Japan differ considerably from those in regions such as North America, Europe and Africa. The correlation between HIV and mycobacterial infections in Japan may also differ from that in countries where research on AIDS-related diseases is well developed. Survey of the occurrence and clinical profiles of these infections is important for the development of countermeasures against mycobacteria and HIV coinfection. In this study, we analysed the current prevalence, clinical features and epidemiologic findings of mycobacterial infection associated with HIV infection in Japan.

Materials and methods

Bacterial isolates and clinical data

From 1996 to 2003, 86 clinical mycobacterial isolates were obtained from eight hospitals in Japan: the International Medical Centre of Japan (IMCJ) (Tokyo); Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association (JATA) (Tokyo); National Tokyo Hospital (Tokyo); Tokyo Metropolitan Komagome Hospital (Tokyo); Social Insurance Central General Hospital (Tokyo); National Nishi-Kofu Hospital (Yamanashi); National Osaka National Hospital (Osaka) and National Kyushu Medical Centre (Fukuoka). Clinical information on individual patients was obtained by the physicians in charge with questionnaire on mycobacterial isolation date, history of previous mycobacterial infection, microscopic observation of

Table 1 Nationality and sex of HIV-positive patients with mycobacterial infection in Japan

Nationality	No. of patients	Male:female	Mycobacteria species
Japanese	33	31:2	*M. tuberculosis*: 21 *M. avium*: 11 *M. chelonae*: 1
Non-Japanese	16	9:7	*M. tuberculosis*: 9 *M. avium*: 6 *M. chelonae*: 1
Unknown[a]	37	33:2, unknown[a]:2	*M. tuberculosis*: 18 *M. avium*: 19 *M. chelonae*: 0
Total	86	74:10, unknown[a]:2	*M. tuberculosis*: 48 *M. avium*: 36 *M. chelonae*: 2

[a] Nationality or sex of these patients was not disclosed due to the ethics code of the corresponding hospital.

sputa, sites of infection (pulmonary or extrapulmonary), peripheral blood $CD4^+$ lymphocyte number, chemotherapeutic regimens and standard demographic data. The Ethics Committees in each hospital approved this study (IMCJ-H13-54) and all patients gave a written informed consent.

As a control for *M. tuberculosis* genotyping, 87 clinical isolates from adult HIV-seronegative tuberculosis patients without any serious complication at IMCJ were used. Since other hospitals, except JATA, have no ward for TB patients and the RFLP patterns of *M. tuberculosis* isolates from JATA and IMCJ were variable, and showed no obvious clustering among the isolates.

Mycobacterial culture and identification of strains

Bacteria were grown on egg-based Ogawa medium (Kyokuto Pharmaceutical Co., Ltd, Tokyo, Japan) for 3-5 weeks. Cultured organisms were applied to a polymerase chain reaction (PCR) kit for *M. tuberculosis* diagnosis (Amplicor *Mycobacterium tuberculosis* Test, Roche Diagnostic Systems, Inc., Branchburg, NJ), and PCR-negative organisms were further applied to an identification kit for mycobacterial species that uses DNA-DNA hybridization (DDH Mycobacteria, Kyokuto Pharmaceutical Co., Ltd).

Drug sensitivity testing

Drug sensitivity of *M. tuberculosis* strains was tested by two agar proportion methods, one with Middlebrook 7H10 agar medium, as recommended by the U.S. Public Health Service,[23] and the other with egg-based Ogawa medium, as recommended by the Japanese Society for Tuberculosis (Vit Spectrum-SR™, Kyokuto Pharmaceutical Co., Ltd).

DNA fingerprinting

Chromosomal DNA from mycobacterial isolates was prepared as described previously[24,25] but with slight modification. The DNA was precipitated in isopropanol, and the precipitates were redissolved in 20 μl 0.1X TE buffer.

For IS6110- and $(CGG)_5$-restriction fragment length polymorphisms (RFLP)[26] of *M. tuberculosis*, DNA was digested overnight with restriction enzymes *Pvu*II and *Alu*I (Takara Bio, Inc., Shiga, Japan), respectively. The digested fragments were separated by electrophoresis on 1% agarose gels. A 1-kb DNA ladder (Promega Corp., Madison, WI) was used as a marker. The agarose gels were stained with ethidium bromide, and the results were recorded photographically. DNA fragments were transferred onto N^+ Hybond membrane (Amersham Biosciences, Little Chalfont, UK), and the DNA was fixed to the membrane by UV illumination. The IS*6110* probe was a 245-bp DNA fragment amplified by PCR as described previously.[25] The 15-mer oligonucleotide $(CGG)_5$ was synthesized by Nippn Techno Cluster, Inc., Tokyo, Japan. The probes were labelled with horseradish peroxidase by the ECL Direct™ System (Amersham Biosciences). Hybridization was conducted with the ECL Direct™ System, according to the recommendations of the manufacturer. Autoradiographs were obtained by exposing the membranes to X-ray film.

For IS*1245*-[27] and IS*1311*-RFLP[28] of *M. avium*, DNA was digested overnight with *Pvu*II. The IS*1245* and IS*1311* probes were 427 and 200-bp DNA fragments, respectively, and were amplified by

PCR as described previously.[27,28] Briefly, the oligonucleotides for IS*1245*, 5′-GCCGCCGAAACGATCTAC-3′ and 5′-AGGTGGCGTCGAGGAAGAC-3′,[27] and for IS*1311*, 5′-GTCGGGTTGGGCGAAGAT-3′ and 5′-GTGCAGCTGGTGATCTCTGA-3′,[28] were used to amplify the fragments prepared from purified chromosomal DNA from *M. avium* ATCC 25291 by PCR.

Analysis

Fingerprinting patterns of *M. tuberculosis* or *M. avium* were analysed with Molecular Analyst Fingerprinting Plus Software, version 1.6 (Bio-Rad Laboratories, Inc., Hercules, CA). To facilitate comparison of the fingerprinting patterns, normalization was performed relative to the molecular-weight markers. Each dendrogram was calculated according to the unweighted-pair group method with average linkage according to the supplier's instructions.

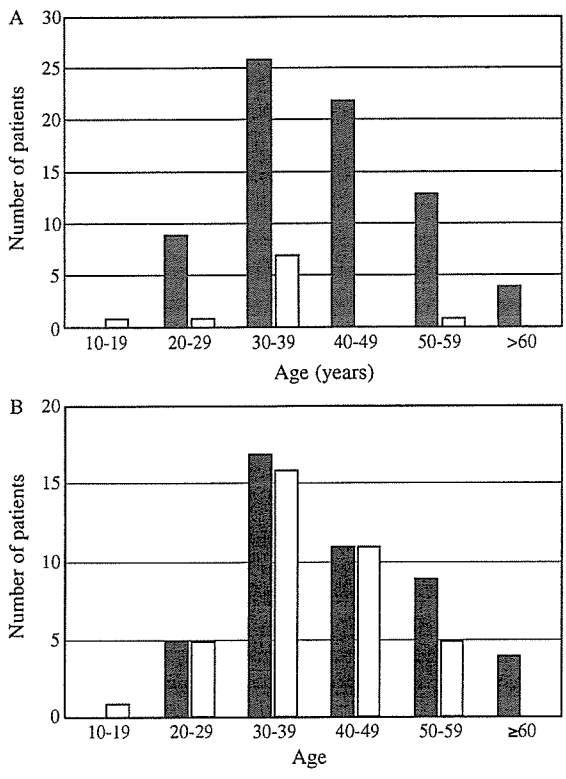

Figure 1 Distribution of 84 mycobacterial infections in HIV-seropositive patients. Panel A: age (years) and sex distribution. Filled bars, male; open bars, female. Panel B: age (years) and pathogenic agent distribution. Filled bars, tuberculosis patients; open bars, non-tuberculous mycobacterial-infected patients.

Results

Mycobacterial infection in HIV-seropositive patients

From 86 HIV-seropositive patients, 48 (55.8%) *M. tuberculosis*, 36 (41.9%) *M. avium*, and 2 (2.3%) *Mycobacterium chelonae* isolates were identified (Table 1).

Nationality and sex are also listed in Table 1. Mean age was 40.5±12.2 years, ranging from 11 to 68 years. Most mycobacteria and HIV coinfected patients were aged 30-39 years (Fig. 1). The most frequent route of HIV infection was sexual transmission (90%); other routes were infection by blood products (5%), drug abuse (5%), mother-to-child infection (1%) and unknown (1%). With respect to mycobacterial infection, 48 and three individuals had primary and recurrent infection, respectively. There was no corresponding record for the remaining patients.

Profile of HIV-seropositive patients with *M. tuberculosis*

In 46 of the 48 tubeculosis patients, the ratio of males/females was 43/3 (Table 1). Mean age was 42.7±11.9 years, ranging from 22 to 68 years. Twenty-five patients had combined pulmonary and extra-pulmonary infection, mainly due to miliary tuberculosis. A total of 56.3% of the 48 patients had pulmonary tuberculosis, as evidenced by positive microscopy smears. Peripheral blood $CD4^+$ cell counts at the time of TB diagnosis ranged from 6 to 331/mm^3, and the median was 62/mm^3.

According to drug sensitivity testing, 43 isolates (89.6%) were sensitive to anti-tubercular drugs, 3 (6.3%) were resistant to a single drug, and 2 (4.2%) were resistant to 2 and 5 drugs, respectively.

In 87 HIV-seronegative TB patients with tuberculosis, 82 were Japanese and five were non-Japanese. The ratio of males/females was 56/31. Mean age was 53.3±20.5 years (56.1±19.0 years for males and 48.6±22.0 years for females), ranging from 18 to 95 years (18-90 for males and 18-95 for females) and patients over 40 years of age accounted for 66.7% of the total. According to drug sensitivity testing, 75 isolates (86.2%) were sensitive to anti-tubercular drugs, 6 (6.9%) were resistant to a single drug, and 6 (6.9%) were resistant to 2 and 6 drugs, respectively.

RFLP analysis of *M. tuberculosis*

To determine whether specific strain(s) of tubercular bacilli prevail among HIV-seropositive patients in Japan, we analysed DNA fingerprints of the isolates by RFLP analysis. Thirty-three of the 48 *M. tuberculosis* clinical isolates were analysed by RFLP, and the patterns are shown in Fig. 2.

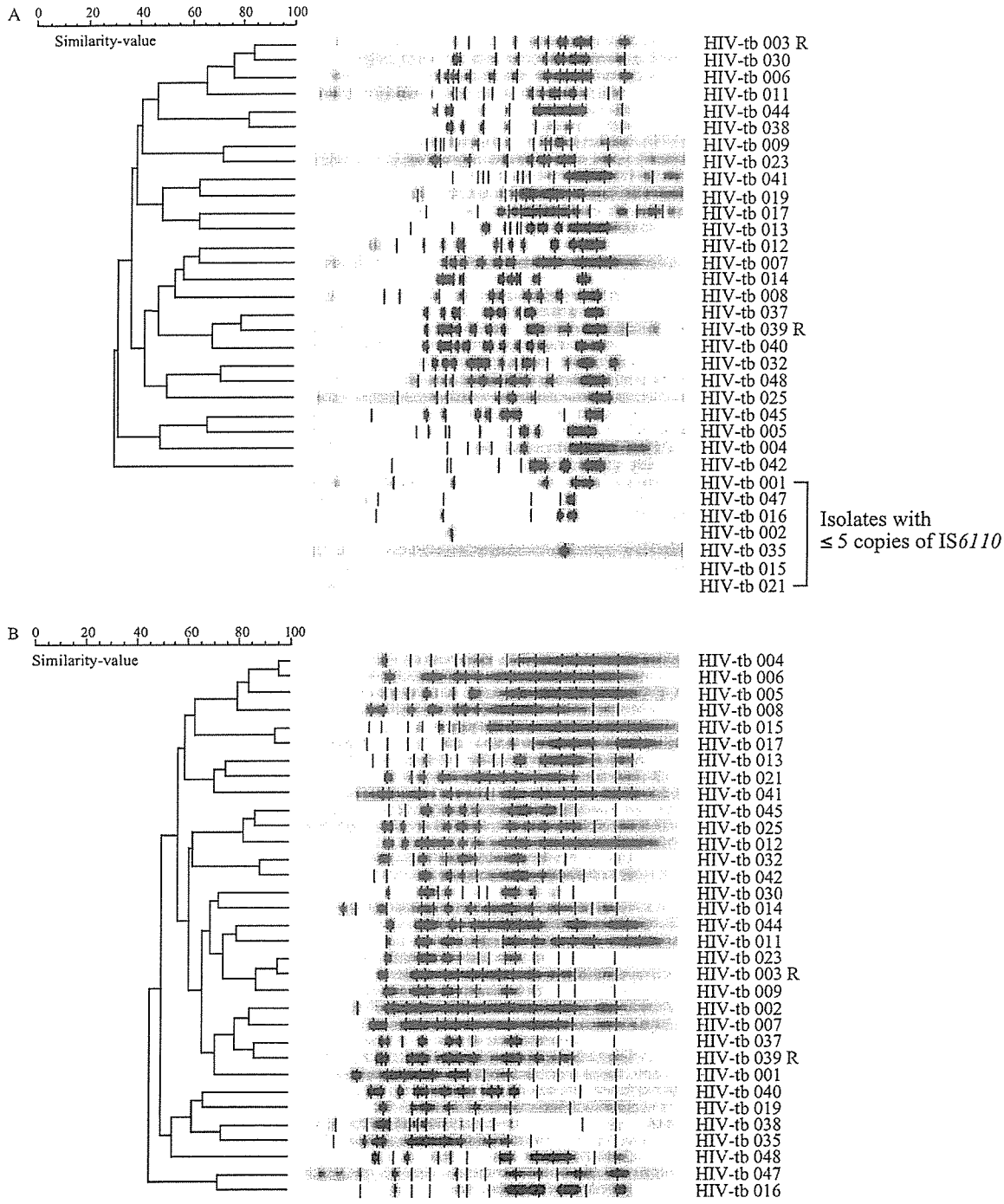

Figure 2 IS*6110*- and $(CGG)_5$-probed DNA fingerprinting patterns of *M. tuberculosis* clinical isolates from HIV-seropositive patients and corresponding dendrograms. The fingerprint patterns are ordered by similarity. The corresponding dendrograms are to the left of the patterns. The position of each IS*6110* (A) or $(CGG)_5$ (B) band is normalized so that the patterns for all strains are comparable. In the IS*6110*-probed DNA fingerprint patterns, isolates with five or fewer copies are indicated in Panel A. The isolates are named as follows: a prefix of 'HIV-tb' indicates an HIV-seropositive patient-derived isolate, and a suffix of 'R' indicates a drug-resistant isolate. For example, HIV-tb 003 R is an HIV-seropositive patient-derived isolate.

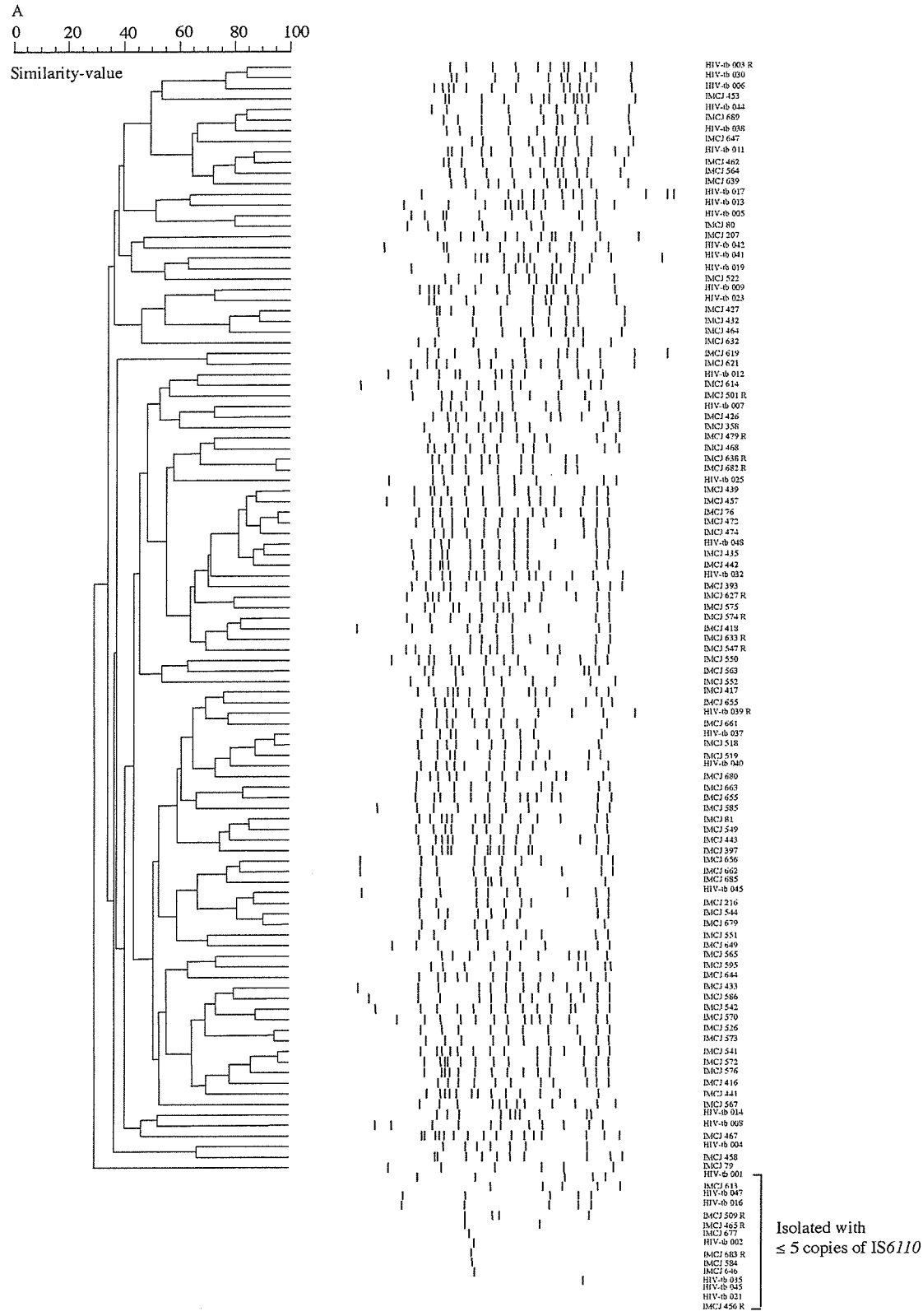

Figure 3. IS*6110*- and $(CGG)_5$-probed DNA fingerprinting patterns of *M. tuberculosis* clinical isolates from HIV-seropositive and HIV-seronegative patients and corresponding dendrograms. The fingerprint patterns are ordered by similarity. The corresponding dendrograms are to the left of the patterns. The position of each IS*6110* (A) or $(CGG)_5$ (B) band is normalized so that the patterns for all strains are comparable. In the IS*6110*-probed DNA fingerprint patterns, isolates with five or fewer copies are indicated in Panel A. The isolates are named as follows: a prefix of 'HIV-tb' indicates an HIV-seropositive patient-derived isolate, a prefix of 'IMCJ' indicates an HIV-seronegative patient-derived isolate, and a suffix of 'R' indicates a drug-resistant isolate. For example, IMCJ 627 R is an HIV-seronegative patient-derived isolate.

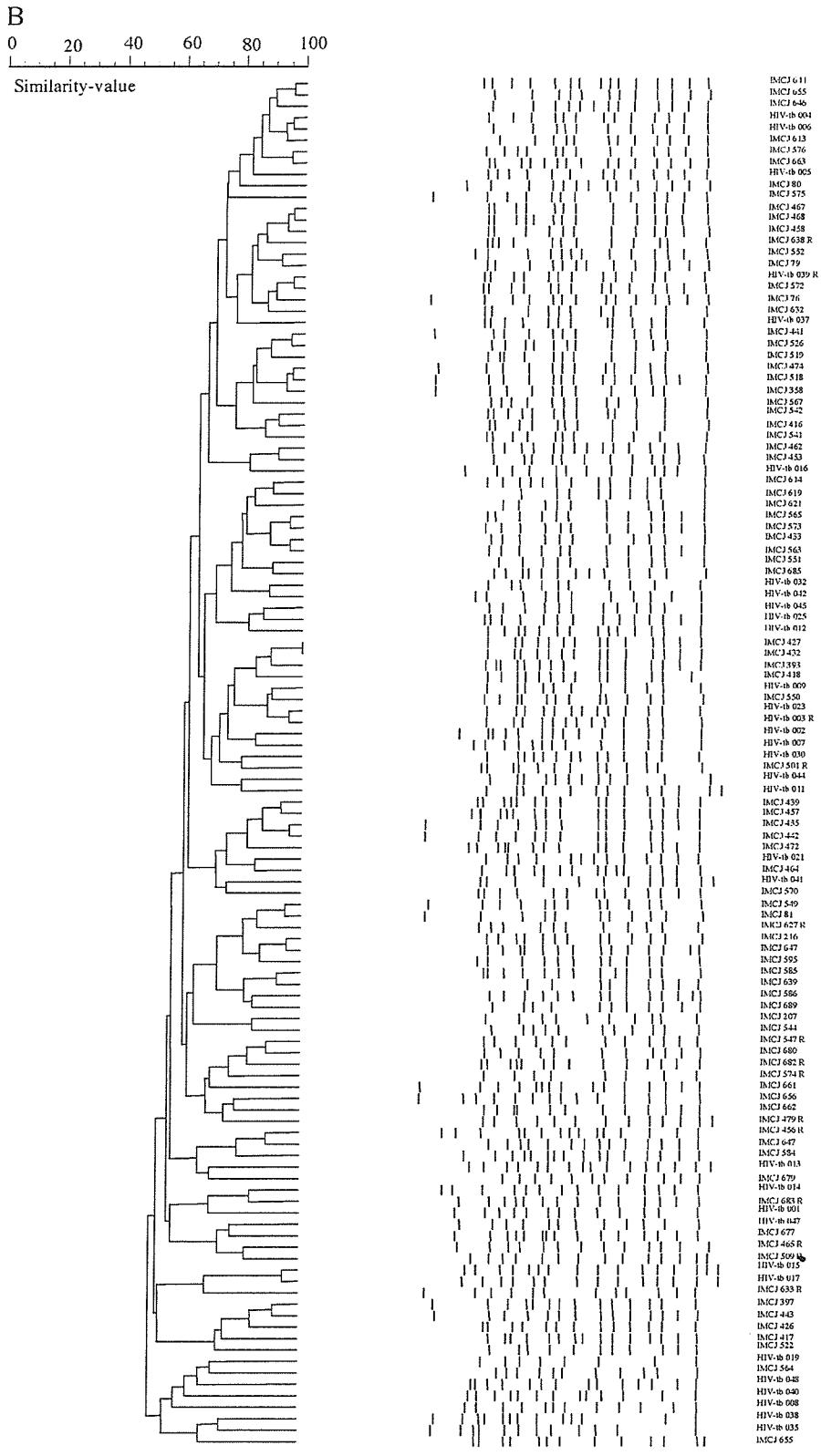

Figure 3 (*continued*)

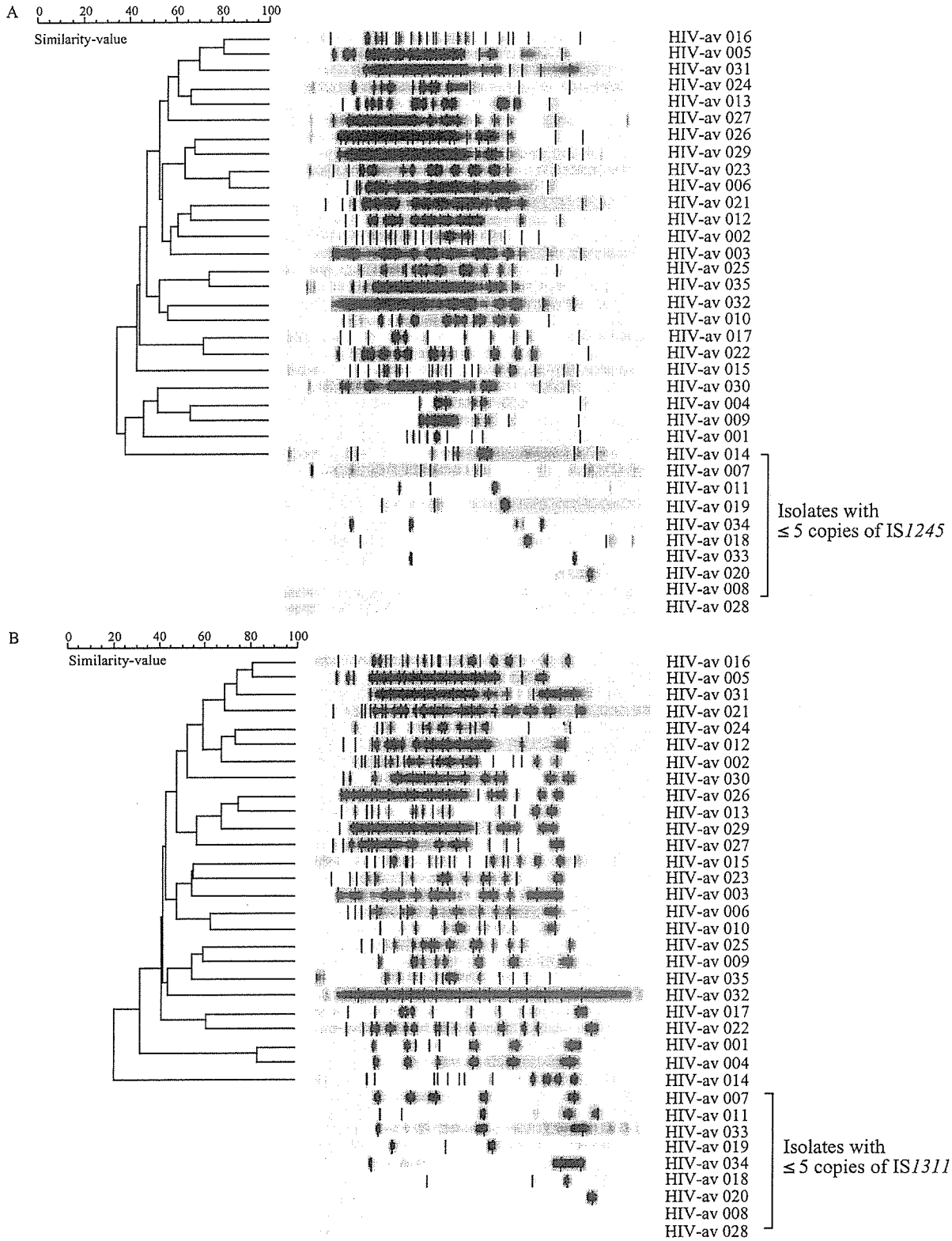

Figure 4 IS*1245*- and IS*1311*-probed DNA fingerprinting patterns of *M. avium* clinical isolates from HIV-seropositive patients in Japan and corresponding dendrograms. The fingerprint patterns are ordered by similarity. The corresponding dendrograms are to the left of the patterns. The position of each IS*1245* (A) or IS*1311* (B) band is normalized so that the patterns for all strains are comparable. In both the IS*1245*- and IS*1311*-probed DNA fingerprint patterns, isolates with five or fewer copies are indicated. The isolates are named as follows: a prefix of 'HIV-av' indicates an HIV-seropositive patient-derived isolate.

The number of IS*6110* bands in the isolates ranged from 0 to 15 (Fig. 2A). Thirty-one different IS*6110* fingerprinting patterns were observed in the isolates. Seven isolates (21.2%) showed 0-5 IS*6110* bands, these isolates were insufficient in band number for cluster analysis. Identical patterns were not found among the isolates (Fig. 2A).

The number of $(CGG)_5$ bands of the copy isolates ranged from 8 to 16 (Fig. 2B). Thirty-three different $(CGG)_5$ fingerprinting patterns were observed in the isolates. Identical patterns were not found among the isolates (Fig. 2B). Three pairs of isolates (HIV-tb 004 and HIV-tb 006, HIV-tb 015 and HIV-tb 017 and HIV-tb 03 and HIV-tb 023) were closely related, with 90% or more similarity. However, the corresponding patients did not show any linkage such as hospital or date of sample isolation.

Comparison of RFLP patterns between HIV-seropositive and -seronegative TB patients

To assess whether the same kinds of mycobacteria prevail in HIV-seropositive and -seronegative patients, RFLP patterns of *M. tuberculosis* clinical isolates were investigated in both groups. In IS*6110*- or $(CGG)_5$-patterns from both groups, the patterns from both HIV-seropositive and -seronegative patients did not consist of apparent clusters and appeared to segregate randomly in the dendrograms (Fig. 3).

Profile of HIV-seropositive patients with *M. avium* infection

The number of HIV-seropositive patients with *M. avium* infection was 36 (Table 1). Mean age was 37.4 ± 9.9 years, ranging from 11 to 56 years. Most of the *M. avium*-infected patients (58.3%) suffered from disseminated infection, and the sputa of 88.9% patients were culture-positive but smear-negative upon preliminary mycobacterial examination. Almost all the *M. avium* isolates were resistant to all anti-TB drugs. Peripheral blood $CD4^+$ cell counts of 34 patients (unknown: 2) at the time of *M. avium* diagnosis ranged from 0 to 202/mm^3, and the mean $CD4^+$ cell count was 38.6 ± 60.4/mm^3. In 26 of 34 patients (76.4%), the $CD4^+$ cell counts were less than 50/mm^3.

RFLP analysis of *M. avium*

The RFLP patterns of 35 of 36 *M. avium* isolates were investigated (Fig. 4). The number of IS*1245*- and IS*1311*-bands ranged from 0 to 25 and from 0 to 23, respectively, and analysis showed 33 different patterns of each. Nine isolates (25.7%) showed 0-5 bands; these isolates were insufficient for cluster analysis because of few numbers of IS*1245* or IS*1311* bands. Among the isolates, identical patterns were not found. Cluster analysis revealed no clusters. These results indicate that no particular strain of *M. avium* prevailed among HIV-seropositive patients.

Discussion

We analysed mycobacterial isolates obtained from HIV-seropositive patients and found that *M. tuberculosis* and *M. avium* accounted for a large proportion of HIV-associated mycobacterial infection in Japan. Although *Mycobacterium kansasii* is also known to be associated with AIDS,[29,30] it was not isolated in this study. Two isolates of *M. chelonae* were obtained from stool specimens of patients.

It has been suggested that recurrent TB is responsible for most cases of HIV-associated TB, particularly in countries with high-level of transmission.[31] Kanazawa et al.[32] reported that the majority of HIV-positive Japanese patients with TB (83%) were more than 40 years of age and had recurrent TB. In the present study, the age of HIV-seropositive patients shifted to the 30s, suggesting that TB incidence among HIV-positive patients in Japan is transforming from recurrence in older persons to primary infection in younger persons.

With respect to drug resistance, 10.4% of the strains obtained from HIV-seropositive patients showed resistance to one or more anti-TB drugs. Abe et al.[33] reported that 10.3% of *M. tuberculosis* isolates from patients in Japan were resistant to one or more of the four first-line anti-TB drugs: isoniazid, rifampin, streptomycin and ethambutol. A 1996 report noted that the drug resistance rate in New York City was 33%.[34]

We found that both the IS*6110* and $(CGG)_5$ fingerprinting patterns of *M. tuberculosis* isolates from HIV-seropositive patients in Japan differed from those of a TB outbreak in New York City[12,18] and of isolates from the patients in Lima, Peru.[16] Comparing RFLP patterns of *M. tuberculosis* isolates from HIV-seropositive patients with those from HIV-seronegative patients, we found that the DNA fingerprints did not distinguish between these two TB patient groups. These data indicate that TB transmission in Japan occurs via HIV-seronegative TB patients rather than via HIV-seropositive TB patients. The epidemiological studies in Botswana[17] and Tanzania[19] showed no clustering any particular

pattern of DNA fingerprints. These findings are consistent with our present results.

Patients infected with *M. avium* suffer from chronic lung disease. In patients with HIV-associated *M. avium* infection, it is thought that pulmonary symptoms will develop when $CD4^+$ lymphocyte counts fall below $100/mm^3$. The median $CD4^+$ lymphocyte count at *M. avium* diagnosis was $10/mm^3$, and at that time the majority of patients showed disseminated *M. avium* infection. Almost all *M. avium*-infected patients in the present study were in advanced stages of AIDS. *M. avium* organisms can be isolated from environmental sources such as water or soil.[35-37] Because they are capable of causing infection in animals, e.g. birds and pigs, it has been postulated that the source of human infection is either the environment or from animals. Ichiyama et al.[38] searched sources of soil, water and dust in Japan and found *M. avium* isolates in 68.0% of dust samples tested. It is believed that the most frequent mode of *M. avium* infection in humans occurs by inhalation or by deglutition of the agent from environmental sources.[37,39,40] To prevent infection with this agent in HIV-seropositive patients, further studies are needed to identify original sources and to further elucidate infectious routes.

In conclusion, the number of HIV patients in Japan is increasing; according to the latest report,[7] the number is over 10 000. The number of TB patients in Japan remains higher than in other developed countries.[41] However, the number of HIV-infected patients with mycobacterial infection in Japan is limited. With respect to TB, no outbreak among HIV-seropositive patients was found. Further monitoring of mycobacterial infection associated with HIV infection in Japan should be continued.

Acknowledgements

The paper is dedicated to Iwao Ojima, the leader of the International Projects on Anti-Tuberculosis Drug Discovery, in honor of his 60th Birthday, June 5, 2005. We thank M. Nakano (Jichi Medical School, Tochigi Prefecture, Japan) for comments on the manuscript. This study was supported by the Health Sciences Research Grants from the Ministry of Health, Labour and Welfare and by a grant from the Research on Health Sciences focusing on Drug Innovation (KH11008) from the Japan Health Sciences Foundation.

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Development of a nosocomial outbreak investigation database

K. Makimoto[a,*], N. Ashida[b], N. Qureshi[b], T. Tsuchida[a], A. Sekikawa[c]

[a]*Osaka University, Graduate School of Medicine, Division of Nursing, 1-7 Yamadaoka, Suita, Osaka, 565-087,1 Japan*
[b]*Osaka University, Graduate School of Medicine, Division of Medical Informatics, Japan*
[c]*School of Public Health, University of Pittsburgh, USA*

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KEYWORDS
Outbreak;
Investigation;
Epidemiology;
Internet; Database

Summary A pilot web-based database was created to facilitate epidemiological investigation of nosocomial outbreaks. The database provides highly structured abstracts in a case study format to serve as a guide for investigations. Problems encountered in abstracting over 330 published reports included missing information and classification of study methods. The database offers a new way to review outbreaks, for example, in terms of their impact measured by various combinations of database fields, such as the number of cases, attack rate, pathogens, service/ward and mode of transmission. Feedback from users of the database suggests its usefulness. Creation of a large web-based database seems to be both desirable and feasible.

Introduction

A literature search plays an important role when initial investigations have failed to identify the source of an outbreak. Only a few relevant databases are available for reference searches. One is PubMed, a web-based database offering references and abstracts. The other is a commercially available database[1] containing summaries of >3000 reports on outbreaks. Although these databases help to pinpoint articles of interest, obtaining the articles is difficult for hospitals with limited resources.

To address this issue, we have developed a pilot outbreak database that provides highly structured abstracts with detailed investigation procedures and control strategies. The purpose of this study was to examine the feasibility of a large-scale database and to identify problems associated with the development of such a database.

* Corresponding author. Tel./fax: +81-6-6879-2541.
E-mail address: makimoto@sahs.med.osaka-u.ac.jp

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Methods

Medline is a major source of articles on epidemiological investigations of nosocomial infection outbreaks. Articles written in English were selected according to the following keywords: nosocomial, hospital, infection, outbreak and epidemic. Abstracts were used to find relevant outbreak investigations. Articles that dealt exclusively with molecular epidemiology were excluded.

A standardized data entry spreadsheet was developed. This comprised four categories with 22 fields: (1) information regarding publication; (2) descriptions of outbreaks; (3) methods; and (4) results and discussion/control strategies. Table I displays the fields and their definitions.

Web-based database

A homepage was created for a web-based database search. The homepage presents study background, instructions for use, and search menus. Search capability features include: (1) pathogens; (2) infection sites; (3) modes of transmission; (4) types of investigation; and (5) ward/service. The URL for the outbreak investigation

Table I Categories and field definitions for the database

Categories	Fields	Contents/definitions
Journal information	Author	
	Title	
	Journal	
	Year published	
Description of outbreak	Pathogen	Pathogens identified as a cause of outbreak
	Duration of outbreak	
	Dates of investigation	
	Country	
	No. of beds	
	Hospital	Name of the hospital and affiliation (university hospital, community hospital, etc.)
	Ward/unit	NICU, ICU, transplant, surgical, internal, etc.
	Type of investigation[a]	Basic epidemiological investigation: investigation of cases only (case report, case series, etc.). Major epidemiological investigation: case-control, matched or unmatched, case definition and control definition; cohort
	Source of infection/mode of transmission	Airborne, droplet, contact, foodborne, waterborne, carrier, contamination, pseudo-epidemic, other (specify)
	Infection sites/specimens	Infection sites: surgical-site infections/surgical wound infections; bloodsteam infections; bacteraemia; pneumonia/ventilator-associated pneumonia; urinary tract infections; other (specify)
		Specimen obtained: blood, urine, sputum, tracheal aspirate, stool, other (specify)
	No. infected/attack rate	Attack rate=number of infected/number of exposed
	No. of deaths/ case fatality rate	Case fatality rate=number of deaths/number of infected
Methods	Case definition/selection	Definite and probable case, provisional and confirmed case
	Control definition/selection	For case-control study
	Investigation procedure	Identification and description of the outbreak, case finding methods, and details of investigation methods
	Results	Results of investigation and/or major findings
Results/discussion	Discussions/control strategies	Implications of the findings and outbreak control strategies

NICU, neonatal intensive care unit; ICU, intensive care unit.
[a] Classification based on Dixon RE. In: Bennett JV, Brackman PS, editors. *Hospital infections*, 3rd ed. Boston: Little Brown and Company; 1992. p. 109-133.

database search is: http://www.health-db.net/infection/index.asp

Results

The Medline search yielded >600 articles published between 1970 and 2000, 330 of which dealt with epidemiological investigations and were abstracted. Only a small number covered all the fields specified for the database. A few outbreaks due to non-infectious origin were included, such as acute onset of diminished vision and hearing in dialysis patients,[2] as were pseudo-outbreaks.

Information regarding facilities

Most reports were from the USA (49%), followed by the UK (14%) and European countries such as Ireland, France and the Netherlands. Countries where English is the first language accounted for 72% of the reports. Most articles included the name of the facility, but only one-third of articles mentioned the number of hospital beds. The mean number of beds was 638 (range 3-3500), with a median of 510. Four articles reported outbreaks involving multiple facilities.

Descriptions of outbreaks

Case definition

With the exception of case-control studies, most articles did not include a case definition. The other exception was investigations of *Legionella pneumophila* outbreaks, which tended to have detailed case definitions because of the difficulties of differential diagnosis. These investigations classified cases into either definite or probable cases.[3,4] Only one study presented both a provisional and a confirmed case definition (an investigation of a scalded skin syndrome outbreak).[5]

Number of cases, attack rates, number of deaths, and case fatality rates

The smallest number of cases reported was one[6,7] and the largest was 635.[8] The single case involved *Legionella pneumophila* which was isolated from a patient and an ice machine.[6] The largest outbreak was caused by a Norwalk-like virus that primarily affected healthcare workers in a tertiary-care centre in Toronto, Canada.[8] Some articles reported the number of deaths and whether deaths were related to the outbreak.[9]

Study methods

It was difficult to distinguish between basic and major epidemiological studies. Some reports indicated the extensiveness of the investigation although they did not use the case-control approach.[10,11] Thus, the differentiation of basic and major epidemiological investigation was omitted, and only the classification of studies into case-control and cohort studies was entered into the database.

Results and discussions/control strategies

Most of the articles gave detailed information on the process of investigation and effectiveness of control and prevention strategies. Several articles reflected the increasing complexity of investigations in recent years due to reduced length of stay and increased intrahospital, interhospital and international transfers.

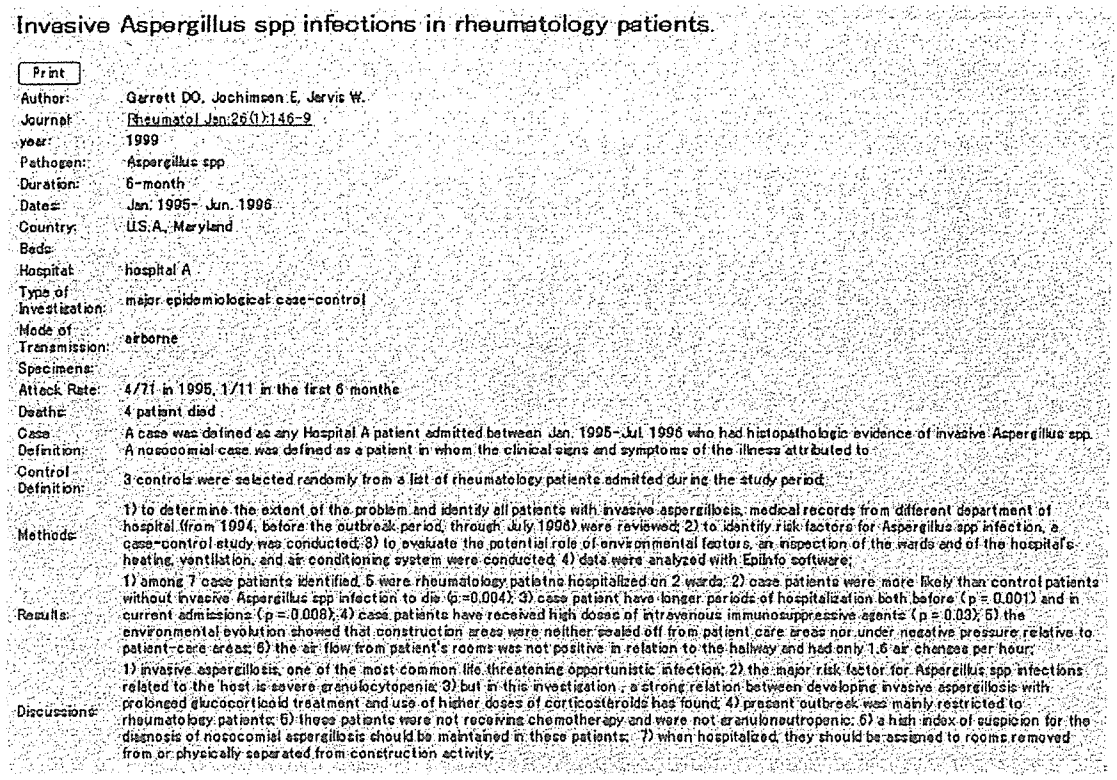
Setting up a homepage

A homepage was created for the database search. Figure 1 shows one of the abstracted data sets. This homepage was submitted to a few list servers including the Hospital Infection Society and the Society for Epidemiologic Research.

Discussion

This database was developed to aid epidemiological investigations by providing instructions in a structured case study format. Database search output presents essential information such that even novice investigators can initiate investigations before they familiarize themselves with the literature. If a similar outbreak is found in the database, it shows how to detect cases and the variables that need to be examined in order to identify common exposures. The database is especially useful for planning case-control studies because it provides case definitions and information on the difficult topic of control selection.

The other feature of the database is provision of indicators used to measure the impact of outbreaks, i.e. number of cases, attack rates, number of deaths, and case fatality rates. These are often missing from reports. Our database offers flexible ways to review the literature in various combinations of infection site, mode of transmission, size



Invasive Aspergillus spp infections in rheumatology patients.

Print

Author:	Garrett DO, Jochimsen E, Jarvis W.
Journal:	Rheumatol Jan;26(1):146-9
year:	1999
Pathogen:	Aspergillus spp
Duration:	6-month
Dates:	Jan. 1995- Jun. 1996
Country:	U.S.A., Maryland
Beds:	
Hospital:	hospital A
Type of Investigation:	major epidemiological case-control
Mode of Transmission:	airborne
Specimens:	
Attack Rate:	4/71 in 1995, 1/11 in the first 6 months
Deaths:	4 patient died
Case Definition:	A case was defined as any Hospital A patient admitted between Jan. 1995-Jul. 1996 who had histopathologic evidence of invasive Aspergillus spp. A nosocomial case was defined as a patient in whom the clinical signs and symptoms of the illness attributed to
Control Definition:	3 controls were selected randomly from a list of rheumatology patients admitted during the study period.
Methods:	1) to determine the extent of the problem and identify all patients with invasive aspergillosis, medical records from different department of hospital (from 1994, before the outbreak period, through July 1996) were reviewed; 2) to identify risk factors for Aspergillus spp infection, a case-control study was conducted; 3) to evaluate the potential role of environmental factors, an inspection of the wards and of the hospital's heating, ventilation, and air conditioning system were conducted; 4) data were analyzed with EpiInfo software;
Results:	1) among 7 case patients identified, 5 were rheumatology patients hospitalized on 2 wards; 2) case patients were more likely than control patients without invasive Aspergillus spp infection to die (p=0.004); 3) case patient have longer periods of hospitalization both before (p = 0.001) and in current admissions (p = 0.008); 4) case patients have received high doses of intravenous immunosuppressive agents (p = 0.03); 5) the environmental evolution showed that construction areas were neither sealed off from patient care areas nor under negative pressure relative to patient-care areas; 6) the air flow from patient's rooms was not positive in relation to the hallway and had only 1.6 air changes per hour;
Discussions:	1) invasive aspergillosis, one of the most common life threatening opportunistic infection; 2) the major risk factor for Aspergillus spp infections related to the host is severe granulocytopenia; 3) but in this investigation , a strong relation between developing invasive aspergillosis with prolonged glucocorticoid treatment and use of higher doses of corticosteroids has found; 4) present outbreak was mainly restricted to rheumatology patients; 5) these patients were not receiving chemotherapy and were not granulocytopenic; 6) a high index of suspicion for the diagnosis of nosocomial aspergillosis should be maintained in these patients; 7) when hospitalized, they should be assigned to rooms removed from or physically separated from construction activity;

Figure 1 Search output screen for case-control study.

of the facility, and/or type of services. We hope that it will help to establish a standardized reporting format for outbreak investigations, leading to improvements in the quality of reports.

The inclusion of pseudo-outbreaks was thought to be helpful for differentiating pseudo- from real outbreaks, and for learning how to examine the former. This could help to identify problems with quality control in laboratories and with specimen handling.

Our database focuses on epidemiological methods, so reports dealing exclusively with molecular epidemiology were excluded. Although molecular epidemiology has made great advances in identifying outbreak pathogens, epidemiological investigations remain essential to determine the mode of transmission and to identify prevention strategies.

Our compilation of outbreak reports is limited to journals published in English. We are planning to translate the database for non-English-speaking health workers (such as many of those in Japan). The database could also be integrated into a training programme for infection control staff.

One suggestion we received was to create a central depository like the Cochrane Library to report investigations. If international collaboration could be attained, expansion of the database would be easier, although abstracting articles is highly labour intensive. Most of the feedback we received indicated that the database was useful for infection control professionals, even those with easy access to a good library, because our information was considered to be well organized and concise.

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A development of an efficient information collecting and retrieval system using an Agent Technology –for infectious disease-

Takemura T, Ashida N, Makimoto K, Kirikae T, Okamoto K, Kuroda T, Nagase K, and Yoshihara H
Department of Medical Informatics, Kyoto University Hospital,
Department of Medical and Welfare, Koshien University,
School of Allied Health Sciences, Osaka University,
Research Institute, International Medical Center of Japan

I. Introduction

At present, a lot of information exists on World Wide Web (WWW) because of spreading of the Internet. These information is, naturally, useful or not a users has an information need. It is very difficult that we judge information that we search is most effective on WWW and we always catch up the newest information if we find a web site including effective information. Moreover, we can not prospect of information retrieval system's updating and providing newest information, even if we can check the useful web site every day.

However, a method of using software agent system is proposed to these problems. The agent is a program that performs some information gathering or processing task autonomously. The internet agent is just as "agent", collect, retrieve, check objective information and so on. The Internet agent has to have agentive, autonomous and adaptive, but there is no internet agent systems that has all specification sufficiently, and multi-purpose agent system hasn't shown up yet.

However, we can consider that multi-purpose agent is not only easy-to-use. If an agent system have gather and check up some web sites decided, the agent system will be very useful.

By the way, infection disease information is very important to prevent the spread of disease. This information includes epidemiological, pathogenic, serum and so on, and it must be provided public as knowledge base that many people can use. Especially, it is desirable that physician, nurse or person that devises a countermeasure of infection disease can read it on time. The internet shows a big effect in this case as well and Infectious disease surveillance center, National Institute of Infectious Diseases in Japan provides a lot of infection disease information in the country level. Moreover prefectures health care center also provide the information in prefectures and smaller level, and it is using the infection disease prevention and the strategy of the control.

However, these sites hold and update information individually, so persons who want to get the information of infection disease have to check and search the every site. Therefore, if the internet agent system works as their "agent", it is very effective for persons who want the information.

In Japan, the research of "a development of hospital infection prevention network" has been conducted to reduction to infection disease information scattering.

In this paper, we developed the collect and retrieval system using an agent technology for the infection disease information efficiently.

Concretely, user can get the infection disease information from keyword retrieval and update page list based on several agents. The agents have three functions mainly as follows.

1. An agent judges information about the infection disease or not automatically.
2. It is as early as possible to catch up the updating information, so collected data make always update.
3. Automatic downloading.

II. Methods

1. The characteristics of infection disease information on WWW

The infectious disease information on WWW is provided by National Institute of Infectious Diseases, Ministry of Health, Labor and Welfare, prefectures health center, division of Hospital Infection control team and so on, as administrative service, or large hospital provide information of method of measures infection. Several web sites have other issue different from infectious disease. And it is difficult that user grasp the update information because almost every site is very large-scale.

Therefore we consider that we list several web sites is judged useful for infectious disease and we have to deal with the information relates to it in these sites.

Figure 1 shows general structure of web sites.

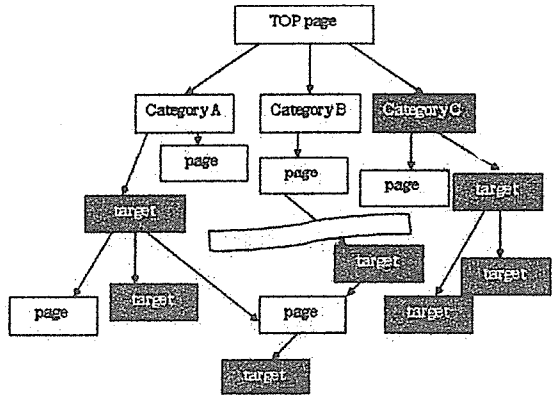

Figure 1. web site structure

2. Agent framework

The basic concept of the agent in this research is three agents work in interaction. One agent is "download agent". This agent's main task is downloading from objective pages according to new URL that given other agents. Second agent is "search agent". This agent rounds web sites according to URL list and confirms the page is updated or checked new pages. Third agent is "judgment agent". This agent judges the web page that "download agent" get, is infection disease page or not. This concept is shown in figure 2.

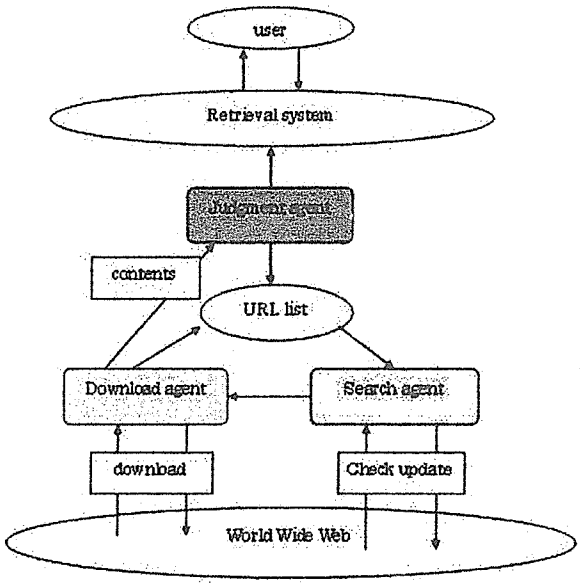

Figure 2. a concept of agent framework

3. Automatic judgment of "judgment agent"

In this concept, it is necessary that the judgment agent judge whether a download page related to infectious disease or not. This judgment ability is very important that if the agent include too mach download pages, the gathering information contains a lot of noises, on the contrary if it underestimate, useful information falls off.

We adopted the technique of Support Vector Machine (SVM) on this time, though there were various methods to judge the contents of a certain document mechanically. SVM is one of the pattern recognition techniques and is expanded by the method which is called a kernel trick is used as the mathematical technique to construct the distinction function of the non-line form. So SVM is one of the best learning models in other many recognition techniques well known. SVM is, to put briefly, a learning method to distinguish two classes. If many objects which have each attributes (attribute A and attribute B) can be expressed by a vector as some amounts of characteristics and mapped on the vector field, SVM choices the nearest elements in two attribute space, and draws a boundary line so that a distance from that both may be the longest. This boundary line makes divide any elements into either. Conceptual figure shows as follows.

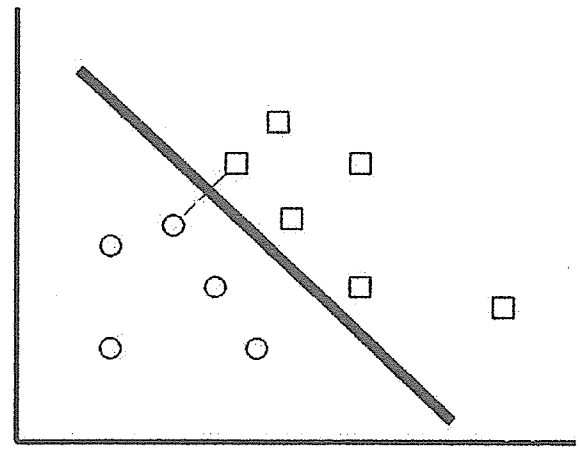

Figure 3. Support Vector Machine

On this time, we made SVM learn medical document or not from the point of medical terminology of which the documents consisted, and SVM judges an objective document whether infections or not with the knowledge. And the knowledge has constructed continuously by learning correct answers judged by human, the data which the download agent corrects, to put it concretely, are judged by us, the judgment agent improve the precision of judgment because the it learn the data with SVM.

III. Result and Discussion

We developed the system that can collect and judgment the only infection information with an agent technology. This system can collect all web sites which contain infection data without omission, and we can efficiently use the information which is scatted and loss on WWW.