FIG. 3. Percentage of biovolumes represented by filamentous cells within various *L. pneumophila* Knoxville-1 cultures. Black bars represent growth at 25°C, white bars represent growth at 37°C, and gray bars represent growth at 42°C. The arrow indicates the point at which detachment of biofilms at 37°C and 42°C occurred. Double brackets indicate days when only 25°C biofilms were assessed. Values are presented as means ± SD of triplicate data. Statistical analysis was performed using Student's *t* test to compare the differences between groups, and *P* values of <0.05 were considered statistically significant.

enhanced ability by *L. pneumophila* to form biofilms on glass, PS, and possibly other surfaces would likely increase its chance of association with humans. On the other hand, the fact that biofilm formation is favored on PP at 25°C also suggests that the competitive edge conferred may be dependent on a combination of the temperature and the type of attachment surfaces.

It is interesting to note that the properties of *L. pneumophila* biofilms formed at 25°C differ considerably from those formed
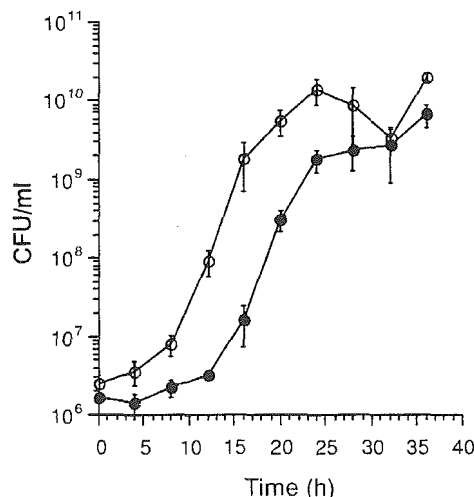

FIG. 4. Growth of *L. pneumophila* Knoxville-1 in BYE. The culture was shaken at 37°C and seeded from filamentous cells of a 37°C biofilm at day 4 (open circles) and rod-shaped planktonic cells of a 37°C culture shaken for 30 h (black circles). Data are means ± SD from three independent experiments.

at 37°C and 42°C. At higher temperatures, the biofilms are formed faster, thicker, and spread wider. Most interestingly, at higher temperatures, they are mycelial mat like in structure. Bacterial biofilms reported to date commonly show structures of polymeric matrices interspersed with water channels (32). For rod-shaped bacteria, e.g., *Pseudomonas* spp. and *Vibrio cholerae*, cells within biofilms remain rod shaped (60), with only a rare appearance of filaments (38). In rivers and on riverbeds, mixed-community biofilms may have filamentous components (bacteria or fungi) among rods and cocci (35), but biofilms composed primarily of extensive meshwork of filaments, to our knowledge, have only been reported for *Thiothrix* spp. in cases of biofouling (6) and *Methanosaeta* spp. in anaerobic sludge granules (29). Furthermore, although filamentation in rod-shaped bacteria with physiological abnormalities has been reported (13, 28, 48, 66), temperature-regulated filamentation in a biofilm context is highly unusual.

One question that may arise with this novel form of biofilm is, can we truly consider it a "biofilm"? The definition given by Donlan and Costerton (16) states that a biofilm is "a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription." For our mycelial mat-like structure, the criteria of attachment is certainly satisfied; furthermore, a thin layer of ruthenium red-stainable substance (possibly exopolysaccharide) (24) coats the filaments (data not shown). The filamentous cells have a growth profile (Fig. 4) that indicates an altered phenotype from the planktonic, rod-shaped form and are expected to have considerable modification in gene expression compared to expression in a binary fission mode. Our current analysis of

the transcriptional profiles of the biofilm filamentous versus planktonic *L. pneumophila* cells also substantiates this point (Z. Piao, unpublished data). Thus, all the criteria seem to have been met for the mycelial mat-like structure to be referred to as a biofilm.

L. pneumophila have long been known to be pleomorphic (31), varying its morphology with physiological conditions (36, 43) or infectious cycle phases (61). Species of *Legionella* have also been reported to form varied microcolonies within Vero cells, some appearing filamentous, although the septation status has not been addressed (44). *L. pneumophila* also "differentiates" into a few extracellular or intracellular forms with varying degrees of infectivity for HeLa cells (25), which can be distinguished by the ultrastructural properties of their cell envelopes (21). In our study, the electron micrograph of the filamentous *L. pneumophila* cell (Fig. 2G) shows cell wall structure that is consistent with that of the extracellular stationary phase rod forms reported by Faulkner et al. (21), confirming its "extracellular" status.

Extensive filamentation by *L. pneumophila* has previously been described in conditions of physiological stress such as exposure to antibiotics (19, 59) and nutrient limitation (63). In other rod-shaped bacteria, filamentation by mutants of global regulator (28) or cell division genes (13, 48) and when exposed to high salt concentrations (66) has also been reported. We considered, by analogy with the phenotype of an *E. coli* mutant (10), that our filamentation phenomenon could be due to the regulatory action of the *recA* gene. The thermosensitive mutation *recA441* (formerly *tif-1*) of *E. coli* is known to convert cells to multinucleate, aseptate filaments at 40°C through the constitutive expression of the *sulA* gene that encodes an inhibitor of the cell division protein FtsZ (34). However, this possibility was ruled out for *L. pneumophila* because disruption of the *recA* homologue was found to be without effect on the phenotype. Another immediate possibility, suggested by analogy with *E. coli ftsZ* mutants (28, 47), is that *L. pneumophila* FtsZ protein might be thermosensitive. We have not yet been successful in substantiating this idea, but we intend to continue to dissect the mechanism of regulation in this novel form of biofilm. The recently completed *L. pneumophila* genome sequences of strains Philadelphia-1 (12) and Paris (11) (http://genome3.cpmc.columbia.edu/~legion/seq_anno.html) indicate that the organism possesses not only *recA* and *ftsZ* but also homologues of other *E. coli* genes, e.g., *ftsA* and *mreB*, involved in cell division and maintenance of cell morphology (48). We are currently looking into how these genes are regulated and correlated with the biofilm phenotype of *L. pneumophila*.

Filamentation is often a consequence of some stress-induced disruption of normal cell division functions. However, in rare cases, it serves as a strategy to counter specific types of stress. Elongation of cells by nutrient-deprived *Pseudomonas aeruginosa* has been proposed to be a strategic response to enlarge their nutrient collection surface without substantially changing the surface area-to-volume ratio (60). Temperature-regulated filamentation in *L. pneumophila* biofilm is unusual in that it is clearly not a simple case of cell division inhibition by sublethal heat stress, e.g., as seen with *Listeria monocytogenes* (53). Induction of filamentation at 37°C or 42°C was not observed for *L. pneumophila* cells outside of the biofilms or in a shaken liquid medium. Instead, only surface-associated cells, i.e.,

within biofilm or on agar, exhibited filamentation (Fig. 3). Some other signal(s) besides temperature, e.g., attachment to surfaces, is possibly involved, and it is conceivable that filamentation is a strategic regulated response rather than an accidental by-product of stress. Our growth experiment showed that cell proliferation of the biofilm filamentous form is faster at the initial stage than the planktonic rod-shaped form (Fig. 4). During this initial stage, the multinucleate filamentous cells have converted into rod-shaped forms, after which no difference in growth rate could be observed. The filament-turned-rod-shaped cells therefore do not differ in terms of growth rate from the normal rod-shaped cells. Presumably, the time and energy required by a filamentous cell to increase in number involve only the formation of cell septa, whereas binary fission involves DNA replication and biomass generation, in addition to septa formation. Hence, filamentation may represent a novel strategy utilized by *L. pneumophila* to rapidly increase its population.

It is worth noting that the experimental system of our study made use of a rich medium. In low-nutrient medium, very little formation of biofilm could be observed (C. C. Sze, unpublished data). In the environment, the bacterium more often encounters low-nutrient status, so one may wonder at the relevance of this particular study. However, it should not be forgotten that the environment is dynamic and that bacteria generally experience cycles of "feast and famine." The survival of a bacterium, therefore, not only involves living through a famine but also hinges on how well it exploits the rare but valuable encounter of a feast. *L. pneumophila* is a slow grower compared to other bacteria such as *E. coli*, *P. aeruginosa*, and *V. cholerae* and may seem to be in danger of losing out to these fast growers in nature. It has, in part, made up for its shortcomings by finding a niche as an intracellular parasite of free-living protozoans (4, 41, 54, 55). By forming mycelial mat-like biofilms, *L. pneumophila* may have hit upon another strategy to compete with fast growers in the environment, which allows it to (i) anchor itself at a location transiently flooded with nutrients, (ii) increase rapidly in biomass while able to access the nutrients by eliminating the process of septum formation, and then (iii) proliferate and disperse in great numbers when conditions are appropriate. We intend to explore this phenomenon of filamentous biofilms further, from both genetic and ecological points of view.

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Isolation and Identification of *Methylobacterium* Species from the Tap Water in Hospitals in Japan and Their Antibiotic Susceptibility

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Abstract: Contamination of tap water by *Methylobacterium* species has become a serious concern in hospitals. This study was planned to examine the distribution of *Methylobacterium* species inhabiting tap water used in Japanese hospitals and antibiotic sensitivity of the isolates in 2004. Species identification of 58 isolates was performed based on the homology of a partial sequence of 16S rDNA. The dominant *Methylobacterium* species in hospital water were *M. aquaticum* and *M. fujisawaense*. To examine the biochemical properties of these isolates, a carbon source utilization was tested using an API50CH kit. The phenotypic character varied widely, and was not necessarily consistent with the results of phylogenic analysis based on the partial 16S rDNA sequence, suggesting that the biochemical properties are not suitable for identification of *Methylobacterium* species. The isolates were also subjected to antibiotic sensitivity tests. They were resistant to 8 antibiotics, but highly sensitive to imipenem ($MIC_{90}=1$ μg/ml) and tetracycline ($MIC_{90}=8$ μg/ml). These findings concerning the isolates revealed the presence of *Methylobacterium* species with resistance to multiple antibiotics in hospital tap water.

The investigations of the water used for handwashing before surgery have revealed the absence of a significant difference on the effects of handwashing using sterilized water and tap water (5). Consequently, there has been a move to save on the costs for infection control by removing equipment installed for sterilized water and switching to the use of tap water. Although the chlorine sterilization of tap water is mandatory in Japan, *Methylobacterium* species resistant to the residual chlorine of disinfectants widely inhabit tap water piped through water supply facilities such as water tanks of high-rise buildings (7, 11). In 1990, Furuhata et al. (6) isolated *Methylobacteritum* strains from hospital tap water in high frequency, showing that hospital tap water is not free from the contamination.

The genus *Methylobacterium* was initially proposed by Patt et al. in 1976 (16), and 19 species have been reported so far (4). This pink pigment-producing bacteria is commonly isolated from various natural environments, including aqueous environments. The *Methylobacterium* strains may be environmental indigenous bacteria, and have been considered to have weak pathogenicity.

However, *Methylobacterium* species have also recently been attracting attention as opportunistic pathogens in the clinical field (19), and isolation from clinical materials has been reported (12, 14). Hospital tap water is considered to be a source of *Methylobacterium* infection, and sufficient monitoring of this genus is necessary. Under these circumstances, the expansion of *Methylobacterium* infection due to the change of water for washing hands from sterile water to tap water is a concern.

With the aim of contributing to hygienic control, this study investigates the distribution of *Methylobacterium* species in hospital tap water, and their drug sensitivity.

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Materials and Methods

Bacterial strains and cultivation. In 2004, 271 samples of tap water in hospitals in 13 prefectures of Japan were cultured on R2A agar medium (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at 30 C for 7 days. Totally 58 colonies with pink pigmentation which were formed by Gram-negative rod-shaped bacteria were collected and stored at −80 C. The 58 strains were confirmed to belong to genus *Methylobacterium* according to the definition given by Patt et al. in 1976 (16). The stored strains were cultured on R2A agar medium at 30 C for 7 days and used for further study. Residual free chlorine concentrations in tap water was simultaneously assayed by the colorimetry method with the Aquacheck LC (Nissan Chemical Industries, Ltd., Tokyo).

Biochemical tests. Forty-nine biochemical properties were tested using API50CH (bioMériux, Marcy-l'Etoile, France) according to the attached instructions. AUX medium (bioMériux) was used as the test medium, and positive reaction was judged based on the turbidity of the medium after incubation at 30 C for 14 days. The results were analyzed using analytical software, Bio Numerics 3.5 (Applied Maths, Sint-Martens-Latem, Belgium), and a phylogenetic tree was prepared using the UPGMA method (18).

Identification by partial 16S rDNA sequencing. Genomic DNA was extracted and purified using the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) following the protocols of the manufacturer. Using the extracted DNA solution as the template for PCR, of a 5′ end partial region about 500-bp of the 16S rRNA gene was amplified by PCR using a MicroSeq 500 16S rDNA PCR Kit (Applied Biosystems, Foster City, Calif., U.S.A.). The PCR products were purified using PCR Kleen Spin Columns (Bio-Rad Laboratories, Hercules, Calif., U.S.A.). The sequencing reactions of the PCR products were performed using a MicroSeq 500 16S rDNA sequencing Kit (Applied Biosystems) and the reaction products were purified with AutoSeq G-50 (Amersham Pharmacia Biotech, Inc., Uppsala, Sweden). A model ABI PRISM 310 Genetic Analyzer (Applied Biosystems) was used for sample electrophoresis and data collection. The obtained sequence data were compared with reference data from GenBank, and a phylogenetic tree was prepared by the neighbor-joining method of Saitou and Nei (1987) (17). Identification of the species was performed by the more than 99% similarity of the partial sequence of 16S rDNA.

Susceptibility testing. Drug sensitivity tests were performed using E-test (AB BIODISK, Dalvägen,

Solna, Sweden) according to the kit instructions. Antibiotics tested were ampicillin (ABPC), cefuroxime (CXM), gentamicin (GM), erythromycin (EM), vancomycin (VCM), tetracycline (TC), imipenem (IPM), chloramphenicol (CP), ofloxacin (OFLX) and fosfomycin (FOM). A bacterial suspension of each test strain (0.5 ml) was dripped on R2A agar medium (Wako Pure Chemical Industries, Ltd.) (60 ml in a 150-mm dish (Corning, Inc., U.S.A.)) and smeared over the surface using a Conradi stick, and an E-test strip was closely attached to the medium. The plates were cultured at 30 C for 7 days, and the growth inhibition zone formed around the strip was observed. MIC was judged by macroscopically reading the graduation at the point where the end of the growth inhibition zone and the strip crossed.

Results

Diversity of Biochemical Properties of Tap Water-Derived Methylobacterium *Strains*

The free residual chlorine concentrations of hospital tap water where 58 strains were isolated are shown in Table 1. The 58 strains were tested for 49 biochemical properties (Table 2). Fifty-four strains were positive for

Table 1. The free residual chlorine concentrations of hospital tap water where 58 strains of *Methylobacterium* spp. were isolated

Free residual chlorine (mg/liter)						Total
0	0.1–0.2	0.3–0.4	0.5–0.6	0.7–0.8	UN[a]	
6	13	17	2	2	18	58

[a] Unknown.

Table 2. Positive characteristics of *Methylobacterium* spp. isolated from the tap water in hospitals using the API50CH test

No.	Characteristics	No. of positive strains (%)
1	Glycerol	42 (77.8)
2	Erythritol	3 (5.6)
3	D-Arabinose	14 (25.9)
4	L-Arabinose	23 (42.6)
5	Ribose	1 (1.9)
6	D-Xylose	17 (31.5)
7	L-Xylose	4 (7.4)
10	Galactose	12 (22.2)
11	Glucose	9 (16.7)
12	Fructose	31 (57.4)
16	Dulcitol	3 (5.6)
37	Glycogen	1 (1.9)
41	D-Lyxose	13 (24.1)
43	D-Fucose	17 (31.5)
44	L-Fucose	5 (9.3)
47	Gluconate	31 (57.4)
48	2-Ketogluconate	2 (3.7)

$n=54$ strains.

some properties, and 4 strains were negative for all properties. Positive reaction was detected only in 17 (34.7%) of the 49 biochemical properties, and the number of positive properties was generally low. The properties shared by most strains were the utilization of glycerol (42 strains, 77.8%), followed by fructose and gluconate (31 strains each, 57.4%), and L-arabinose (23 strains, 42.6%), D-xylose and D-fucose (17 strains each, 31.5%), D-arabinose (14 strains, 25.9%), D-lyxose (13 strains, 24.1%), galactose (12 strains, 22.2%) and glu-
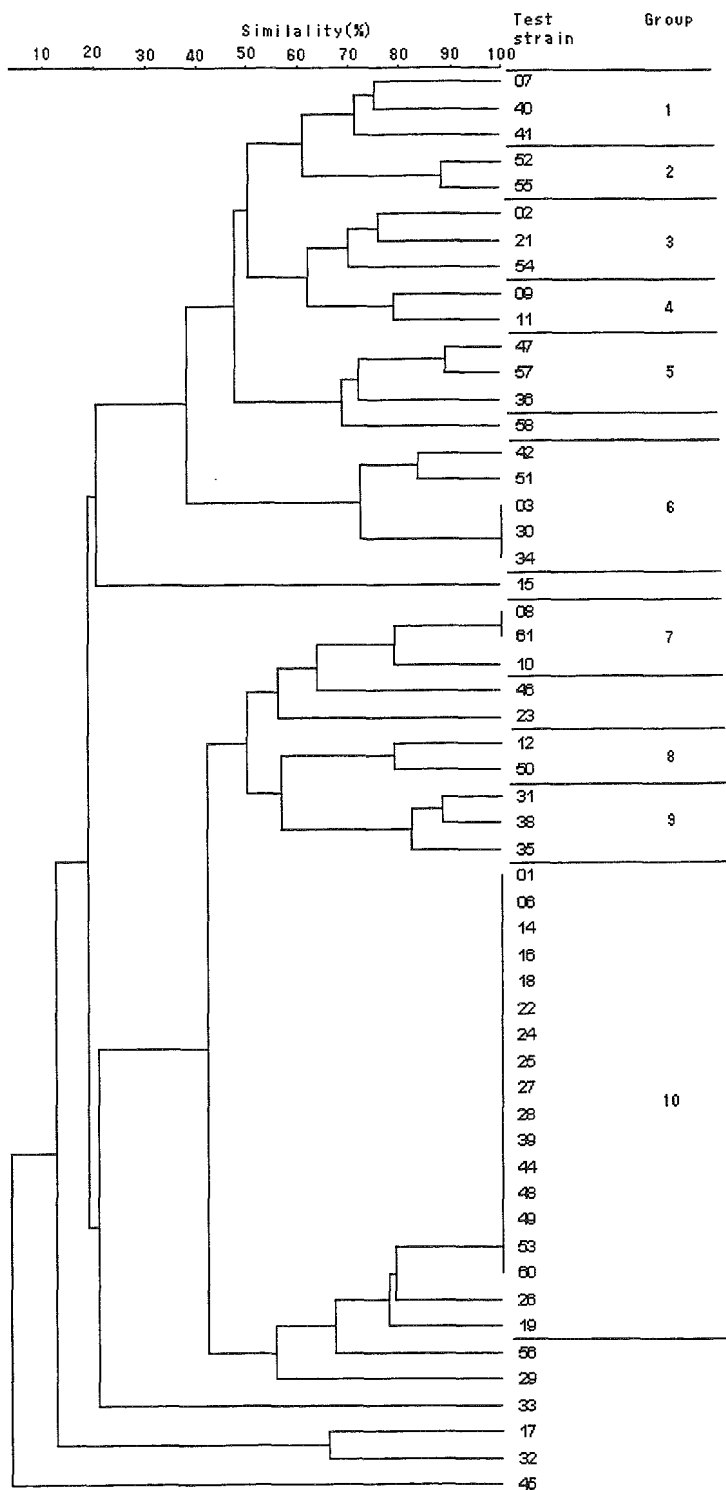

Fig. 1. Dendrogram of 54 *Methylobacterium* strains isolated from hospital tap water based on 49 biochemical characteristics.

cose (9 strains, 16.7%). In addition, positive reaction for L-fucose was detected in 5 strains (9.3%), L-xylose in 4 (7.4%), erythritol and dulcitol in 3 each (5.6%), 2-ketogluconate in 2 (3.7%), and ribose and glycogen in 1 each (1.9%).

The results of cluster analysis based on the biochemical properties are presented as a dendrogram in Fig. 1. When the strains were grouped at a similarity of 70%, 48 strains (82.8%) were grouped into 10 clusters (Groups 1 to 10), but 10 strains (17.2%) were not grouped with any strains, showing considerable diversity. The highest number of strains (18 strains, 33.3%) was included in Group 10, and the similarity of characteristics was 100% in 16 of these strains. Five strains (9.3%) were included in Group 6, and the similarity was 100% in 3 strains.

Identification of Tap Water-Derived Methylobacterium by 16S rDNA Sequence Analysis

From the results of the similarity analysis based on the partial sequence of 16S rDNA, 36 of the test strains (62.1%) were identified as *Methylobacterium* species (Table 3). *M. aquaticum* and *M. fujisawaense* were most frequently identified (11 strains each, 19.0%), followed by 6 strains (10.3%) each of *M. mesophilicum* and *M. radiotolerans*. One strain (1.7%) each were identified as *M. aminovorans* and *M. hispanicum*. Multiple candidate species were considered for 13 strains (22.4%), and determination based only on the results of this study was difficult. *M. rhodinum* and *M. sumiense* were candidates for 3 strains (5.2%).

The results of phylogenic analysis of the isolates compared with sequence data of known species are shown in Fig. 2. Arbitrarily, the species were divided into Group A, with very high homology with allied species, and Group B with low homology with allied species. Group A consisted of 12 species: *M. zatmanii, M. thiocyanatum, M. rhodesianum, M. lusitanum, M. populum, M.aminovorans, M. suomiense, M. rhodinum, M. chloromethanicum, M. extorquens, M. dichlorometanicum* and *M. organophilum*, and 15 isolates (25.9%). Group B consisted of 6 species: *M. hispanicum, M. radiotolerans, M. fujisawaense, M. nodulans, M. aquaticum* and *M. mesophilicum*, and 43 isolates (74.1%).

Because the similarities of 9 strains (15.5%) (Strain No. 6, 8, 18, 22, 23, 26, 30, 31, 61) to the sequences of type strains (Fig. 2) were low (less than 99%), the possibility of their being new species was considered for these unidentified strains.

On comparison of these identification results with the above biochemical property clusters, strains identified to be the same species belonged to multiple biochemical property clusters (Table 3), showing that identification based on biochemical properties alone is difficult.

MIC Distributions of Tap Water-Derived Methylobacterium Species

Table 4 shows the ranges of MIC, 50% MIC values (MIC_{50}), and 90% MIC values (MIC_{90}) of 10 antibiotics for the test strains. On comparison of the MIC_{90} of the drugs tested, IPM showed the highest antibacterial activity (1 µg/ ml) among the 10 drugs, followed by TC (8 µg/ml) and GM (128 µg/ml), but the MIC_{90} of the other drugs was the highest concentration tested (>32 µg/ ml, >256 µg/ml, >1,024 µg/ml).

The range of MIC of TC, EM and GM was wide and the distribution was unimodal. The susceptibility was also various in ABPC, VCM, CP, and OFLX, and the number of strains in the highest concentration of MIC tested was 16 strains (>256 µg/ml, 27.6%), 41 strains

Table 3. *Methylobacterium* species identified by 16S rDNA sequence analysis, and their groups by biochemical properities

Species	No. of strains (%)	Groups of characteristics[a]
M. aquaticum	11 (19.0)	3, 10, UC[b], NG[c]
M. fujisawaense	11 (19.0)	1, 2, 4, 5, 6, 8, 9, UC
M. mesophilicum	6 (10.3)	1, 2, 6, NG
M. radiotolerans	6 (10.3)	3, 4, 5, 6, UC
M. aminovorans	1 (1.7)	10
M. hispanicum	1 (1.7)	7
M. rhodinum or M. suomiense	3 (5.2)	10
M. lusitanum or M. populum or M. rhodesianum or M. thiocyanatum or M. zatmanii	9 (15.5)	8, 10
M. extorquens or M. chloromethanicum or M. dichloromethanicum	1 (1.7)	UC
Methylobacterium sp.	9 (15.5)	6, 7, 9, 10, UC
Total	58 (100.0)	

[a] Groups in Fig. 1.
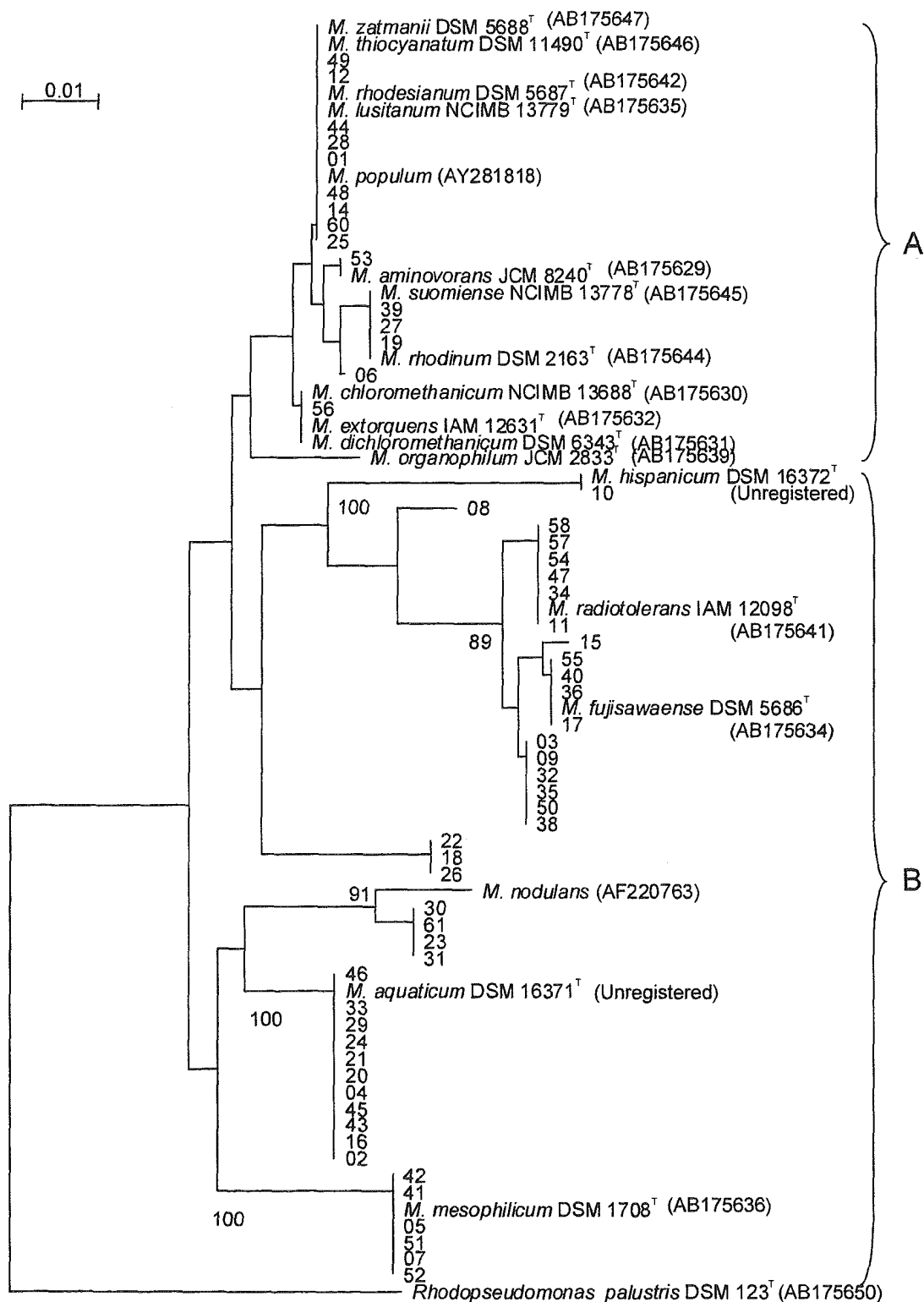[b] Unclassified.
[c] No growth.

Fig. 2. Distance matrix tree showing phylogenetic relationships among the *Methylobacterium* strains within the type strains. *Rhodopseudomonas palustris* was used as a member of outgroups. The scale bar indicates the number of substitutions per nucleotide position.

Table 4. Susceptibility of *Methylobacterium* strains isolated from the tap water in hospitals to 10 antimicrobial agents using the E-test

Antibiotic	MIC (μg/ml)		
	Range	MIC_{50}	MIC_{90}
Ampicillin	8 – >256	128	>256
Cefuroxime	1 – >256	128	>256
Gentamicin	1 – >256	16	128
Erythromycin	0.032 – >256	4	>256
Vancomycin	8 – >256	>256	>256
Tetracycline	0.064 – 32	2	8
Imipenem	0.032 – 8	0.25	1
Chloramphenicol	1 – >256	256	>256
Ofloxacin	0.25 – >32	8	>32
Fosfomycin	2 – >1,024	64	>1,024

$n=58$ strains.

(>256 μg/ml, 70.7%), 21 strains (>256 μg/ml, 36.2%) and 20 strains (>32 μg/ml, 34.5%) respectively, showing high resistance. In contrast, IPM, CXM and FOM showed bimodal MIC distribution. Regarding the number of drugs for which the MIC value was the highest concentration tested, there were 7 drugs for 1 strain (1.7%), followed by 6 drugs for 3 strains (5.2%), 5 drugs for 2 strains (3.4%), 4 drugs for 9 strains (15.5%) and 3 drugs for 8 strains (5.2%), showing that many strains were multidrug resistant. As a result, the susceptibility varied in all drugs, and sensitivities varied markedly among the strains. Excluding IPM and TC, the cumulative MIC distribution of the other drugs was considerably shifted to the resistance side (higher concentration of MIC).

The drug sensitivities of these test strains were not related to species. There was no association between the drug sensitivity and the site of isolation or the free residual chlorine concentration in the tap water from which the bacteria were isolated.

Discussion

The objective of this study was to elucidate which species of *Methylobacterium* inhabit tap water in hospitals. Since *Methylobacterium* species were isolated from various samples, including aqueous environments (11, 15), it was showed that the bacteria were widely distributed in natural environments. Because of the diversity in the properties among the strains, new species have been reported continually since 2000 (1, 8, 13). Currently, 19 species belong to the genus *Methylobacterium* (4). This study showed that various *Methylobacterium* species inhabit hospital tap water in Japan, and that *M. aquaticum* and *M. fujisawaense* are dominant. *M. aquaticum* was isolated from tap water in

Spain by Gallego et al. (8) and reported as a new species in 2005. *M. fujisawaense* was proposed as a new species by Green et al. in 1988 (10), and it was found that these bacteria were isolated in Fujisawa, Kanagawa Prefecture, Japan, by Kouno and Ozaki.

In the phylogenetic analysis, since the strains belonging to Group B formed 74.1%, it became clear that there are many strains belonging to Group B in tap water, and it was in agreement with results reported by Hiraishi et al. (11). These isolates were investigated with regard to the relationship with the isolated areas and the residual chlorine concentration in tap water, but no marked tendencies were noted in geographical differences or with regard to chlorine resistance.

Since the strains belonging to Group A had a high homology with the partial sequence of 16S rDNA, more detailed investigation is necessary for identification of the species. The 9 strains (No. 6, 8, 18, 22, 23, 26, 30, 31, 61) suggested to be new species could be divided into 4 groups.

Excluding strain 12 and 56, all other strains belonging to Group A were included in phenotypic Group 10 in Fig. 1, suggesting that the phenotypes of Group A species are similar. Although 5 strains (No. 16, 18, 22, 24, 26) belonging to Group B were also included in phenotypic cluster 10, no 16S rDNA group-specific or species-specific phenotypic characteristics were found. Based on the above findings, it may be difficult to identify *Methylobacterium* species based on the biochemical properties alone.

The isolation of *Methylobacterium* species from clinical materials has been known from the past (9). Because *Methylobacterium*-associated sepsis has recently been noted occasionally in AIDS patients (20) and bone marrow-transplanted patients (2), it has attracted attention as an etiological agent of opportunistic infection (19). Thus, information on the drug sensitivity of clinical isolates is important for therapy. Brown et al. (3) reported that in drug sensitivity tests of 15 clinical isolates and 3 standard strains, 100% of the strains were sensitive to amikacin and gentamicin even at a low concentration, but 9 isolates (60%) were resistant to β-lactams due to β-lactamase production. Zaharatos et al. and ourselves performed a similar drug sensitivity test of clinical isolates with imipenem and meropenem, using an E-test, and the MIC of imipenem was 0.25–1 μg/ml, showing high sensitivity, but the MIC of meropenem was >32 μg/ml, showing very strong resistance (21).

In the present study, the tap water-derived isolates exhibited strong resistance to 8 drugs, including the β-lactams except for imipenem and tetracycline. Regarding imipenem, the MIC_{90} was 1 μg/ml, showing high

sensitivity, as reported by Zaharatos et al. (21). However, the MIC$_{90}$ of gentamicin was 128 μg/ml, showing low sensitivity, in contrast to the results of Brown et al. (3). Brown et al. obtained MIC using the agar plate dilution method, but we used the E-test, and the difference may have been due to methodological differences. Since a high resistance to β-lactams such as ampicillin and cefuroxime was noted, β-lactamase production by the test isolates was investigated using the disc method. β-Lactamase production was detected in 51.7% of the test isolates (data not shown), and it was in agreement with results reported by Brown et al. (3). Since *Methylobacterium* strains were multidrug resistant, it will also be necessary to elucidate the tolerance mechanisms.

As mentioned above, since the classification of *Methylobacterium* species is unclear in many regards, it is thought that a reconsideration of the taxonomy based on gene techniques will be even more necessary from now on.

From the fact that *Methylobacterium* species which are pathogenic bacteria of opportunistic infection inhabit hospital tap water in Japan, careful follow-up study is necessary to draw a conclusion that tap water can be used in stead of sterilized water for handwashing before surgical operations.

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IV. 研究成果の刊行物・別刷

2. レジオネラの除菌と感染防止に関する研究

〈原　著〉

病院給湯設備におけるレジオネラ汚染とその除菌

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Legionella Contamination of Hot Water Supply Systems in a Hospital and Control Measures for Eradication

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要　　旨

レジオネラによる院内感染の主な感染源は病院の給水・給湯設備である．しかし，我が国では病院給湯設備のレジオネラ汚染と除菌についての詳細な報告はなく，その実態さえ不明である．産業医科大学病院において 2003 年 7 月に病棟の特別浴槽シャワーヘッドより Legionella pneumophila が検出され，追加調査で貯湯槽からも L. pneumophila が検出された．中央循環式の給湯設備であることより設備全体の汚染があると判断し，1 年間に渡り汚染調査と除菌作業を繰り返した．この調査・対策期間中に合計 52 箇所でのべ 119 回の培養検査を行い，迅速な除菌対策のため必要に応じ PCR 法も併用した．培養検査で 15 箇所のべ 18 検体から汚染が検出され，その内訳は貯湯槽 3 箇所，末端給湯栓 8 箇所，シャワーヘッド 4 箇所であった．これらからの分離株はパルスフィールド電気泳動により 3 つの遺伝子型にしか分類できず，汚染が給湯水の循環により施設全体に拡がっていたことが示唆された．除菌対策として(1)給湯水を 75℃ で 24 時間循環させながら末端給湯栓類(983 箇所)で放水を 1 年に 1 回行うこと(2)貯湯槽の清掃(3)給湯水温を 66℃ に上げて維持管理することを実施した．その結果，汚染は検出限界以下(5 CFU/100 mL)に除去できた．この期間中にレジオネラ肺炎の院内発生は認めず，水道料金や灯油料金の負担が除菌対策に伴って増えることはなかった．給湯水の昇温循環運転と末端給湯栓類からの放水作業は安価で有効な除菌法であった．

Key words：レジオネラ，院内感染，給湯水

はじめに

レジオネラ属菌はグラム陰性の通性細胞内寄生菌でヒトに急性肺炎(レジオネラ肺炎，在郷軍人病)やインフルエンザようの熱性疾患(ポンティアック熱)を引き起こす病原性を持っている．本属菌は空調用冷却塔水，給湯水，修景用水，循環式浴槽水などの人工水環境に混入・増殖し，それらが感染源になることが明らかにされている[1]．米国での 1980 年から 1998 年までの調査[2]によるとレジオネラ症の 25〜45％ が院内感染であり，その感染源の多くは給水・給湯設備である[3,4]．我が国では，1981 年に斎藤ら[5]が本邦初のレジオネラ症を報告し，同年には柏木ら[6]により最初の院内感染集団発生事例も報告された．厚生省レジオネラ研究班が行った調査[7]によると，1979 年から 1992 年の 14 年間に我が国でレジオネラ症と診断された患者数は 86 名であり，詳細な情報が得られなかった 6 例を除いた 80 名のうち 19 例

（23.7％）は院内感染であった．この報告以後，全国規模の調査は行われていないが，1996年に大学病院の新生児病棟において4例のレジオネラ肺炎が発生し，うち1名が死亡した事例が報告された[8]．病棟の水環境調査により起炎菌と同一血清群の*Legionella pneumophila*が複数の給湯水，室内加湿器，ミルク加温器から検出された．また，2000年には名古屋の大学付属病院の循環式浴槽水が感染源の院内感染例[9]が，2003年には岡山の大学付属病院で給湯水が感染源と推定された院内感染例が報告されている[10]．このような国内外における給湯設備が感染源または感染源と推定されるレジオネラ院内感染の発生を受け，産業医科大学病院では2003年7月に臨時にレジオネラ検査を行った．その結果，シャワーヘッド拭き取り調査にて*L. pneumophila*が検出され，同年同月に行われた定期検査で貯湯槽水からも*L. pneumophila*が検出された．病院給湯設備全体のレジオネラ汚染が疑われたため，給湯水の昇温と末端給湯栓類からの放水（フラッシング）による除菌を実施した．我が国では病院給湯設備のレジオネラ汚染と除菌についての詳細な報告は見当たらず，汚染の実態さえ不明である．今回の報告の目的は，医療関係者に病院の中央循環式給湯設備におけるレジオネラ汚染とその除菌の実例を具体的に提示することで，汚染と除菌に関する知見を共有し，本邦におけるレジオネラ院内感染の発生を防止することである．

材料と方法

1. 病院の概要

産業医科大学病院は本館（地上10階地下1階），東別館（地上2階），西別館（地上4階地下1階）の3つの建物で構成されており，延べ床面積は54916.5 m² である．病床数は618で，21の診療科よりなる特定機能病院である．

2. 給水・給湯設備の概要

給水は北九州市の供給する水道水と産業医科大学が掘削した井戸よりの井水を併用している．これらを受水槽に引き込んで貯留し，揚水ポンプで病院本館屋上の高架貯水槽に揚水した後，重力により各部署に供給している．給水の残留塩素濃度は毎日測定・記録されており，0.6〜0.8 ppm に維持管理されている．貯湯槽への補給水はこれらの貯水槽より供給されており，給湯方式は中央循環式である．その概要を表1に示した．高層階系統と低層階系統の2系統により，病院全体に給湯されており，高層階系統は本館4階から10階，東別館，そして西別館を，低層階系統は本館地下1階から3階までを担っている．配管方式は下向き複管方式で，上層階から順次下層階に給湯され，それぞれ返湯管により高層階系および低層階系の貯湯槽へ返湯される．配管内の流速

表1　給湯設備の概要

系統数	2
貯湯槽の容量と数	高層階系統 2400 L，2基（横型）
	低層階系統 3300 L，2基（横型）
貯湯槽の材質	SUS 304
配管方式	下向き式　複管式
配管材質	耐熱性塩化ビニルライニング鋼管 HTLP
加熱方式	蒸気による間接加熱

は滞流水を防止するため約15分で一循環する速度（高層階系は毎分130 L，低層階系は230 L）に調節されている．また，膨張管は高架貯水槽に接続されており，膨張槽は設置されていない．病院各部署で使用されている末端の給湯栓類は単純給湯栓，湯水混合栓，および温度調整弁（温調弁）を使用した自動栓である．

3. 試料採取

シャワーヘッドの拭き取りは滅菌綿棒を使用して行なった．給湯水試料は初流水を放流後，温度計で湯温が一定になったことを確認・記録した後，滅菌ボトルに約400 mL 採取した．貯湯槽水試料は貯湯槽近傍（1メートル以内）の給湯管と返湯管のドレン管よりそれぞれ採取した．本院では貯湯槽本体のドレン管は排水管に直結されており，貯湯槽内の貯留湯水を直接採取することが出来なかった．検水の残留塩素濃度とpHの測定は携帯型デジタル水質計（ハイドロクオント 501，東西化学産業株式会社，大阪）で行った．給水・給湯水ともにpHは7.4〜7.6の範囲に維持されていた．給湯水試料では残留塩素の検出が無かったため，塩素中和剤であるチオ硫酸ナトリウムの添加は行わずに培養検査に供した．高架貯水槽内の貯留水はドレン管から適当量を放水した後に採取し，水温，pH，残留塩素濃度を測定した．指針[1]に従いチオ硫酸ナトリウムを添加した後，培養検査に供した．

4. 培養検査

拭き取り試料は，WYOα（栄研化学株式会社，東京）またはGVPC寒天培地（日本ビオメリュー株式会社，東京）に直接塗布した．その後37℃で10日目まで培養した．給水・給湯試料は指針[1]に準じ，遠心またはろ過濃縮・酸処理後，0.1 mL ずつ2枚のWYOαまたはGVPC寒天培地に塗布した．37℃で10日目まで培養を続け，増殖してきたレジオネラと疑われる灰白色・浸潤な集落を計数した．2枚の培地で得られた集落数より平均を算出し，試料水100 mL あたりの集落数（CFU/100 mL）を算出した（検出限界は5 CFU/100 mL）．レジオネラと疑われる集落は，各培養平板から1検体当たり5集落まで釣菌し，指針に準じシステイン要求性を調べ，この結果に従って必要があれば集落数の集計に反映させた．菌種および血清群の同定には抗血清（デンカ生研株

式会社，東京）を使用した．一部の試料の培養検査は（財）
北九州生活科学センター（北九州市戸畑区）に委託し，レ
ジオネラが検出された場合は菌株の供与を受け，パルス
フィールド電気泳動法に供した．

5．PCR法

迅速な対策を講ずるため，必要に応じ LEG225 と
LEG858 プライマー[11]を使用して PCR 法を行った．
培養開始 3～4 日目のレジオネラと疑われる微小集落
を釣菌し，滅菌水 50 μL に懸濁した．この菌液を熱
湯中で 10 分間煮沸した後，20000 G，4℃ で 2 分間遠
心し上清を回収した．この 5 μL を鋳型 DNA として用
い，以前に報告した条件[11]で一段階目の PCR のみ行
なった．陽性対照には *L. pneumophila* Philadelphia-1
（ATCC33152）を用いた．電気泳動で陽性対照と同じ位
置（654 塩基対）に PCR 増幅産物が観察された場合は供
試菌をレジオネラと判断した．

6．パルスフィールド電気泳動

「ジーンパス　グループ 5　試薬キット」（日本バイオ
ラッド，東京）を使用し，添付手順書に従い SfiI で
DNA を切断した．切断された DNA を 1％アガロース

ゲルで CHEF mapper システム（日本バイオラッド）を
使用して電気泳動した．疫学的に関連のない対照株とし
て Philadelphia-1 株を使用した．

7．給水量と灯油使用量

2002 年度と 2003 年度の病院全体で使用した給水量
および灯油量は，月別集計簿より転記した．給湯水とし
て使用された給水量は給湯設備の維持管理に関する日報
から月別の給湯水量を集計した．また，使用用途ごとの
灯油量は記録されていなかったため，給湯水量を給湯温
度に昇温するために必要とした熱量を算出し，灯油量に
換算した（8450 kcal/L）．これを給湯ボイラーに使用さ
れた灯油量とした．但し，貯湯槽への補給水温は測定さ
れていなかったので，便宜的に 4 月～10 月の給水温を
20℃，11 月～翌年 3 月を 10℃ として必要熱量を概算し
た．

成　　績

今回の調査・除菌対策実施期間中に合計 52 ヵ所（の
べ 119 回）のレジオネラ検査を行なった．その概要は表
2 に示した．表 2 には示していないが，この期間中に定

表 2　産業医科大学病院給湯水のレジオネラ検査結果とその対策

年月	試料の種類	試料数	陽性試料数（重複試料数）*	レジオネラ菌数範囲，CFU/100 ml	菌種（血清群）	対　　策
2003. 7	シャワーヘッド	11	5(1)	2-124**	*L. pneumophila*(1)	汚染シャワーより放水 シャワー（ホースを含む）の交換
	貯湯槽水	4	3	25-500	*L. pneumophila*(1, 5, 6)	
8	シャワーヘッド	4	0			
8						貯湯槽設定温度を 66℃ へ変更
9						高層階系貯湯温度を 75℃ で 24 時間運転 高層階の給湯栓類（381 ヵ所）の放水
9	高層階系貯湯槽水	2	0			
	病棟給湯水	12	1	40	*L. pneumophila*(1)	汚染給湯栓より放水
10	病棟給湯水	1	0			
10						低層階系貯湯温度を 75℃ で 24 時間運転 低層階の給湯栓類（474 ヵ所）の放水
10	シャワーヘッド	6	0			
	低層階系貯湯槽水	2	0			
	病棟・外来給湯水	9	2	95, 320	*L. pneumophila*(1)	汚染給湯栓より放水
10						貯湯槽の清掃
11	病棟・外来給湯水	2	0			
2004. 2	シャワーヘッド	6	0			
	貯湯槽水	4	0			
	病棟給湯水	20	2	75, 3000	*L. pneumophila*(5, 6)	汚染給湯栓より放水
3	病棟給湯水	5	5(2)	100-2860	*L. pneumophila*(5, 6)	
3						高層階系貯湯温度を 75℃ で 24 時間運転 4, 5 階の給湯栓類（128 ヵ所）の放水
3	4, 5 階病棟給湯水	16	0			
5	シャワーヘッド	4	0			
	貯湯槽水	3	0			
	病棟・外来給湯水	8	0			

* 同一箇所より異なる日時に試料を採取，** CFU/拭き取り試料

期検査として行われた空調冷却塔水，加湿器水，人工呼吸器加湿水の培養検査ではレジオネラは検出されなかった．また，レジオネラ肺炎の院内発生は認めなかった．

1. 特別浴槽シャワーヘッドの汚染

2003年7月17日に10階病棟の一般浴室と特別浴槽のシャワーヘッド拭き取り検査を臨時に行った．特別浴槽の1本のシャワーヘッドよりレジオネラと疑われる微小集落の形成が培養3日後に認められた．PCR法でレジオネラであることが確認されたので7月22日に，当該シャワーおよび特別浴槽の使用を禁止した．また，汚染シャワーの放水を30分間行った（実測温55℃）．汚染が判明したことより，追加調査として同日に10階病棟の特別浴槽シャワーヘッド全て（4本）と一般浴室のシャワーヘッド（1本），8階，5階，及び4階病棟の特別浴槽シャワーヘッド（それぞれ1本）の検査を実施した．その結果，10階病棟の特別浴槽シャワーヘッドの4本全てから再度レジオネラが検出された．しかし，10階病棟の一般浴室のシャワーヘッドと他の病棟の特別浴槽シャワーヘッドでは汚染が認められなかった．このことから，10階病棟の特別浴槽シャワーヘッドに限局したレジオネラ汚染と考え，汚染していた4ヵ所のシャワー（ホースを含む）を新品と交換した．

2. 貯湯槽水の汚染

7月28日の定期検査により2系統の貯湯槽水にレジオネラ汚染があることが判明した．高層階系貯湯槽の給湯温度は62℃であったが，返湯水の実測温は51℃であった．また，低層階系貯湯槽も給湯温度は62℃であったが，返湯水の実測温は52℃であった．給湯温度の低下が汚染の原因と考え，8月14日に返湯水の実測温が55℃以上になるように設定温度を4℃あげ，66℃とした．これにより補給水の供給により湯温が最も低下する時間帯（16時頃）でも返湯温が実測温で55℃以上に維持できた．なお，貯水槽の貯留水検査ではレジオネラは検出されず，補給水のレジオネラ汚染の可能性は低かった．

3. 昇温と給湯栓類よりの放水による除菌

貯湯槽水でレジオネラ汚染が検出されたことより，病院給湯設備全体のレジオネラ汚染が危惧された．そこ

で，貯湯槽設定温度を75℃に上げて24時間運転し，その間に末端給湯栓から放水を行うことで給湯設備全体の除菌を試みた．その概略は表3に示した．高層階系統は9月9日，低層階系統は10月10日のそれぞれ0時から24時まで昇温運転し，この間に給湯栓からの放水を実施した．単純給湯栓の放水は2人1組で巡回して行い，2分以上放水し，湯温が一定になってから温度を記録した．記録した温度で20秒以上の放水作業を行った．60℃以上の湯温での放水を目的に，同時に開放する栓は5ヵ所までとし湯温の低下を極力避けた．温調弁のある自動栓からの放水は専門技術を必要としたため業者に委託して行った．放水作業は病棟では7〜9時に，厨房，中央材料部，手術部，ICU，中央臨床検査部などはそれぞれの部署の担当者により業務に支障が少ない時間を狙い，8〜18時の間にそれぞれ行われた．高層階系では381ヵ所，低層階系では474ヵ所の合計855ヵ所の末端給湯栓から放水が行われた．放水実測温は高層階系統で最高71℃，最低59℃で平均湯温は66℃であった．60℃未満の湯温の給湯栓は1ヵ所であった．一方，低層階系統では最高71℃，最低45℃で平均湯温は64℃であった．60℃未満の湯温の給湯栓は80ヵ所あった．これらの給湯栓は外来診察室や放射線部撮影室などに集中していた．

昇温循環中も給湯水の使用を禁止しなかったので，患者と病院職員の火傷を防ぐため，昇温循環中には全ての給湯栓設置個所に給湯配管の熱湯消毒中である旨の警告文を貼付し，注意の喚起をはかった．合計3回の昇温・除菌対策を実施したが，火傷等の事故の発生はなかった．また，昇温運転による給湯配管の膨張に起因する漏水事故も発生しなかった．

4. 除菌対策後のレジオネラ検査

放水作業中に湯待ち時間が長く，また湯温が低いことが判明した末端給湯栓類を中心に合計31ヵ所から採水し，培養検査を行った．その結果，高層階系統では20ヵ所中1ヵ所（8階病棟）から，低層階系統では11ヵ所中2ヵ所（地下1階）からレジオネラが検出された．高層階系，低層階系ともに貯湯槽水からはレジオネラが検出されなかったことより，末端給湯栓に限局した汚染と

表3 昇温除菌作業の概要

	除菌対象場所	昇温運転実施日時（2003年）	末端給湯栓放水日時	放水給湯栓数	放水実測温（平均）
高層階系統	本館4階〜10階 東別館1階 西別館2階	9月9日0〜24時	9月9日7時〜9時	381	59〜71℃（66）
低層階系統	本館地下1階〜3階	10月10日0〜24時	10月10日7時〜18時	474	45〜71℃（64）
高層階系統（追加）	本館4，5階	*3月5日21時〜3月6日21時	3月6日8時〜9時	128	53〜70℃（60）

* 2004年

考えた．汚染給湯栓のみで放水作業を1時間行った．その後の検査（2003年，10月3日及び11月4日）ではレジオネラは検出されなかった．

5．4階病棟給湯栓の広範囲な汚染と除菌

2004年2月9日の定期検査（20ヵ所）で高層階系統の2ヵ所（4階病棟）よりレジオネラが検出された．高層階系統の除菌は「湯量が少ない」「湯がでない」などの状況も無く，湯温が高い状態で行われていたため，この原因を調査した．その結果，汚染給湯栓が見つかった病棟は昇温除菌作業時に給排水配管改修工事のため病棟が閉鎖されていたこと，そのため末端給湯栓からの放水作業が実施されていなかったことが判明した．即刻，汚染給湯栓と同じ配管により給湯されている給湯栓全ての放水を約1時間行い，汚染給湯栓は使用禁止とした．3月1日に汚染給湯栓およびその給湯栓と同じ配管の最も上流（4階医師当直室）と下流（4階医師控室）および5階病棟の給湯栓の合計5ヵ所よりそれぞれ採水し，再検査を行った．培養開始，4日後の3月5日にレジオネラと疑われる集落が全ての検水で観察され，PCR法でレジオネラであることが確認された．菌数が多いこと，全ての検体でレジオネラが検出されたことより早急に昇温循環と放水作業を行った（表3）．3月10日に4,5階病棟の16ヵ所で採水し検査したところ，いずれの検水からもレジオネラは検出されなかった．

6．除菌の確認

2003年7月から2004年3月までの間に，汚染が検出された給湯栓（8ヵ所），シャワーヘッド（4ヵ所），貯湯槽水（3ヵ所）の合計15ヵ所について2004年5月24日に培養検査を行った．いずれの試料からもレジオネラは検出されず，検出限界以下に除菌できたと判断した．

7．分離菌株の遺伝子型別

給湯水より分離された菌株から分離場所，日時，血清群などが異なる15菌株を選んで遺伝子型別を試みた．表4に示したように15菌株は3つの遺伝子型に分類できた．第1は血清群1に属するシャワーヘッド分離株（図1レーン1から3），高層階貯湯槽への返湯水分離株（レーン4）であった．第2は血清群6に属する高層階返湯水から分離された菌株（レーン5），4・5階病棟の給湯水分離株（レーン9, 10, 12から15）であった．第3は血清群5に属する低層階貯湯槽の給湯水分離株（レーン6），返湯水分離株（レーン7），4階病棟給湯水から分離された菌株（レーン8と11）であった．対照として使用したPhiladelphia-1株（レーンC）はどの遺伝子型にも属さなかった．

8．昇温に伴う給水と灯油使用量の変化

表5に給湯水の昇温による給水，給湯水，灯油使用量の変化を示した．貯湯槽水の設定温度が62℃であった2002年度と2003年度の4〜7月期の月別平均使用量が

表4　給湯水由来 *L. pneumophila* の遺伝子型別

| 遺伝子型	菌株(血清群)	分離年月日*	由来(給湯系統)
I	UOEH101(1)	2003年7月17日	10階シャワーヘッド(高層)
	UOEH104(1)	2003年7月22日	10階シャワーヘッド(高層)
	UOEH109(1)	2003年7月22日	10階シャワーヘッド(高層)
	UOEH111(1)	2003年7月28日	貯湯槽返湯水(高層)
II	UOEH113(6)	2003年7月28日	貯湯槽返湯水(高層)
	UOEH123(6)	2004年2月9日	4階病室(高層)
	UOEH125(6)	2004年2月9日	4階病室(高層)
	UOEH128(6)	2004年3月1日	4階病室(高層)
	UOEH130(6)	2004年3月1日	4階医師控室(高層)
	UOEH132(6)	2004年3月1日	4階医師当直室(高層)
	UOEH134(6)	2004年3月1日	5階医師控室(高層)
III	UOEH114(5)	2003年7月28日	貯湯槽給湯水(低層)
	UOEH117(5)	2003年7月28日	貯湯槽返湯水(低層)
	UOEH120(5)	2004年2月9日	4階共有スペース(高層)
	UOEH126(5)	2004年3月1日	4階共有スペース(高層)

* 試料採取年月日を分離年月日とした．
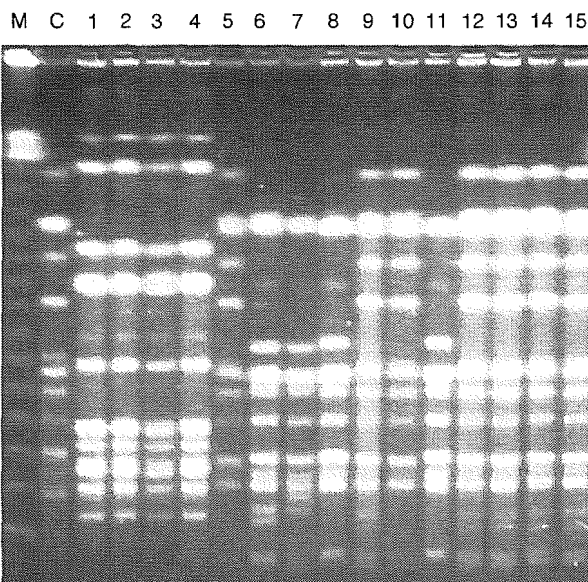
図1　給湯水由来株のパルスフィールド電気泳動像．
菌株の由来は表4に記載．レーン M; Lambda ladder, C; Philadelphia-1, 1; UOEH101, 2; UOEH104, 3; UOEH109, 4; UOEH111, 5; UOEH113, 6; UOEH114, 7; UOEH117, 8; UOEH120, 9; UOEH123, 10; UOEH125, 11; UOEH126, 12; UOEH128, 13; UOEH130, 14; UOEH132, 15; UOEH134.

表5　給湯水の昇温による給水，給湯水，灯油使用量の変化

	月別平均使用量(m³)						前年同期補正比[c]
	2002年度4〜7月[a]	2003年度4〜7月[a]	(前年同期比)	2002年度8〜3月[a]	2003年度8〜3月[b]	(前年同期比)	
灯油(給湯ボイラー)	11429	10719	0.938	13657	12143	0.889	0.948
灯油(病院全体)	264500	264000	0.998	336000	332000	0.988	0.99
給湯水(貯湯槽水)	2300	2157	0.938	2373	1967	0.829	0.884
給水(病院全体)	46279	45628	0.986	49603	46127	0.93	0.943

[a] 貯湯槽設定温度62℃，[b] 貯湯槽設定温度66℃，[c] 2002年度の4〜7月期と2003年度の4〜7月期の月別平均使用量が同じ(前年同期比が1)と仮定した場合の2002年度8〜3月期と2003年度の8〜3月期の前年同期比

それぞれ同じ(前年同期比が1)と仮定して，貯湯槽の設定温度を4℃上げて66℃で運転した2003年8〜3月期と設定温度が62℃であった2002年8〜3月期を比較した．その結果，給湯温度を4℃あげても給湯ボイラーで使用された灯油量は前年度に比べ約5％減少していた(表5)．給湯温度を上げたにもかかわらず給湯ボイラーの灯油使用量が減った理由は給湯水使用量が約12％減少したためであった(表5)．この減少の原因として，病棟において給湯温が上昇しているため湯温を下げるために使われる給水の混合量が増えていることが疑われた．しかし，病院全体の給水量の増加は認められなかった(表5)．除菌作業及び給湯温度を上げて維持管理することで水道料金および灯油料金の負担が増えることはなかった．

考　察

　レジオネラ属菌発見の端緒となった1976年の米国フィラデルフィアにおける大規模な集団発生は空調冷却塔水が感染源であった[12]．そのため，空調冷却塔水のレジオネラ汚染に注目が集まり，本邦でも実態調査や除菌対策が精力的に行われてきた[1]．また，欧米では空調冷却塔が稼働していない冬期を含め，年間を通じてレジオネラによる院内感染が発生することから，院内感染に関しては，給湯水のレジオネラ汚染が空調冷却塔水と同等に重視され，多くの研究が行われてきた[13-18]．しかしながら，我が国では病院給湯水のレジオネラ汚染に関する報告が非常に少なく[10,19]，その実態さえよくわからない状況にある．

　今回の調査・除菌対策実施期間中に合計52ヵ所で検査が行われ，15ヵ所(29％)から汚染が検出された．レジオネラ汚染が見つかりやすい湯待ち時間が長く，湯温の低い給湯栓を選んでの調査であったので，この汚染率は病院給湯設備全体の汚染率を示しているわけではないが，貯湯槽水の汚染は設備全体の汚染につながるため最も深刻な問題であった．低層階系統の貯湯槽給湯水から分離された株と返湯水から分離された株の遺伝子型が同一であったことは汚染が低層階全体に拡がっていたこと

を示している．Wadowskyら[18]，金子ら[20]は熱源の位置や設定温によっては，貯湯水に温度成層が形成され貯湯槽底部の湯温がレジオネラの増殖可能温度になり，配管の汚染とその拡大の主な原因になる可能性を示している．本院では低層階に湯の使用量が多い厨房があるため，高層階より貯湯量の多い貯湯槽を使用している．そのために貯湯槽内に温度成層が形成されやすく，また，給湯温度も低かったために貯湯槽内でレジオネラの生存を許したことが疑われる．しかし，低層階系の給湯水を汚染していたこれらの菌株の遺伝子型が高層階系統由来の株と同一であった理由は不明であった．また，10階の特別浴槽シャワーヘッドより分離された株と4，5階病棟の給湯水から分離された株は，それぞれ高層階系貯湯槽の返湯水からの分離株と遺伝子型が一致していた．高層階系貯湯槽の給湯水からは菌が検出され無かったことより考えて，末端給湯栓の汚染が返湯水を介して貯湯槽を汚染することが示された．しかし，10階シャワーヘッドと4階病棟は同じ給湯系列であるにもかかわらず検出菌株が異なっていた．今回の調査では1検体あたり5集落しか釣菌・精査しなかったので，試料中の優占株のみが検出されやすくなったことが原因と思われる．遺伝子型別により4階医師当直室が4・5階病棟の配管系統の最も上流に位置していたため，当直室の汚染が同一配管系統全ての汚染につながったことも明らかとなった．4階病棟共有スペースは4階病棟病室と給湯支管が異なっていたため，同じ階でありながら異なる菌株が分離されたと思われる．今回の遺伝子型別検査の結果より中央循環式の給湯設備では末端給湯水の汚染であっても貯湯槽の温度管理を含めた維持管理が適切になされないと容易に設備全体の汚染につながることが示唆された．末端給湯水の汚染が判明した場合はその汚染を除去するだけでなく，貯湯槽水の検査も行い，維持管理を確認し，必要に応じ変更することが大切と思われる．

　末端給湯水の汚染の最大の原因が給湯水の停滞であることはよく知られている[1]．一旦汚染がおこると汚染給湯栓局所での通常の給湯温度(55℃程度)での放水作業では除菌は困難で，昇温循環と放水作業が必要であっ

た．特別浴槽のシャワーは，一般浴槽のシャワーに比べ使用頻度が低く，シャワーヘッド内に給湯水が長時間停滞しやすいことが汚染の原因と疑われた．医師当直室の汚染が高度であった原因も一般病室における給湯水使用に比べ，当直室では給湯水の使用が少なく，横枝管内に給湯水の停滞がおこりやすくなっていたことが考えられた．また，4・5階病棟給湯水の広範囲の汚染は，給湯水の昇温運転時に放水作業が行われていなかったことによると思われた．循環ループ内の給湯水の昇温循環だけでは不十分で，放水作業により枝管内の停滞水を排出することが汚染の防止と除菌に重要と考えられた．病院内で給湯水の停滞がおこりやすい施設・場所は特別浴槽シャワーヘッド，医師当直室，外来診療部門，放射線部撮影室であることが明らかとなった．これらの場所は使用頻度が極端に少ない給湯栓が多数あり，湯待ち時間が長く，湯温の低い給湯栓も多かった．これらの給湯栓では定期的な放水作業による汚染防止がもっとも重要と考えられる．これらの場所はレジオネラの末端汚染を定期的に監視する採水場所として有用で，汚染監視の基準点に最適と考えられる．

古畑ら[21]は，一旦給湯系に定着したレジオネラは長期間に渡り生残，増殖すること，このような場合には貯湯槽の清掃と給湯水を70℃で20時間循環させることが有効であることを報告している．我々は除菌対策として給湯水の75℃での昇温運転（24時間）と末端給湯栓類からの放水作業，そして貯湯槽の清掃を行った．それらに加え，貯湯槽水の設定温度を4℃上げて66℃で維持管理した．このことにより前年度に比べて水道料金や灯油料金の負担が増えることが予想されたが，負担増は無かった．これは給湯水の利用量が減ったことに起因していた．今回の除菌方法は病院全体としての費用負担の増加もなく実施できるもので非常に有効であった．現在，病室や医師当直室の給湯栓での停滞水を防止するため，病院清掃業者に依頼して，毎日の洗面台清掃時に給湯水の放水を実施している．また，今回の除菌放水作業により湯が出ない給湯栓類が病院内に66ヵ所存在することが判明した．これらの給湯栓類は蛇口近傍で止水されていたので，横枝管を含めた給湯栓の撤去を予定している．末端給湯栓の汚染が施設全体の汚染につながる中央循環式の給湯設備では貯湯槽の維持管理に加えて停滞水の防止が非常に重要と思われる．

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