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Distribution of legionellae in a hospital water system: prevalence of immunologically and genetically related *Legionella pneumophila* serogroup 6 isolates

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1. SUMMARY

A hospital warm water system was monitored for the presence and distribution of legionellae. Subtyping of ten selected *Legionella pneumophila* isolates, originating from four different sites in the system by using serogroup specific antisera in an indirect immunofluorescence test, revealed that nine of the ten isolates belonged to serogroup 6, while the remaining one was serogroup 10. Two monoclonal antibodies (mAbs) specific for a subgroup of serogroup 6 strains were further used for characterization. None of the strains reacted with these mAbs. Genome analysis by elaborating *NotI* profiles using the pulsed field gel electrophoresis (PFGE) technique revealed that nearly all serogroup 6 isolates derived from different sites, including a new building connected

by a ring pipe, were identical according to restriction fragment patterns. The patterns were distinguishable from those of the two *L. pneumophila* serogroup 6 reference strains, and from that of the *L. pneumophila* serogroup 10 isolate. These data argue for a relatively homogeneous *L. pneumophila* serogroup 6 population in the entire water system.

2. INTRODUCTION

The natural habitats of legionellae are domestic water systems. They are also found in ponds and rivers, but never in salt water biotopes [1]. Among the various species of the genus *Legionella*, *L. pneumophila* is the most prevalent isolate, which can be distinguished serologically, leading to a categorization of 14 different serogroups. The serogroup-specific antigens are most probably due to the variation of lipopolysaccharide structures [2]. Several studies have shown

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that especially elderly patients hospitalized in care units are highly susceptible to an infection with legionellae, leading in most cases to severe pneumonia, often with a lethal outcome [1,3]. The so-called Legionnaires' disease originates from waterborne legionellae, which live intracellularly in free-living amoebae and are capable of infecting immuno-compromised patients, when legionellae are inhaled in aerosolized form [1,4]. Nosocomial infections by legionellae account for a high percentage of pneumonia cases in different countries [1,3,5]. Measures for eliminating legionellae from the domestic water systems by superheating or chlorination had not been as successful as expected, since the bacteria survive in cysts of amoebae, which are resistant to such treatment [6]. Numerous other factors influence the colonization of water systems by legionellae, such as oxygen concentration, pH, and even the material of the pipe systems [7-9].

In this study we monitored the distribution of legionellae in a hospital water system. The previously established method of pulsed-field gel electrophoresis (PFGE), which had been shown to be a powerful tool for the differentiation of legionellae [5,10], was applied in addition to immunological methods for subtyping, to get some insight

into the composition of the legionellae population.

3. MATERIALS AND METHODS

3.1. Cultivation of legionellae from water samples

Water samples were collected from different sites of the water system of a hospital building complex, which is fed by a hot water tank, with an adjusted temperature of 64°C (see Table 1). Unless stated otherwise, after stagnation of at least 12 h, 0.5-l water samples were collected and concentrated by centrifugation at 5500 × g for 20 min. The pellet was suspended in 1 ml of distilled water, from which 0.1 ml were plated on BCYE agar (Oxoid, FRG). The plates were incubated for 3 days at 37°C in 5% CO₂ atmosphere. Colonies exhibiting the typical growth morphology of legionellae [11] were picked and subcultured on BCYE and LB blood agar. Those isolates which did not grow on LB were chosen for further identification.

3.2. Serotyping of legionellae

Among the isolates which were identified as legionellae according to internationally accepted

Table 1

Immunological analysis of *L. pneumophila* strains isolated from different sites of the water system

Designation (in Fig. 1)	Serogroup	Reactivity to mAbs ^a 4-5 and 4-6	Site of collection
(1) WMe 2/1	6	-	Ward A, water tap
(2) WMe 3/1	6	-	Ward B, water tap
(3) WMe 4/1	6	-	Office, water tap (after stagnation for 4 days)
(4) WMe 4/3	6	-	Office, water tap (after stagnation for 4 days)
(5) WMe 4/5	10	-	Office, water tap (after stagnation for 4 days)
(6) WMe 4/6	6	-	Office, water tap (after stagnation for 4 days)
(7) WMe 5/1	6	-	Office (as above), but after 1 min water flow
(8) WMe 5/2	6	-	Office (as above) but after 1 min water flow
(9) WMe 7/1	6	-	New building (connected with a ring pipe), major reverse flow
(10) WMe 7/3	6	-	New building (connected with a ring pipe), major reverse flow
(11) Chicago-2	6	+	Reference strain (ATCC 33215)
(12) Dresden-37	6	-	Reference strain ^a

^a cf. ref. [10].

criteria [11], ten arbitrarily chosen strains collected at four different sites of the water system were further analysed by using adsorbed rabbit sera raised against all 14 serogroups of *L. pneumophila* by the indirect immunofluorescence test [10,12]. They were further characterized with monoclonal antibodies specific for serogroup 6, as described recently [12].

3.3. Pulsed-field gel electrophoresis (PFGE)

For genome analysis, DNA was prepared from the isolates after growth for 3 days (see above) and subjected to PFGE after *NotI* cleavage, essentially as recently described [5,10]. PFGE was performed by using the CHEF DrII System (BioRad, FRG) at 200 V with an increasing pulse time from 60 to 90 s over a period of 22 h and hereafter at a constant pulse time of 90 s for 3 h. Molecular mass standards used were yeast chromosomes (YNN 295; BioRad, FRG) and lambda ladders (Pharmacia, FRG).

4. RESULTS

4.1. Analysis of water samples collected at different sites of the water system

Water samples were collected at five different sites of the warm water system (see Table 1). Samples from a water tap at ward A on the 2nd floor contained approximately 10^2 colony forming units (cfu) per liter, nearly two-thirds of which displayed the typical legionella colony morphology. One of the colonies was chosen for further subtyping. At ward B on the 2nd floor, we found exclusively legionellae at a concentration of 20 cfu/l, collected from a water tap. One of the isolates was analysed in detail. At an office on the 1st floor, we took a water sample from a tap which had not been used for 4 days. The content was 10^4 cfu/l and 2×10^3 cfu/l by collection after 1 min water flow, which were exclusively legionellae according to colony morphology [11]. From this site we chose six isolates for further investigation. At a newer part of the building complex, which is connected to the water system by a ring pipe, we did not detect any bacteria in

the sample from a water tap, but in the water sample taken from the major reverse flow we found 5×10^2 cfu/l all exhibiting the typical colony morphology of legionellae. Two isolates were analysed in detail.

4.2. Immunological analysis of selected *L. pneumophila* isolates

The ten isolates were analysed by the indirect immunofluorescence assay (IFAT) using polyclonal antisera raised against the 14 serogroups of *L. pneumophila*. All the isolates reacted very strongly with serogroup 6 specific antiserum, with the exception of strain WMe 4/5 which reacted with the serogroup 10 specific serum (Table 1). Two monoclonal antibodies (mAbs) specific for a subgroup of serogroup 6 strains were used to subtype the isolates. mAbs 4-5 and 4-6 were shown to react with the serogroup 6 reference strain Chicago-2, but not with a prevalent group of isolates analysed previously in Dresden [10]. The features of the isolates from the hospital building complex were identical to the reaction pattern of strain Dresden-37, as they also did not react with these monoclonal antibodies (Table 1).

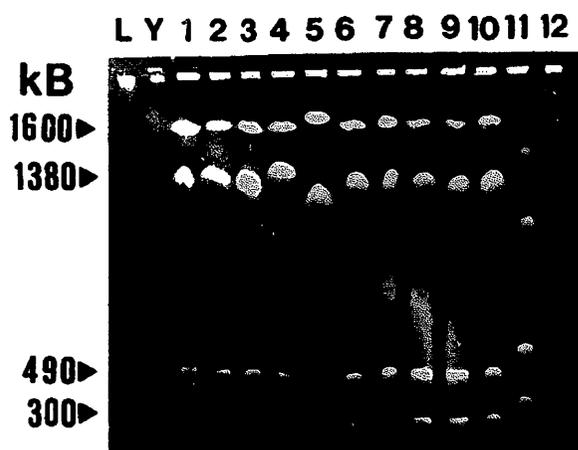


Fig. 1. Pulsed-field gel electrophoresis of *NotI* cleaved genomic DNA of *L. pneumophila* isolates. For strains see Table 1. As reference strains *L. pneumophila* serogroup 6, Chicago-2 and Dresden-37 (lanes 11, 12) were applied. DNA size markers are indicated as L (lambda ladders) and Y (yeast chromosomes). Relevant DNA sizes are given.

4.3. Analysis of the *NotI* restriction fragment pattern obtained by pulsed-field gel electrophoresis (PFGE)

In a further attempt to distinguish the isolates we prepared genomic DNA of the strains for pulsed-field gel electrophoresis. The DNA was cleaved by *NotI*, a rare cutting enzyme for *L. pneumophila*, and electrophoresed by PFGE. It can be seen from Fig. 1 that all isolates, except WMe 4/3 (lane 4) and WMe 4/5 (lane 5), were identical according the *NotI* profile, but different from that of the reference strains (lanes 11, 12), which were also dissimilar. Four fragments could be seen, ranging from 1600 kb to 300 kb. The majority of the isolates displayed fragments of 1600 kb, 1380 kb, 490 kb, and 300 kb, among which the 1600-kb and the 300-kb fragment was also found in the immunologically related strain Dresden-37 (lane 12), while the Chicago-2 strain (lane 11) displayed fragments of completely dissimilar size. Strain WMe 4/3 (lane 4) is nearly identical according the *NotI* pattern, differing only slightly in the size of the second large fragment, whereas the serogroup 10 strain WMe 4/5 (lane 5) shares only one common fragment (300 kb) with the remaining isolates of this investigation.

5. DISCUSSION

L. pneumophila serogroup 6 strains are often isolated from domestic water systems as shown previously [10,13-15]. Our results also show that this serogroup is prevalent in the warm water system investigated in this study. While serogroup 1 strains can be differentiated by monoclonal antibodies into 12 different groups [16], for serogroup 6 strains only two groups can be distinguished immunologically [12,14]. The reference strain Chicago-2 reacts with two monoclonal antibodies, as described recently [10], while other serogroup 6 isolates do not. Therefore genome analysis elaborating *NotI* profiles by pulsed-field gel electrophoresis had been used for differentiation. Especially for serogroup 6 strains it could be shown that this technique is highly efficient for subtyping [10].

The serogroup 6 isolates analysed in this study were identical to serogroup 6 strains isolated from a water system in Dresden by using monoclonal antibodies and were different to the Chicago-2 reference strain in this respect. Genome analysis, however, revealed that the isolates of this study are different not only to the Chicago-2 strain but also to the strain from Dresden, underlining the discriminating power of the *NotI* profile. By analysing the isolates collected at four different sites in the water system, it became obvious that most of the serogroup strains were identical not only in their monoclonal antibody reaction but also according to the *NotI* profile. Only one of the prevalent serogroup isolates displayed a slightly different pattern. These data argue for a relatively homogeneous composition of the *L. pneumophila* population in the water system. Samples collected at a new building which was connected by a ring pipe also contained *L. pneumophila* of the same *NotI* profile type, arguing for a colonization of the system by the prevalent population.

Since nosocomial *L. pneumophila* infections account for a high percentage of pneumonia [3], it is necessary to monitor water systems. Various subtypes of *L. pneumophila* serogroup 1 have shown to be highly virulent, while others rarely occur as infectious agents [1]. A clear insight into the *Legionella* population of a water system is helpful for risk evaluation. Serogroup 6 strains which seldom cause disease [1] were found in our survey, while the more virulent serogroup 1 strains could not be detected. Although we analysed only ten strains in detail, such a monitoring is worthwhile in making decisions for further surveys. Studies in the last decade dealing with the analysis of the composition of *Legionella* populations in domestic water systems were based on immunological criteria for subtyping [1,15]. These techniques are useful for determination of antigenically diverse serogroups but do not discriminate enough for analysis of serogroup 6 strains. Other methods have been established to overcome this problem, including electrophoretic typing of alloenzymes and rDNA hybridizations [16,17]. In this study, we used the rather new method of *NotI* profiling for subtyping legionel-

lae, which was shown to be very useful for this purpose.

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