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Legionella pneumophila in a hospital water system following a nosocomial outbreak: prevalence, monoclonal antibody subgrouping and effect of control measures

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SUMMARY

Swabs and water samples from a hospital water system were cultured for legionellae over an extended period. *Legionella pneumophila* serogroup 1, including outbreak associated strains, were isolated in small numbers from approximately 5% of these samples despite implementation of the current DHSS/Welsh Office regulations. No cases of nosocomial legionnaires' disease were proven during the study. Physical cleaning and chemical sterilization of taps, and replacement of washers with 'approved' brands did not eradicate the organisms. Eradication of legionellae in hospital water supplies appears to be unnecessary in preventing nosocomial legionnaires' disease provided the current DHSS/Welsh Office recommendations are implemented.

INTRODUCTION

Showers, other outlets of domestic water systems and cooling towers have been implicated previously as sources of legionella infections (Tobin *et al.* 1980; Fischer-Hoch *et al.* 1981; Helms *et al.* 1983). In a nosocomial outbreak of legionnaires' disease involving the main block of a hospital, strains of *Legionella pneumophila* serogroup 1 (SG1) which appeared identical were obtained from two of the victims and from a shower on a ward where one of them was a patient (Palmer *et al.* 1986), but only one of the cases had showered before the onset of symptoms. Legionellae were not isolated from the hospital cooling tower at the time of the outbreak. The outbreak appeared to have been controlled by re-elevating the hot water temperature to maintain levels of at least 50 °C at outlets; the lowest actual recording in the main block during the studies was 56 °C and most were between 60 °C and 64 °C.

In order to monitor control measures the domestic water supply in the hospital was investigated to find the prevalence of legionellae. Legionella strains were

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recovered. An attempt was then made to measure the persistence of legionellae in the system and to evaluate the effect of various eradication measures. In particular the effect on isolation rates from the system of substituting 'approved' washers was assessed since it had been suggested that certain types of washers might be important in supporting the growth of legionellae (Colbourne *et al.* 1984).

Watkins *et al.* (1985) had described a monoclonal antibody subgrouping scheme for *L. pneumophila* SG1. This defines three major subgroups – Pontiac, Olda and Bellingham, each further sub-divisible into minor subgroups. The Pontiac subgroup contains most of the strains causing outbreaks and corresponds to the la group of McKinney *et al.* (1983), the other subgroups tend to be associated only with single sporadic cases. The human isolates in the outbreak and the isolate from the shower were Pontiac 2a strains, a different subgroup from those obtained from the hospital environment at the time of a small outbreak 3 years earlier. Monoclonal antibodies were used to subgroup strains isolated during the prevalence and intervention studies to compare them with each other and the strains isolated previously.

MATERIALS AND METHODS

The standard techniques for isolating legionellae from water at the time of the outbreak required collection of five-gallon samples, but following the report of Helms *et al.* (1983) small-volume samples and tap swabs were used in this investigation.

Prevalence survey

Swabs were taken, usually in mid morning, from mixer taps from at least one site in each of 19 wards in the main block over 2 days. From the remaining two wards, in one of which an infected patient had been staying, all 42 outlets were sampled. A smaller number of individual hot and cold water taps from baths and sinks and shower roses were also swabbed. Repeat swabs were taken from sites positive on initial investigation. The J-tubes of three taps were physically cleaned following isolation of legionellae and these were re-swabbed repeatedly over the subsequent period.

Small-volume (50 ml) hot-water samples were collected, usually prior to swabbing, from 48 of the taps swabbed (26 on the 2 principal wards and 22 on the others). Complementary cold-water samples were taken from each of the sites on the 19 wards. Additional five-gallon hot-water samples were collected from 8 of the sites and five-gallon cold-water samples from 2 showers on the 2 principal wards. Furthermore 25 ml samples from all 12 individual hot and cold taps on each of these 2 wards were collected. A single five-gallon sample was collected from the calorifier. Duplicate samples were obtained from a small number of outlets.

Intervention study

Following the prevalence survey 20 mixer taps in each of 6 wards in one wing of the main block were investigated. The wards were randomly assigned to three groups. In two wards washers in the tap fittings were replaced with WRC approved washers (nitrile rubber washers (Barking-Grohe Ltd.) WRC reference number

7904526), and in two others washers were replaced and the inside of the J-tubes physically cleaned with brushes. In the remaining two the taps were kept as controls. Samples were collected at approximately 1-month intervals in two batches from each tap by opening the hot supply and collecting the first 75 ml of water.

Microbiology

Water samples. Large-volume samples were filtered through Sartorius 142 mm diameter cellulose acetate membranes, pore size 0·45 μm, using a Sartorius stainless steel filter holder. Each membrane was cut into small pieces and put into 30 ml of filtrate in a sterile jar which was vigorously shaken. Ten millilitres of the suspension was transferred to a universal container which was centrifuged at 2500 r.p.m. for 20 min, and approximately 9 ml of the supernatent was discarded. The deposit was resuspended in the remaining supernatent. Small volume samples were centrifuged directly in 25 ml aliquots. Each supernatent was discarded, and the deposit resuspended in the remaining fluid. The three suspensions from each sample were pooled. From each pooled suspension aliquots of 0·1 ml were used to inoculate two agar plates, one containing antibiotic.

Nine millilitres of acid buffer (see below) were added to each residual deposit with thorough mixing and the mixture left to stand for 5 min, after which 0·1 ml was used to inoculate a further agar plate.

Swabs. Swabs were inoculated directly onto agar plates (as above). They were then washed in 9 ml of acid buffer for 5 min. A 0·1 ml aliquot of washings was used to inoculate a further agar plate.

Acid buffer. This was made by adding 3·9 ml of 0·2 M hydrochloric acid and 25 ml of 0·2 M potassium chloride and adjusting the pH to 2·2 with 1M potassium hydroxide.

Media. The agar plates contained Legionella CYE agar base (Oxoid CM655) or occasionally Legionella BCYE base (Gibco Europe Ltd. 152-0900) with Legionella BCYE Supplement (Oxoid SR110) or a home-made equivalent. The antibiotic containing plates had initially Legionella selective supplement (Oxoid SR100) and later BMPA α-selective supplement (Oxoid SR111) added.

Incubation. The plates were incubated in a humid carbon dioxide enriched atmosphere (a candle jar containing a universal container of water) for 7 days at 37 °C.

Presumptive legionellae were identified by their characteristic cultural and microscopic appearance and an inability to grow on ordinary blood agar.

Monoclonal antibody subgrouping specific for strains of *L. pneumophila* SG1 was performed on suspected isolates as described by Watkins *et al.* (1985).

RESULTS

Prevalence survey

Swabs were collected from 64 sink mixer taps, 12 sink and bath hot taps, 12 sink and bath cold taps and 5 shower roses. Legionellae were isolated from 4 (6·3%) of the swabs from mixer taps but no others. All 4 isolates were obtained from the 42 sites sampled in the 2 intensively studied wards.

Table 1. *Legionellae isolated on repeated swabbing of taps found to be positive*

Growth of legionella in first positive sample (Day 0)		Growth of legionella in subsequent samples								
Tap	Growth/*Legionella pneumophila* serogroup 1 subgroup	Day/Growth/*Legionella pneumophila* serogroup 1 subgroup								
A	+ P4e	9+ P2a	14− ↓	14± P4e	17−	21−	28−	35−*	42−	76−
B	++ P4e	9+ P2a	14− ↓	14−	17−	21−	22−	28−	36−	42−
C	+ P2a	8+ ↓ P4e	12−	19−	33−	42−				
D	± O2b	13± P2a	47−†							

* A P2a subgroup strain was isolated from a water sample taken from this tap on day 35.
† P4e subgroup strains were isolated from a water sample and a swab taken from within the fitting on day 47
±, Very scanty growth; +, scanty growth; ++, moderate growth.
P, *Legionella pneumophila* serogroup 1 Pontiac subgroup.
O, *Legionella pneumophila* serogroup 1 Olda subgroup.
↓, Indicate that the outlets of mixer taps were physically cleaned between specimens.

The results of repeat swabbing of the positive sites are shown in Table 1.

Table 2 shows the results of water samples collected during the prevalence study. Legionellae were found only in hot water samples. One sample of hot water from a mixer tap was taken on the same occasion as a cold water sample from the same tap. Legionellae were isolated from the hot water only. One small-volume hot-water sample yielded legionellae when sampled at the same time as a five-gallon sample from the same outlet which did not. Positive tap swabs had previously been obtained from this outlet (Tap A – see Table 1).

On Day 47 tap D was dismantled and swabs and water samples taken from various sites within the fitting. Legionellae were isolated from one of the water samples and one of the swabs, but not from the washers removed from the tap at the time.

Repeat sampling from the (separate) hot taps from which water samples containing legionellae had been obtained initially yielded a further positive result from one accompanied by a positive swab from the same tap.

Intervention study

In this phase to assess the effect of intervention, 1667 water samples were collected from 120 taps over 14 months. Twenty (16·7%) of the taps yielded legionellae on at least one occasion and overall 73 (4·4%) of the samples collected were positive. The results by nature of intervention and time are shown in Tables 3 and 4. The maximum number of colonies isolated on any one plate was 50, which represents about 5000–10000 orgs/l; usually between 1 and 10 were obtained, representing 100–2000 orgs/l.

Many taps produced negative samples throughout the whole intervention study. Indeed one entire ward never produced a positive sample then, although it

Table 2. *Legionellae isolated from water samples collected in prevalence survey*

Sample	Sites tested	Samples collected	Number positive (% of sites/% of samples)
50 ml hot water from mixer taps	48	60*	2† (4·2/3·3)
50 ml cold water from mixer taps	22	22	0
5 gallons hot water from mixer taps	8	16‡	0
5 gallons cold water from showers	2	4	0
25 ml water from hot-water taps	24	24	2 (8·3/8·3)
25 ml water from cold-water taps	24	24	0
5 gallons from calorifer	1	1	0

* Four sites were sampled twice and four three times.
† One was the second of two samples from one site.
‡ Each site was sampled twice.

Table 3. *Legionellae isolated from water samples collected during intervention study – by nature intervention*

Intervention	Sites sampled	Sites positive (%)	Samples cultured	Samples positive(%)
Control	40	9 (22·5)	559	29 (5·2)
Washers changed	80	11 (13·8)	1108	44 (4·0)
Washers changed only	40	2 (5·0)	557	4 (0·7)
Washers changed plus fittings cleaned	40	9 (22·5)	551	40 (7·2)

has done so subsequently. Of the taps that yielded legionellae some did so occasionally whereas others did so regularly.

Monoclonal antibody subgrouping

All the isolates which survived for subgrouping during these investigations reacted with the monoclonal antibody panel used and were therefore *L. pneumophila* SG1.

Of the 17 strains obtained during the prevalence study, 3 died before testing but 14 gave recognized patterns – 1 Olda 2b and 13 Pontiac (7 P2a and 6 P4e) (Watkins *et al.* 1985). Details of subgroups isolated on repeated tap swabbing are shown in Table 1. The two strains isolated initially from the hot-water tap samples both belonged to the Pontiac 2a subgroup.

During the intervention phase legionellae from 33 different samples were referred for subgrouping. For part of this phase multiple colonies (up to five) were typed from each positive sample. Altogether 67 strains were tested. One failed to survive, 34 belonged to the Pontiac subgroup (2 P1a, 3 P2a, 12 P2c, 9 P4c and 8 P4e) and 32 to the Olda subgroup (20 O1a, 7 O1b and 5 O2a). As in the prevalence study different subgroups were sometimes found in water from the same outlet at different times. On some occasions all the colonies tested from a particular plate showed the same monoclonal typing pattern, but on others distinct differences were found (Table 5).

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Table 4. *Legionellae isolated from water samples in intervention study – by time*

	Sampling: number/weeks													
	1 0/2	2 4/6	3 8/10	4 12/14	5 16/20	6 23/25	7 27/29	8 31/33	9 35/38	10 40/42	11 45/48	12 50/52	13 54/56	14 58/61
	No samples positive/No. samples tested (%)													
Total (six wards)	6/119 (5·0)	3/120 (2·5)	5/120 (4·2)	8/117 (6·8)	9/119 (7·6)	1/118 (0·8)	7/118 (5·9)	7/119 (5·9)	8/119 (6·7)	9/119 (7·6)	4/120 (3·3)	3/120 (2·5)	1/120 (1·7)	1/119 (0·8)
	Method of intervention													
Control (two wards)	3/40 (7·5)	2/40 (5·0)	3/40 (7·5)	4/40 (10·0)	4/40 (10·0)	1/40 (2·5)	3/39 (7·7)	1/40 (2·5)	2/40 (5·0)	4/40 (10·0)	1/40 (2·5)	1/40 (2·5)	0/40 (0)	0/40 (0)
Washers changed (four wards)	3/79 (3·8)	1/80 (1·2)	2/80 (2·5)	4/77 (5·2)	5/79 (6·3)	0/78 (0)	4/79 (5·1)	6/79 (7·6)	6/79 (7·6)	5/79 (6·3)	3/80 (3·7)	2/80 (2·5)	2/80 (2·5)	1/79 (1·3)
Washers changed only (two wards)	0/39 (0)	0/40 (0)	0/40 (0)	1/39 (2·6)	0/40 (0)	0/39 (0)	1/40 (2·5)	0/40 (0)	1/40 (2·5)	1/40 (2·5)	0/40 (0)	0/40 (0)	0/40 (0)	0/40 (0)
Washers changed and J-Tubes cleaned (two wards)	3/40 (7·5)	1/40 (2·5)	2/40 (5·0)	3/38 (7·9)	5/39 (12·8)	0/39 (0)	3/39 (7·7)	6/39 (15·4)	5/39 (12·8)	4/39 (10·3)	3/40 (7·5)	2/40 (5·0)	2/40 (5·0)	1/39 (2·6)

Table 5. Legionella pneumophila *serogroup 1 subgroups isolated from various sites at different times during the intervention study*

Outlet reference	Sample reference	*Legionella pneumophila* serogroup 1 subgroups detected
1	1	O2a
	2	P4c
2	1	P1a + P4e
	2	O1b
	3	O2a
	4	O1a
	5	P2c + P4c + P4e + O1a
	6	P4e + O1a
	7	P2a
3	1	P2a + P4c + O1b
	2	P4e
	3	P4c
	4	P2a + O1a
	5	P2c + P4c + O1a

P, Pontiac subgroup; O, Olda subgroup.

DISCUSSION

The DHSS (1980) and Welsh Office (1980) have recommended that domestic cold water should be kept and distributed at a temperature below 20 °C or as near to 20 °C as possible and that hot water should be stored at a temperature of 60 °C and distributed at a temperature of not less than 50 °C.

Following the outbreak of nosocomial legionnaires' disease and the implementation of these recommended control measures, our prevalence survey indicated that legionellae were colonizing 1 in 16 of the mixer taps in the hospital's main ward block and could be isolated from approximately 1 in 30 of small-volume hot-water samples collected from such taps. This order of prevalence was confirmed in the control samples collected during the intervention study. Furthermore we found legionellae in more than 8% of similar samples from separate hot water taps.

The isolation of legionellae from hot-water samples was in keeping with earlier reports of hospital and hotel supplies. (Dennis *et al.* 1982; Fischer-Hoch, Smith & Colbourne, 1982; Helms *et al.* 1983). Fischer-Hoch and her colleagues (1982) have suggested that the calorifier may be the important reservoir of legionellae in hospital plumbing systems. Although the organism was not cultured from the calorifier sample we tested, it was obtained from the hot-water side of the system fed by the calorifier. Possibly calorifiers act as reservoirs of legionellae and seeding to more distal parts of the system occurs from this source. However, it is not yet clear whether legionellae need to colonize and multiply at distal sites in order for transmission to people to occur, or whether there are sufficient organisms present already in water reaching the outlets. In either event the likely benefit of routine cleaning and maintenance of the calorifiers is apparent.

In our study colonization of tap outlets by legionellae, as indicated by the positive swabs, was a notable feature as has been described previously (Helms *et al.*

1983). Inspection of outlets revealed a slimy deposit which could have provided a hospitable environment for legionellae. After repeated sampling of positive sites and, in three cases, physical cleaning of outlets to remove such slime, the number of organisms isolated on swabbing decreased and then become undetectable (see Table 1). No recolonization was observed in these taps over the next 2 months. If multiplication at tap outlets is important in outbreaks our data suggest that physical cleaning of the tap would have beneficial effect in the short term by reducing the number of legionellae dispersed by the system. However this would provide only part of the answer as legionellae were also cultured from other internal sites when Tap D was disassembled, and our current work, at a preliminary stage, shows that legionellae can be detected in water prior to entering the tap fitting, in similar numbers to those in water collected through taps. Furthermore four of the taps from which legionella positive samples were obtained most frequently were sterilized recently by ethylene oxide treatment. This has had no apparent effect on the subsequent frequency of legionella isolation.

From the results obtained (Tables 3 and 4), it is apparent that there was little difference between the percentage of positive samples from control and all intervention taps. Paradoxically changing washers with physical cleaning of the taps resulted in more positive samples than when washers were changed without cleaning. Perhaps the physical cleaning was inadequate and might have loosened infected plaques. However, this would only have had a short term effect. Some local variation such as reduction in water temperature or presence of a redundant length of piping might have been a factor. There was no evidence to suggest that changing to 'approved' washers eradicated the organism. Replacement of tap washers alone may not remove all sites for legionella colonization.

There appears to be a progressive decline in the percentage of samples yielding legionellae in each round of sampling at the end of the study (see Table 4). This might have been due to the effect of chlorination as additional chlorine (in the form of chlorine dioxide) up to 0·1–0·4 p.p.m. free chlorine at outlets was being injected into the system at the point of entry to the hospital site from sampling 11 onwards. However, occasional low isolation frequencies had been observed earlier (e.g. sampling 6) and subsequent rounds of sampling, without any decrease in the chlorine concentration, showed a reversion to original frequency. Thus no lasting benefit occurred from adding these levels of chlorine to the system.

It can be seen that there may be distinct differences between the subgrouping patterns of legionellae obtained from the same outlets at different times (Tables 1 and 5). This may have indicated sampling variation, temporal variation in the presence or prominence of the different types or antigenic variants. How much antigenic variation within the major subgroups occurs with changes in temperature and other parameters is not known but it probably can occur (P. J. Dennis, personal communication). Variations were also seen between subgroup patterns when multiple colonies were subgrouped from a given plate. Thus it seems that multiple subgroups are present coincidently. This is not surprising as different legionella species and serogroups of *L. pneumophila* in individual samples have been described previously (Stout *et al.* 1982; Arnow & Weil, 1984).

Altogether 80 strains gave recognizable subgrouping patterns in these investigations. All were *L. pneumophila* SG1, the major human pathogen. Most were

Pontiac like strains including 10 of the Pontiac 2a subgroup, the same as that isolated from two of the cases in the outbreak.

We have shown that despite implementation of the current DHSS (1980)/Welsh Office (1980) recommendations approximately 5% of tap swabs and water samples in these studies yielded legionellae. Although they were isolated usually in small numbers, strains potentially capable of causing outbreaks, including those of the Pontiac 2a subgroup implicated in our outbreak, were present. What action should be taken in these circumstances? Intensive surveillance since the outbreak has revealed serological evidence of only two possible sporadic nosocomial cases of *L. pneumophila* infection since the end of the outbreak in 1983 (I. Zamiri, personal communication). One patient had a rise in titre which was diagnostic of recent infection and the other a high titre which did not rise: no legionellae were obtained from them to identify the infecting strain. Both had been in hospital for only part of the 10-day incubation period of legionnaires' disease. The absence of proven nosocomial cases of legionellosis may be due to a dose effect. If so, our experiences suggest it is not necessary to eradicate legionellae from the hospital water supplies. Indeed it may not be possible to do so. It seems that the current recommendations are adequate to keep the level of legionellae in such systems at such a low level that no hazard to patients exists.

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