ARTICLE 2

DETECTING *LEGIONELLA PNEUMOPHILA* IN WATER SYSTEMS: A COMPARISON OF VARIOUS DENTAL UNITS

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Legionella species may be present in a variety of water systems, including cooling towers, spas, water storage tanks and shower heads.[1,2] *Legionella pneumophila* is the most common cause of legionella pneumonia, with serogroups 1 through 6 being the most frequently implicated in infections of the respiratory tract.[2]

The bacteria enter the respiratory tract by means of fine aerosols[1] and any water system containing legionellae or other bacteria that liberates aerosols into the atmosphere could be considered a potential source of infection. Dental units fall into this category, as the high-speed outlets emit water to cool the dental bur and tooth during drilling procedures. This creates fine aerosols that could be inhaled by patients, dental surgeons, nurses or hygienists.[3]

Many studies have examined bacterial contamination of dental unit water systems, and data show that bacterial levels are significantly higher than those found in normal tap water.[4-9] In early studies, scientists cultured samples on routine, unsupplemented media[7-9]; since legionella has specific growth requirements that ordinary media do not meet, this species was not detected. In more recent investigations, researchers have found *Legionella* species in dental water systems and employed various methods to eradicate them.[10]

ABSTRACT

The authors sampled 194 dental units over a 44-month period to detect the presence of *Legionella pneumophila*. They found *L. pneumophila*, usually in very low numbers, in 25 percent of the units over this time. However, higher counts were collected from 4 percent of the units, primarily from one model. The authors document colony counts collected from nine different models and those collected from air/water syringes vs. high-speed outlets, and they describe the effectiveness of disinfection.

Oppenheim and colleagues[11] found no evidence that the presence of legionella in dental units caused infection, and there have been no reported cases that identify dental units as a source of legionnaires' disease. However, Fotos and colleagues[12] and Reinthaler and colleagues[13] showed that dental personnel do develop raised antibody titer to legionella after varying periods of clinical work, which indicates that exposure to the bacteria is sufficient to initiate an immunological response.

Infection with *L. pneumophila* ranges from the severe and often fatal legionnaires' disease to the milder form, Pontiac fever, which is a self-limiting illness with flu-like symptoms.[2,14,15] It is possible that some Pontiac fever infections pass undiagnosed.

This study assesses the prevalence of *L. pneumophila* in dental unit water systems in a dental hospital and compares the levels in various dental unit models to determine whether colonization is related to the type of unit.

MATERIALS AND METHODS

We sampled 194 dental units (nine different models) in one dental hospital for the presence of *L. pneumophila*. Samples were obtained from all departments including restorative dentistry, pediatric dentistry, primary treatment (oral diagnosis) and oral surgery. We examined units from three to six times over a 44-month period. The time between samples ranged from six to 12 months. Samples were taken directly from the high-speed outlets

TABLE 1

MEAN PLATE COUNTS OF *L. PNEUMOPHILA* CULTURED FROM AIR/WATER SYRINGES.*

MAKE	NO. OF UNITS	Mean Plate Counts cfu/plate			
		0	1-10	11-100	101-1000
Adec Minitrol	42	40	1	1	0
Anthos Pegaso	36	34	1	1	0
Ash Ranger	28	26	1	1	0
Kavo Estetic (1024C)	22	18	2	2	0
Siemens Sirona 55	11	4	0	7	0
Kavo Atlantica (1038A)	15	4	4	7	0
Plan Meca 2000	12	1	0	5	6
Castellini Stilflex 3	17	14	0	3	0
Castellini Area 4	11	9	0	1	1
Total	**194**	**150**	**9**	**28**	**7**

* Water samples collected from air/water syringes over 44 months.

without the handpiece attached and from the air/water syringes. The high-speed outlets on two models were not functioning during the sampling period, so we collected samples only from the air/water syringes from these units. We ran the water for 30 seconds from each outlet before collecting 100-milliliter samples in sterile plastic containers. Each sample passed through a 0.45 micron bacteriological field monitor filter (Millipore Corp.). Half the filter membrane was placed on selective legionella medium SCYE agar, which is charcoal-based agar with added growth and selective supplements. We vortexed the other half of the filter membrane for 30 seconds in 1 mL sterile distilled water with sterile glass beads. We plated 100 microliters of the vortexed suspension onto SCYE agar and 100 µL onto non-selective legionella medium NSCYE agar (CYE agar and legionella BCYE growth supplement). The plates were incubated in moist air at 37 C for seven days.

Examination of plates for presumptive *L. pneumophila*. We examined each plate for typical white to blue-gray or green colonies that had a sticky consistency. The colonies were counted and recorded as total counts per plate. Representative colonies of each morphological type were subcultured onto Columbia blood agar (Difco Laboratories Ltd.) and NSCYE agar. We incubated these plates under the same conditions as described previously and examined them after five days. Colonies that had failed to grow on blood agar but had grown on the NSCYE agar were presumed to be *Legionella* species.

Immunofluorescent antibody test. Presumptive *Legionella* species were further identified using a legionella immunofluorescent antibody test kit (Genetic Systems) according to the manufacturer's instructions. Briefly, this involved preparation of air-dried smears that we then incubated with a monoclonal anti-*L. pneumophila* antibody labeled with fluorescein isothiocyanate. (This monoclonal antibody binds to all known serogroups of *L. pneumophila.*) The incubated slides were air-dried and mounted in buffered glycerol, then examined under a X40 objective. *L. pneumophila* appeared as brightly fluorescing apple-green rods.

We included appropriate positive *(L. pneumophila)* and negative *(Pseudomonas aeruginosa* NCTC 10662) controls in each assay. We assumed that organisms not confirmed as *L. pneumophila* belonged to another *Legionella* species, but we did not further identify these.

Decontamination. Although no specific recommenda-

TABLE 2

MEAN PLATE COUNTS OF *L. PNEUMOPHILA* CULTURED FROM HIGH-SPEED OUTLETS.*†

MAKE	NO. OF UNITS	Mean Plate Counts cfu/plate			
		0	1-10	11-100	101-1000
Adec Minitrol	42	41	0	1	0
Anthos Pegaso	33	33	0	0	0
Ash Ranger	24	22	1	1	0
Kavo Estetic (1024C)	22	19	3	0	0
Siemens Sirona 55	11	9	1	1	0
Kavo Atlantica (1038A)	15	6	3	6	0
Plan Meca 2000	12	3	1	3	5
Total	**159**	**133**	**9**	**12**	**5**

* Water samples collected from high-speed outlets over 44 months.
† High-speed outlets on Castellini Stilflex 3 and Castellini Area 4 not sampled.

tions determine a safe level of legionella in dental unit water systems,[15] any unit showing persistant contamination with legionella was taken out of clinical use. We flushed each of these units with an undiluted hypochlorite solution (14 percent weight per volume available chlorine, Merck BDH) until the solution exited from the outlets. After allowing the unit to stand unused for 20 minutes, we cleared the unit of hypochlorite by flushing it with water until all the chlorine had been eliminated from the unit (determined to be the point at which the solution stopped bleaching blue paper towels).

RESULTS

Of 194 units, 49 (25 percent) harbored *L. pneumophila* in either the high-speed coolant or the air/water syringes on at least one occasion over the sampling period; of the 49 positive units, 23 were positive on more than one occasion. In 145 of the 194 units (75 percent), we did not detect legionella in any of the water samples taken over the 44-month period. We considered the majority of the units from which *L. pneumophila* was isolated as having low levels of contamination (plate counts less than 100 colonies) (Tables 1 and 2). However, seven of the 194 units (4 percent) had plate counts of more than 100 colonies in either or both of the water systems examined. We considered this an unacceptably high level and recommended that these units immediately be taken out of service and decontaminated.

In general, the pattern of contamination appeared to be related to the model of the dental unit rather than to the type of dental procedure generally performed with that unit. Six of the nine models were represented in one department with the same water supply and virtually identical usage, yet differences in colonization were apparent. While *L. pneumophila* was rarely found in some units (for example, Minitrol and Anthos Pegaso), others had high proportions of *L. pneumophila* at least once during the test period (Figure).

We detected *L. pneumophila* colonies at similar frequencies in two models. However, in one of these the numbers of colonies remained very low, while counts from the other model were consistently high. Seven of the nine models had legionella counts that did not exceed 100 on any occasion. In one model, however, six out of 12 units harbored high counts of legionella on four or more occasions (Figure).

During the test period, we detected *L. pneumophila* in 44 of the air/water syringes and 26 of the high-speed outlets. However, in nine of the air/water syringes and in nine of the high-speed outlets, we counted less than 10 colony forming units per plate. Few units showed counts above 100 cfu per plate except for the Plan Meca 2000 units, where consistently high counts were found in six of the air/ water syringes and five of

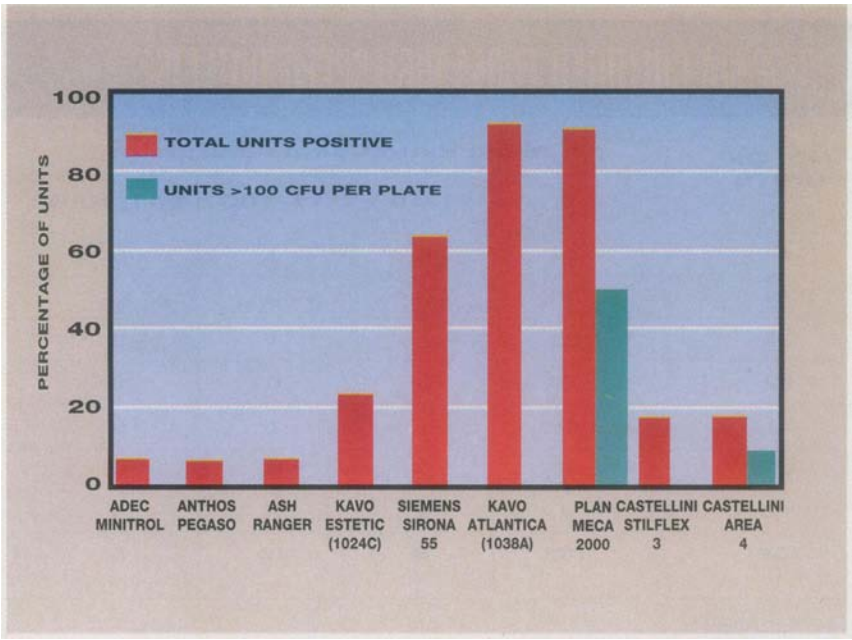

Figure. Detection of *L. pneumophila* in dental units according to unit model. (Number of units assayed is listed in Tables 1 and 2.)

the high-speed outlets. In one of these units, high counts were detected only in the high-speed outlet, and high counts were detected in two of the units' air/water syringes only. One unit of a different model also showed a count of more than 100 cfu per plate in the air/water syringe sample (Tables 1 and 2).

Other *Legionella* species. We conducted preliminary investigations to determine if legionellae apart from *L. pneumophila* may colonize in the dental units. We collected and analyzed data over a two-year period, which showed that of 91 units tested, 21 yielded colonies that presumably were *Legionella* species (gram-negative rods that grew on legionella media but failed to grow on blood agar), but were fluorescent-antibody negative to the *L. pneumophila* monoclonal.

Of the 21 positive units, 11 were of the same model and five gave counts greater than 100 cfu per plate (Table 3). *L. pneumophila* was isolated from some of the units in addition to the non-pneumophila *Legionella* species.

DISCUSSION

Overall, the isolation frequency of *L. pneumophila* was low, with only 25 percent of 194 units being positive over the entire test period and only 4 percent of all units having consistently high counts. The air/water syringes seemed to be more susceptible to contamination with *L. pneumophila* than the high-speed outlets, but both clearly could be affected. Our findings strongly suggest a difference in the susceptibility to contamination with *L. pneumophila* of the nine unit models examined. One model in particular appeared to be consistently susceptible to high levels of contamination, and each unit of this model required decontamination more frequently than the other units. In these units, the decontamination protocol appeared to clear the *L. pneumophila* for three months, but at six months after decontamination, counts had returned to unacceptably high levels.

According to the literature, a number of factors may influence bacterial levels in dental units: the type of materials used in the tubing, the bore size of the tubing and the frequency of use.[16-19] We did not monitor the amount of use of each unit during this study, but most units were used daily, although the number of patients treated with each unit in a dental school, by dental students and staff, was far less than would have been treated in a general practice.

Interestingly, we found that some units that had been unused for relatively long periods did not yield high levels of *L. pneumophila,* while others in daily use did. This suggests that the colonization and detection of *L. pneumophila* depended more on the unit model than on the amount of use. The age of the units did not appear to contribute to the levels of contamination, as some units with low levels of contamination were older than the units that yielded consistently high levels of contamination. There seemed to be no relationship between the isolation of *L. pneumophila* and the department or the type of usage of the dental unit since different models in the same department displayed widely different levels of colonization. All units were supplied with water from the same main supply, and no legionella was detected at the source of entry to each floor.

The quantity of water sampled will affect the chances of isolating bacteria. While the sampling volume in industrial water systems can be large, this is impractical with dental unit

TABLE 3

MEAN COUNTS OF NON-PNEUMOPHILA LEGIONELLA SPECIES CULTURED FROM DENTAL UNITS.*†

MAKE	NO. OF UNITS TESTED	Mean Plate Counts cfu/plate			
		0 (%)	<1-10	11-100	101-1000
Adec Minitrol	29	18(62)	9	0	2
Anthos Pegaso	16	15(94)	1†	0	0
Kavo Estetic (1024C)	12	12†(100)	0	0	0
Siemens Sirona 55	8	6†(75)	1	0	1
Kavo Atlantica (1038A)	6	3†(50)	3†	0	0
Plan Meca 2000	12	9†(75)	2†	0	1
Castellini Stilflex 3	2	2(100)	0	0	0
Castellini Area 4	6	5(83)	0	0	1
Total	**91**	**70**	**16**	**0**	**5**

* Water samples collected from dental units over a 2-year period.
† *L. pneumophila* isolated from one or more units.

water systems; we believe that 100-mL samples were sufficient, and this amount certainly revealed differences between the units. The extent of contamination in the units actually may have been higher than our data indicate since the methods used, including selective media, may have reduced the levels isolated.

Some research suggests that the growth of *L. pneumophila* is influenced by the presence of other aquatic organisms.[20] Our own preliminary investigations indicated that certain units contaminated with *L. pneumophila* also harbored high levels of other bacteria (mostly pseudomonads), but it did not always follow that the units which had high levels of other bacteria were contaminated with *L. pneumophila.* It also is possible that the presence of other aquatic bacteria in the water samples may have reduced the numbers of legionella bacteria isolated.

Many methods have been used to eradicate bacteria, including *Legionella* species, from dental units. Chlorination currently is the most widely used method. Disinfection, however, is only a temporary measure as adherent bacteria may be protected by biofilms that develop on the tubing's surface.[20] We did not assess how quickly the units became recontaminated after disinfection, but this appears to depend on the unit model and the level of contamination. Our preliminary longitudinal sampling suggests that units with initially high levels of contamination became recontaminated more quickly than units that had low levels of contamination, and that within two months, contamination in these units returned to original levels.

Many practitioners flush the water lines on dental units routinely before they use the unit and between each use. Though flushing may eradicate transient oral flora, this practice probably has little effect on legionella levels, as these bacteria are thought to persist on the surface of tubing in the dental unit. In the current study, samples were collected after flushing.

The infective dose of *L. pneumophila* seems to vary, so it is difficult to decide on a "safe" level of the bacteria.[15] Researchers need to determine whether the presence of any legionella in dental unit water systems should be deemed unacceptable or whether colony counts need to reach a certain level to be pathogenic. In making this determination, researchers must consider the mode of transmission. Although the primary mode of legionellae transmission is inhalation of aerosolized organisms, infection occasionally occurs via other routes such as direct inoculation of wounds with a contaminated water supply, in which case small numbers of the bacteria may be significant.[22]

Surveillance studies show

that the presence of legionella in dental unit water systems does not lead to detectable incidences of human infection,[10,11] although seroconversion has been proven.[12,13] Thus, the intermittent isolation of low numbers of this pathogen from these systems may not be clinically relevant. The risk of infection to healthy individuals also remains open to question. Hence, screening large numbers of dental units in the absence of disease may be of little value in the preventing legionella infection. Because the incidence of legionella infection is relatively low despite its widespread presence in the environment, most public health officials do not recommend screening in the absence of disease.[2]

The likelihood of legionella infection seems to depend on several factors,[15] including host susceptibility and aerosolization of the organism. There is a greater risk to elderly or immunocompromised patients who are more susceptible to any type of infection, and cases have been reported in which infection resulted from bacteria originating in dental unit water supplies.[22] With the increase in the treatment of elderly immunocompromised patients, it is more important to ensure that hygiene standards in the dental practice, including water quality, are kept high.

Controlling legionella in dental water systems is both time-consuming and expensive and must be repeated regularly to be effective. Regular maintenance and servicing of water systems can help reduce the bacteria levels, and screening of dental units may be useful in monitoring the effectiveness of disinfection and maintenance programs.

Our study showed that some units had much higher levels of legionella than others; therefore, these units were subsequently tested more frequently. By identifying possible high-risk units and monitoring these specific units we would hope to reduce the risk of legionella infection to staff and patients.

CONCLUSION

We detected a difference in the prevalence of *L. pneumophila* contamination among nine different models of tested dental units. One unit model was more susceptible to contamination with *L. pneumophila* than the others. Contamination did not appear to be related to the type of use of the unit and could take place independently in the air/water syringes and high-speed outlets. We found some evidence that *Legionella* species other than *L. pneumophila* may colonize dental units to differing degrees, but further work is required to confirm this. ■

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