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Isolation of pathogenic *Legionella* species and legionella-laden amoebae in dental unit waterlines

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Summary Legionella released into the air during treatment are a potential source of infection. Water stagnation in dental unit waterlines (DUWLs) creates biofilms and promotes the proliferation of these micro-organisms. This study investigated the presence of amoeba infected with legionella, *L. pneumophila* and other pathogenic *Legionella* species in a dental teaching hospital. Water samples were collected in the morning and afternoon from 99 dental units and 16 taps connected to the municipal water supply. Samples were plated on selective media and tested for legionella using the direct immunofluorescent antibody technique and the latex agglutination test. Legionella were found in 33% of the DUWLs and in 47% of the mains taps supplying these units. Legionella-laden amoebae occurred in one mains tap sample and in 20% of DUWLs in a clinic of the teaching hospital. *L. micdadei* was the predominant species isolated from this clinic. *L. pneumophila* serogroups 2–14 predominated in the mains water, whereas *L. pneumophila* serogroup 1 was found in approximately half of the contaminated DUWLs and mains taps irrespective of the time of sampling. Pathogenic *Legionella* species seeded by municipal water into DUWLs is a potential source of legionella infection for both dental personnel and patients during prolonged dental treatment. This problem is compounded by the presence of legionella-laden amoebae which may contain levels of organism well within the infective dose. The interaction of legionella with amoebae is an important ecological factor that may significantly increase the risk of legionellosis, and thus should be given further consideration in the refinement of risk assessment models.

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Introduction

Bio-aerosols generated during dental procedures are the main source of legionella, the agents that cause both Legionnaires' disease and Pontiac fever.[1-5] Raised levels of antibodies to legionella confirm that dental surgeons, nurses, hygienists and patients are exposed to contaminated aerosols. This may reflect continuous exposure to small numbers of these micro-organisms that may result in mild Pontiac fever or subclinical infections.[2,6-8]

Biofilms formed within the small bore tubing of dental units create an environment that favours the attachment and growth of *Legionella* species. Once colonized, other micro-organisms as well as protozoa may attach to this biofilm.[1,9,10] Under adverse conditions, amoebae will increase their uptake of bacteria and provide a continuous supply of bacteria that are released into the environment.[11] This may be problematic because legionella-laden amoebae may contain infective numbers of organisms.[12] The recognition of amoebae as reservoirs and vehicles for bacterial spread leads to public health concerns.[13]

Many cases of legionella infection are not identified as such.[14] *L. pneumophila* serogroup 1 is responsible for 80% of reported human cases of legionellosis and is usually the only species isolated during routine testing.[15,16] Specialized laboratory methods for identifying other species are not readily available and may be a reason for them not being routinely identified.[3]

Studies have reported the presence of legionella in dental unit waterlines (DUWLs) and water supplying these units, but the presence of legionella in amoebae has been rarely reported.[17] This study investigated the presence of legionella-laden amoebae and other pathogenic *Legionella* species in DUWLs and municipal water supplying the units.

Methods

Water sampling

Water samples were obtained from the six clinics housed in the three buildings of the University of the Witwatersrand Oral and Dental Hospital (Table I). The Maxillo-Facial and Oral Surgery Clinic, Restorative Dental Clinic and Periodontology and Oral Medicine Clinic were located in the Oral Dental Hospital that was opened in 1953. The Prosthetic and Orthodontics Clinics were transferred to University Corner in 1980. The Oral Health Clinic was established in Dental House in 1989 and equipped with dental units that had been in used since 1975.

The hospital is provided with potable water that passes a booster pump before distribution to the dental units. Each dental unit has a water-cooled air turbine drill, a water-cooled conventional speed drill and a three-in-one syringe that produces air and water jets, a spittoon and a drinking water fountain. The water required for each dental unit is supplied by an intricately branched system of narrow-gauge tubing.

Samples of 550 mL water were collected from the three-in-one syringe of 99 dental units, 22 basin taps in the vicinity of the units and 16 mains taps that supplied municipal water to these units (Table I). They were collected aseptically on a Monday morning before the start of the clinical session, and in the afternoon after the clinics had been in operation for 5 h. The samples were placed in sterile plastic bottles treated with 0.5 mL of a 3% solution of sodium thiosulphate and transported to the laboratory for analysis.

Sample processing

Water samples were analysed using a modified International Standard method, ISO/DIS 11731, 1996 (Water Quality—Detection and Enumeration of Legionella). The water samples did not appear to be very contaminated and therefore the acid treatment step of the ISO/DIS 11731 method was omitted. Samples were concentrated aseptically by membrane filtration, using a three-piece PVC manifold (Millipore SA, Johannesburg, RSA) and cellulose Type HA membranes with a pore size of 0.45 μm (Millipore Corporation, Bedford). The concentrated samples were removed by cutting the membranes into four pieces, placing them in sterile containers containing 3 mL of the original sample and sonicating them in an ultrasound tank (Ultrasons-H) for 10 min until the membranes appeared to be clean.

Culture of legionella

Concentrated samples were divided into two aliquots. The first was heat-treated in a water bath at 50 °C for 30 min and the second was left untreated. Both samples were diluted in sterile distilled water using 10-fold dilutions up to 10^6, inoculated on to either buffered charcoal yeast extract agar containing alpha-ketoglutarate agar (αBCYE) (Oxoid, UK) or glycine, vancomycin, polymyxin, cycloheximide agar (GVPC) (Oxoid, UK), and incubated aerobically at 37 °C for 10 days.