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Detection of *Legionella* spp., *L. pneumophila* and *L. pneumophila* serogroup 1 in drinking water system

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Background: *Legionellae* are ubiquitous bacteria in aquatic environments. Exposure to *L. pneumophila* can result in acute respiratory illnesses termed legionellosis with the majority of potentially fatal cases caused by *L. pneumophila* serogroup (sg1) and non-fatal cases caused by other *Legionella* species. Thus the need to evaluate the safety of drinking water (DW) so as to manage public health risks associated with its use. We have developed a protocol and a qPCR array for screening, identifying and confirming *Legionella* spp., *L. pneumophila*, *L. pneumophila* serogroup 1 (sg1), activities of some genes associated with virulence factors and other important functions, based on 16S rRNA gene, the macrophage infectivity potentiator gene (mip), lipopolysaccharide biosynthesis proteins (LPS), entry gene (rtxA), intracellular multiplication gene (cegC1), type IVA secretion system (lvrD) and phosphoinositide phosphatase (sidF). The objective of this study was to increase the coverage of *Legionella* spp. and various *L. pneumophila* strains by modifying 16S rRNA gene- and mipA-based assays, and accuracy of *L. pneumophila* sg1 through those strain-specific gene assays.

Materials: Specific test against 24 bacterial genera, 16 *Legionella* spp. and 5 *L. pneumophila* strains were conducted, while sensitive tests were done against the DNA isolated from three matrices (tap water, DW storage tank sediments and DW bioreactor effluent tubing biofilm) spiked into a serial of dilutions of *L. pneumophila* sg1. Comparing to selective culture methods, 40 water samples were collected from premise tap water, storage tank sediments, hospital tap water, DW plant, and different cooling towers. Samples were analyzed according to the standard plating method as well as by molecular techniques developed in this study. The brief protocol was firstly to screen *Legionella* spp. using the new 16S rRNA gene assay, and then to identify and confirm *L. pneumophila* and *L. pneumophila* sg1 through qPCR array for those samples presented positive signals. Clone-sequencing and phylogenetic analyses were also done for identification of different species.

Results: The newly designed assay target 16rRNA gene was not only specific and sensitive, but also covered more sequences, which included the most known isolates and latest sequences in Genbank, than the previous assays. The assays included in the qPCR array were specific to *L. pneumophila* (mipA) and *L. pneumophila* sg1 (other five assays) and consistent sensitive (1-10 cells per reaction) in all the matrices tested. Nonetheless, they had similar high qPCR efficiency in those matrices tested. For the samples tested, the occurrence of *Legionella* spp. was 12 (30%), and phylogenetic analysis showed high diversity, while the detection positive for *L. pneumophila* and *L. pneumophila* sg1 were 10 (25%) and 8 (20%), and they were comparable to those using selective culture methods, but the former was much sensitive, informative and confirmative, demonstrating its power and usefulness for *Legionella* detection.

Conclusion: The qPCR array and protocol developed in this study are a more practical and useful technique for detecting and distinguishing *Legionella* spp., *L. pneumophila* and *L. pneumophila* sg1 in environmental samples. Detection can be expanded by increasing target gene selections. This technology can be useful in diagnosing infection and molecular epidemiology to determine the distribution of different *Legionella* spp., *L. pneumophila* and *L. pneumophila* sg1, and even important genes.

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