A Rapid Method for the Detection of the Nosocomial Waterborne Pathogens

Legionella spp. and Pseudomonas aeruginosa

by Real-time Quantitative PCR: A Comparison with Standard Culture

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Abstract

Legionella and other waterborne pathogens cause approximately 25% of healthcare-associated infections. Infection prevention depends on determining if the hospital water system is a reservoir for these pathogens. We evaluated the GenoSystems real-time quantitative PCR (qPCR) technology for the rapid detection of Legionella species and Pseudomonas aeruginosa in water samples collected from 18 healthcare facilities and 1 long-term care facility. 100 samples were tested and qPCR results were compared to standard culture. qPCR detected L. pneumophila in 29% of samples and Legionella spp. in 67% of samples compared to 16% and 17% by standard culture. Sensitivity/specificity of the L. pneumophila and Legionella species qPCR (with culture as the standard) was 100%/85% and 100%/100%, respectively. 22% of samples were culture positive for P. aeruginosa. Sensitivity/specificity for culture positive samples was 100%/100% for enriched samples. The Legionella pneumophila and P. aeruginosa qPCR are recommended for evaluating the risk of exposure from water within healthcare facilities.

Background

Legionella environmental surveillance of hospital water systems is important in determining the risk for hospital acquired Legionnaires’ disease. Legionella culture is the gold standard, however, rapid methods like real-time quantitative PCR (qPCR) could be used to obtain a rapid evaluation of Legionella contamination. The GenoSystems qPCR can identify Legionella pneumophila and Legionella bacteria in water samples in 3 hours versus 7-10 days for culture method. While testing has been done using cooling tower samples, evaluation with hospital potable water samples is needed to demonstrate the utility of the technology for routine Legionella analysis. Stenotrophomonas maltophilia, Acinetobacter species, and Pseudomonas aeruginosa are waterborne pathogens which cause significant morbidity and mortality and infection has been linked to hospital water. We also evaluated real-time, qualitative PCR for detection of P. aeruginosa in water samples.

Objectives

1. Evaluate the sensitivity and specificity of PCR vs. culture in detecting the presence of Legionella pneumophila and Legionella species.

2. Evaluate the utility of GenoSystems’ qPCR method for Legionella risk assessment of drinking water by comparison to standard culture method.

3. Evaluate the performance of GenoSystems’ qualitative PCR for detection of P. aeruginosa in drinking water samples.

Materials and Methods

100 potable warm water samples (1 L) were collected from 18 healthcare institutions and one building housing senior citizens and split for testing. Legionella Analysis

qPCR- Total DNA was extracted from 500 µL using the GeneExtract instrument (in approximately 2 h). (GenoSystems, Bruz, France)

Figure 1. Legionella spp. on BCYE

Approximately 200 µL of purified extract is recovered. qPCR were performed with the GeneDisc Cycler with GeneDisc L. pneumophila (sig 1 to 15) and a separate GeneDisc Legionella spp. (approximately 1 h per disc). The genome units of DNA per sample were expressed as GUL. For a 500 mL sample: Level of Detection (LOD) PCR equals: 330 GUL; Level of Quantification (LOQ) equals: 1866 GUL. If the GU was less than the LOD PCR, the sample was reported as ‘Absence’. Culture- Analysis for all Legionella species, including pneumophila, was performed directly and after concentration. Buffered Charcoal Yeast Extract (BCYE) medium and BCYE with dyes, glycine, vancomycin, and polymyxin (DVP) were used for culture isolation. Concentration was achieved by filtering 100 mL of the sample through a 47 mm diameter, 0.2 µm pore size polycarbonate filter membrane. The membrane was transferred to 10 mL of original sample and vortexed to suspend the concentrate. Results were obtained in 7 days after humidified incubation at 36ºC. (1)

Pseudomonas aeruginosa analysis with enrichment

Qualitative PCR- 100 mL of sample was filtered through a membrane filter (mixed cellulose ester,0.45-µm, 47 mm diameter) which was transferred to a 50 mL sterile tube containing 10 mL BCYE broth. After a 15 h incubation, the extraction was carried out with 2 mL of the broth culture. Bacterial cells were isolated after a series of centrifugations and incubations, then bead beating. DNA was recovered by purification with dedicated resin supplied in the lysis buffer. The final DNA solution is analyzed using the utility of the technology for routine Legionella analysis. Stenotrophomonas maltophilia, Acinetobacter species, and Pseudomonas aeruginosa are waterborne pathogens which cause significant morbidity and mortality and infection has been linked to hospital water. We also evaluated real-time, qualitative PCR for detection of P. aeruginosa in water samples

Conclusions

Legionella- L. pneumophila PCR can be used with confidence to evaluate the risk of exposure to L. pneumophila from hospital water distribution systems. We note, however, the differential pathogenicity among the 16 serogroups of L. pneumophila would necessitate serotyping of isolates from cultures to adequately assess the risk of Legionnaires’ disease (2). The low specificity of the Legionella species PCR compared to culture may preclude the meaningful use of the species PCR in evaluating disease risk from Legionella species in potable water samples. This weak correlation was also seen with cooling tower samples (3). The frequency of positive PCR results in the absence of disease suggests that this result should be interpreted with caution. It may be that Legionella species (or genetically-related species) are easy to detect by PCR and more difficult to detect by culture.

P. aeruginosa- Given the excellent correlation between culture and PCR for the detection of P. aeruginosa, PCR could be used as an alternative to culture for environmental surveillance of hospital water systems.

References

