

Background

Microbial contamination of a hospital's water system can be the source of infection with waterborne pathogens such as *Legionella*. Prevention of this mode of transmission can be accomplished by disinfection of the hospital water distribution system. Water treatment with monochloramine is a relatively new strategy for water disinfection of hospital water systems and few studies have evaluated its long-term effects^{1,2}. The changes in microbial populations in a hospital water system following treatment with monochloramine remains largely unknown.

Next generation sequencing technologies have been previously implemented in characterizing the microbial populations in biofilms, water, and aerosols of both water distribution systems and hospitals. Prior studies of monochloramine addition to biofilms³ and a municipal water system⁴ have found strong selective pressures to be exerted by this disinfectant.

Our study is the first to assess the changes in *Legionella* and microbial flora due to chloramination in a hospital's hot water system using next generation sequencing.

Objectives

To characterize the selective pressures of monochloramine treatment on hot water microbial composition using next generation sequencing.

To assess the risks and benefits of this disinfection strategy based upon what pathogenic organisms may be resistant.

Methods

This study was conducted in a hospital complex in Pittsburgh, PA. The complex consists of a 12 story, 495 bed tertiary care hospital and an 11 story administrative building receiving the same chlorinated incoming municipal cold water. The hospital's hot water was treated using on-site generation of monochloramine (Sanipur, Italy) and these hot water samples are designated as "treated". The administrative building acted as a control because it received no supplemental water treatment and these water samples are designated as "control".

Hot water samples were collected from 6 faucets and the hot water tank from each building monthly for 3 months.

DNA was extracted using a bead-beating, phenol-chloroform extraction as described previously^{5,6}. PCR was performed using universal 16S ribosomal RNA primers 515F and 1391R^{6,7}. Sequencing was performed using the Roche 454 FLX platform per manufacturer's instructions.

The Ribosomal Database Project Classifier software was used to make taxonomic assignments⁸. A BLAST database containing bacterial isolates identified in the Silva ARB database (version 104) was used to assign species names to nearly identical sequences⁹. Explicitet was used for microbial community analyses and to identify specific bacteria that were differentially present between groups.

Results

Community composition. Treatment of the hospital hot water with monochloramine dramatically reduced the presence of *Legionella* and produced a starkly different microbial assemblage than in the untreated control water (Figure 1). Bacteria from the order Sphingomonadales and *Limnohabitans* dominated in the treated water while *Flexibacter* and the family Planctomycetaceae predominated in the control samples (Figure 1).

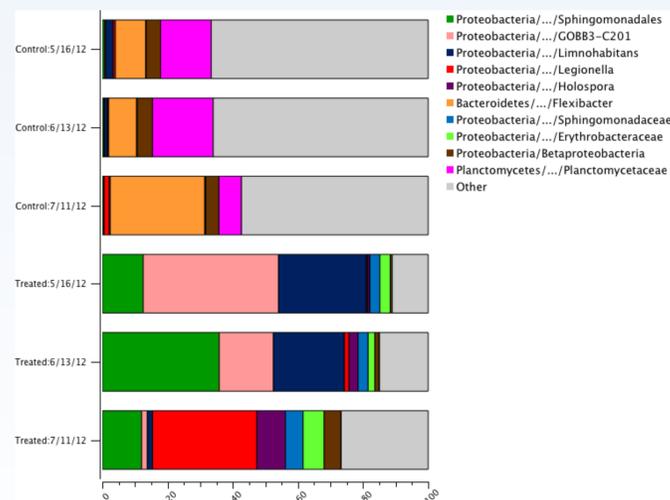


Figure 1: Taxonomic assignments of bacteria in control and treated samples by sampling month. Treated samples had lower bacterial diversity, with the top ten taxa accounting for 75-90% relative abundance clustered by month.

The bacteria from the control water were more diverse with the top ten taxa only accounting for approximately 35-45% relative abundance by month (Figure 1). During the month of July there was an influx of *Legionella* into the monochloramine treated sample as well as slightly in the control (Figure 1).

Community dissimilarity. The bacterial communities found in monochloramine treated and control samples were found to be very dissimilar (Figure 2). Pairwise comparisons between treated and untreated control samples pooled by month and by individual sample showed the most dissimilarity, with a Morisita-Horn Index below 0.09 (Fig. 2). **Comparison of treated and control taxa.** A comparison of bacteria identified in monochloramine treated and control water demonstrated highly significant differences in assemblages for almost all of the bacterial taxa identified in this study (Figure 3).

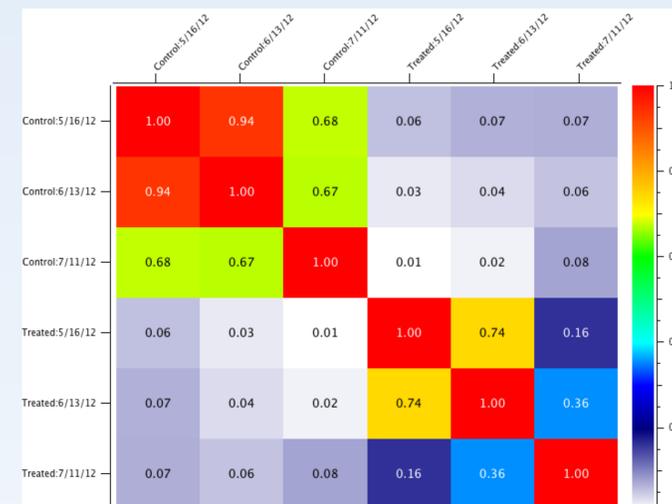


Figure 2: Bacterial community similarity, as calculated by Morisita-Horn, comparing control and treated months. Numbers represent the percent sequence similarity, with 1 being the most similar and 0 being the least (Morisita-Horn Index).

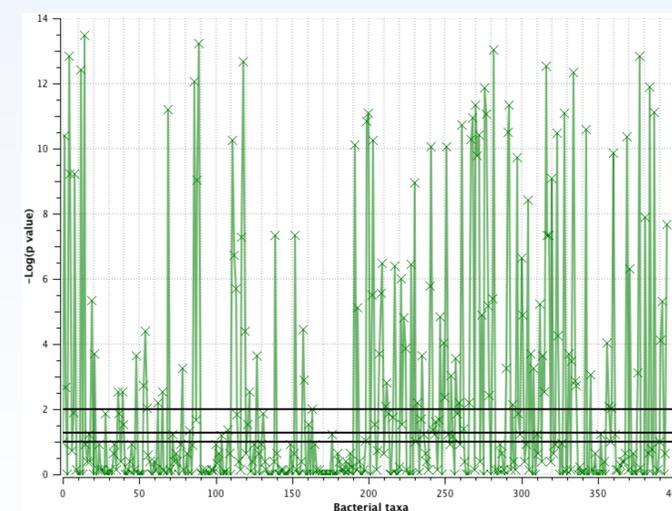


Figure 3: Manhattan plot comparing bacterial taxa identified in treated and untreated samples. Black lines represent p value thresholds of 0.10, 0.05, and 0.01, from bottom to top, respectively.

Conclusion

There was almost no overlap in the bacterial taxa present in the treated and control waters. We found this difference to be statistically significant and due to the monochloramine treatment. There was also a reduction in bacterial diversity in monochloramine treated communities. This may have led to the relative ease for *Legionella* to recolonize the system in July once the disinfectant was not being correctly formed due to degradation of the chlorine.

We did not find an increase in *Mycobacterium* species or nitrifying bacteria in the chloraminated water as some studies have found in municipal water systems.

This study shows a strong reduction in *Legionella* presence and a lack of many issues with chloramination found in municipal water supplies. As with any disinfection system it is important to regularly monitor levels of chemical components and the presence of opportunistic pathogens. Next generation sequencing may be beneficial to this end as it becomes more affordable and faster to perform.

References

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