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## Brief Report

## Risks of exposure to microbial contamination in eyewash stations

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## Key Words:

*Mycobacterium*

Water age

*Enterobacter*

Stagnation

## A B S T R A C T

Emergency eyewash stations are important safety equipment characterized by long water age. Significant increases in microbial contamination were detected in eyewash stations with water ages longer than 1 day. *Enterobacter* and *Mycobacterium* were identified in high abundance in eyewash stations with prolonged water age, suggesting eyewash stations as potential sources of pathogen exposure. Proper eyewash flushing was shown to be an effective practice to mitigate risks of exposure to microbial contaminants from eyewash use.

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Eyewash stations are important safety equipment for mitigating health risks of exposure to hazardous chemicals and infectious agents. One typical installation is plumbed eyewash stations connected to a source of potable water, most commonly tap water. To ensure an eyewash station is functional with clean water in the event of an emergency, weekly flush by running the eyewash for 1–3 minutes is recommended.<sup>1</sup> Accordingly, the water age in eyewashes is expected to be ~7 days. Studies of water quality in premise plumbing, that is, portions of the drinking water distribution system inside buildings, have demonstrated that risks of exposure to microbial contamination increase with prolonged water age.<sup>2–4</sup> Plumbed eyewash stations are essentially extensions of the premise plumbing, yet with considerably longer water age, suggesting elevated risks of microbial contamination in eyewashes. Furthermore, eyewash users could be particularly vulnerable to contaminated water due to existing ocular injuries. Therefore, it is important to evaluate the risks of exposure to microbial contaminants arising from eyewash use during an emergency.

In this study, eyewash stations with various water ages were investigated to determine the level of microbial contamination. Exposure risks were demonstrated by the identification of potential

pathogens with high-throughput sequencing, highlighting the need to develop strategies for reducing exposure to microbial contaminants from eyewash use.

## METHODS

To ensure eyewash stations included in this study were comparable, only plumbed eyewash stations installed in the same building following identical maintenance protocols were selected. To test the impact of water age on microbial contamination, water in triplicate eyewash stations was allowed to stagnate, resulting in water ages ranging from 1 to 7 days. To test the utility of flushing in lowering the level of microbial contamination, water samples were collected from triplicate eyewash stations with 7-day water age after 5, 10, and 20 minutes of flushing. The level of microbial contamination in water samples was quantified as heterotrophic plate counts (HPC) in colony forming units (CFUs) as previously described.<sup>5</sup>

To further identify microbial contaminants present in eyewash stations, water samples of 7-day water age were analyzed with 16S rRNA gene amplicon sequencing.<sup>6</sup> Water samples collected following 20 minutes of flushing, referred to as “fresh controls”, were also analyzed with sequencing as the baseline. Briefly, microbial cells in water samples were harvested with membrane filtration followed by DNA extraction, purification, PCR amplification, and paired-end high-throughput sequencing of 16S rRNA gene amplicons as previously described.<sup>6,7</sup> Bioinformatics analysis was performed with QIIME2 (version 2020.6) and the DADA2 denoising pipeline.<sup>7,8</sup> Raw

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sequences were deposited into the NCBI sequence read archive database (SRA) with the accession numbers SAMN31045930–SAMN31045935.

Proliferation of a specific microbial taxon due to prolonged water age was quantified as “fold change”:

$$\text{Fold Change} = \frac{F_{\text{water age}} \cdot MC_{\text{water age}}}{F_{\text{fresh control}} \cdot MC_{\text{fresh control}}} \quad (1)$$

where  $F$  is the fraction of microbial contaminants attributable to 16S rRNA gene sequences of a specific taxon at a specified water age or in fresh controls;  $MC$  represents the overall level of microbial contamination measured as HPC at a specified water age or in fresh controls.

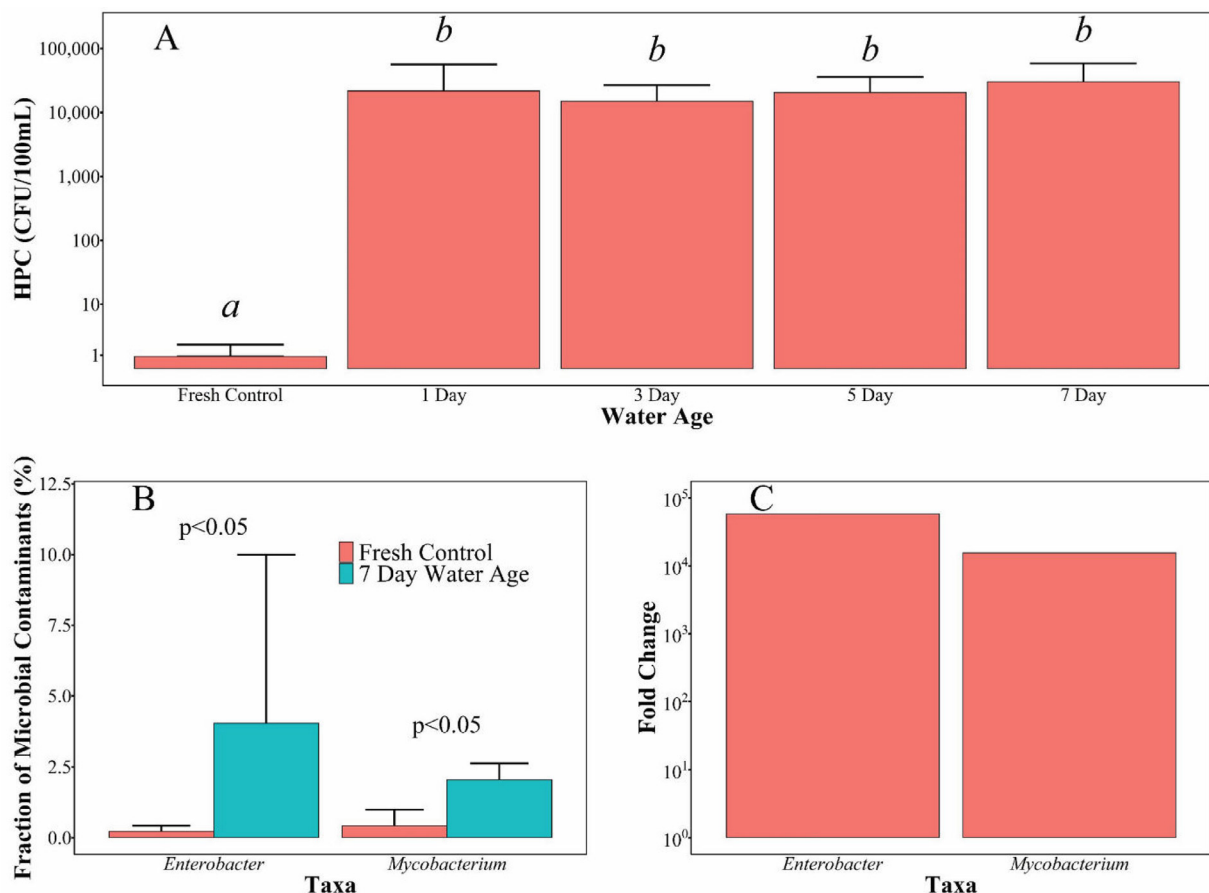
## RESULTS

Prolonged water age resulted in significant increases in microbial contamination in eyewash stations (Fig 1A). When the water age in eyewash stations was extended to 1 day, HPC as a measure of microbial contamination reached  $21,700 \pm 34,277$  CFU/100mL from  $1 \pm 1$  CFU/100mL in fresh controls. Extending the water age up to 7 days, however, did not lead to further increases in HPC (Fig 1A). Since typical eyewash stations have water ages considerably longer than 1 day, it is expected that high levels of microbial contamination in eyewash stations are likely prevalent.

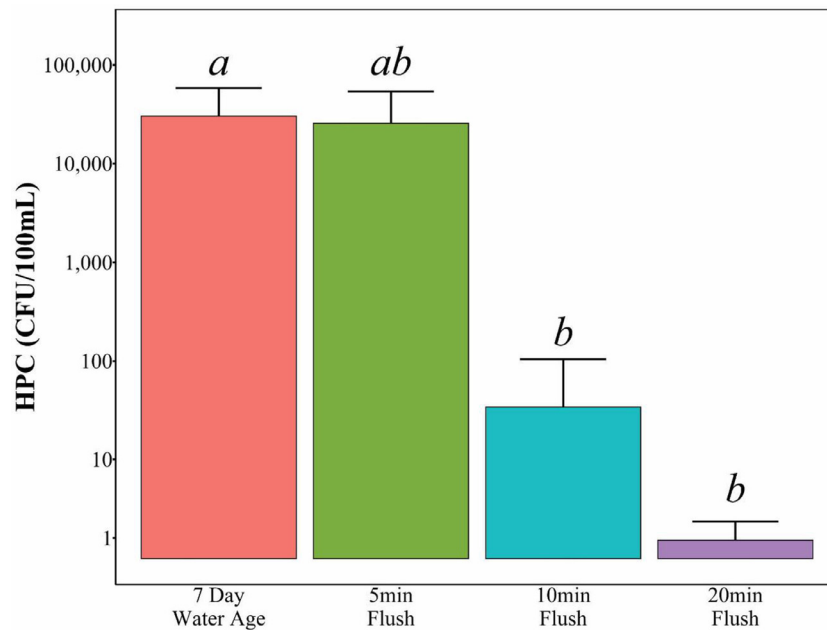
Microbial contaminants in eyewash stations were further profiled with 16S rRNA gene amplicon sequencing. Sequences of 2

potential pathogens, *Enterobacter* and *Mycobacterium*,<sup>9,10</sup> were identified to show significant increases in abundance with prolonged water age (Fig 1B). At 7-day water age, sequences of *Enterobacter* accounted for  $4.04 \pm 5.96\%$  of the microbial contaminants, a significant increase from  $0.22 \pm 0.20\%$  in fresh controls. Similarly, the fraction of *Mycobacterium* sequences in microbial contaminants rose significantly from  $0.42 \pm 0.57\%$  in fresh controls to  $2.05 \pm 0.57\%$  at 7-day water age (Fig 1B). Taking into consideration the significant increases in overall microbial contamination measured as HPC as a result of prolonged water age (Fig 1A), the abundance of *Enterobacter* and *Mycobacterium* experienced increases of 550,000-fold and 150,000-fold, respectively (Fig 1C). The identification of potential pathogens in high abundance is indicative of the potential risks of pathogen exposure during eyewash use and the need for developing practices to lower microbial contamination in eyewash stations.

Eyewash flushing could be a readily implementable practice to reduce microbial contamination. To determine the optimal flushing time, eyewash stations with 7-day water age were flushed for varying durations. Significant decreases in microbial contamination were observed only in eyewash stations flushed for 10 minutes or longer (Fig 2). On average, a 10-min flush reduced HPC from  $30,275 \pm 27,957$  CFU/100mL (7-day water age) to  $34 \pm 70$  CFU/100mL. Extending flushing to 20 minutes further reduced HPC to  $1 \pm 1$  CFU/100mL, evidence that proper flushing is effective in mitigating microbial contamination in eyewash stations.



**Fig 1.** Impact of prolonged water age on microbial contamination in eyewash stations. (A) Changes in HPC as a measure of microbial contamination in eyewash stations with increasing water age; HPC values are not significantly different from each other for water ages labelled with the same italic lowercase letters (i.e., *a* and *b*) (ANOVA with Tukey's Honest Significance test,  $P < .05$ ). (B) Fractions of microbial contaminants in eyewash stations attributable to *Enterobacter* and *Mycobacterium* at 7-day water age and in fresh controls as determined by 16S rRNA gene amplicon sequencing; statistical significance is indicated by  $P$ -values according to paired Student's  $t$  test. (C) Increases in the abundance of *Enterobacter* and *Mycobacterium* due to prolonged water age of 7 days represented by fold change as calculated according to Eq. 1. Error bars represent standard deviations. Fresh controls stand for water samples collected from eyewash stations following 20 minutes of flushing.



**Fig 2.** Impact of flushing on HPC as a measure of microbial contamination in eyewash stations with 7-day water age. Error bars represent standard deviations. HPC values are not significantly different from each other for flushing times labeled with the same italic lowercase letters (i.e., *a* and *b*) according to ANOVA with Tukey's Honest Significance test ( $P < .05$ ).

## DISCUSSION

This study revealed elevated microbial contamination in eyewash stations with prolonged water age, that is, >1 day (Fig 1), which is consistent with observations of significant bacterial growth in drinking water distribution systems due to water stagnation.<sup>2,4,5</sup> Notably, populations of potential pathogens linked to *Enterobacter* and *Mycobacterium*<sup>9,10</sup> were identified in high abundance with extended water age in eyewash stations, suggesting risks of exposure to pathogenic contaminants via eyewash use. As a potential risk mitigation practice, daily flushing for 10–20 minutes was shown to be effective in lowering the level of microbial contamination in plumbed eyewash stations tested in this study, likely due to the depletion of water with prolonged water age and high levels of microbial contamination, followed by the inflow of fresh tap water with minimal microbial contamination. Water disinfection options, such as UV treatment, could be amended to eyewash stations, which however may be a concern of economic feasibility. Therefore, proper flushing remains to be a readily implementable and effective practice to reduce exposure to microbial contamination in eyewash stations. Inclusion of other configurations of eyewash in further investigations is warranted to extrapolate findings from this study.

## CONCLUSIONS

Eyewash stations could be potential sources of pathogen exposure due to elevated levels of microbial contamination due to prolonged water age. Proper eyewash flushing was shown to be an effective

practice to mitigate risks of exposure to microbial contaminants from eyewash use.

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