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Major Article

Efficacy of laundering and tumble-drying in reducing microbial contamination of wastewater treatment plant worker coveralls

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Key Words:
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Background: Evidence implicates textiles in health care as potential reservoirs of pathogens. No similar data exist for the wastewater treatment plant (WWTP) industry. We investigated if coveralls worn by WWTP workers could present occupational infection risk.

Methods: We enumerated heterotrophic plate counts (HPCs), total coliforms, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Clostridium difficile*, and *Acinetobacter* spp on coverall swatches experimentally contaminated with raw, primary, secondary, and final effluent. Contaminated swatches were examined by culture-based methods after laundering, tumble-drying, and storing.

Results: Concentrations of microorganisms and efficacy of decontamination differed depending on the contaminating wastewater matrix and the organism. Laundering was an effective decontamination method for coveralls contaminated with all microorganisms, except HPCs. Tumble-drying resulted in statistically significant decreases for HPCs, *P aeruginosa*, and *Acinetobacter*. Increases in contamination after laundering were seen in *Acinetobacter* spp, in *P aeruginosa* when coverall swatches were contaminated with raw and final effluent, and in HPCs when contaminated with secondary effluent.

Discussion: Results suggest that solely laundering at 60°C for 25 minutes as per ASTM Standard F1449 may not always be an efficient means of controlling microorganisms on coveralls.

Conclusions: Clearer guidelines are needed to better protect WWTP workers.

Although the main route of occupational infection transmission remains person-to-person contact, the role of the environment in the spread of infection should not be underestimated.[1] Environmental transmission is particularly relevant in settings where direct or indirect exposure to pathogens during routine tasks is to be expected, such as health care institutions, food processing environments, and wastewater treatment plants (WWTPs). WWTP workers interact with a medium that inherently presents potential for occupational infections, as it carries varying concentrations of bacteria, viruses, fungi, and protozoa at different stages of treatment.[2-4] Workers and their equipment could become exposed to pathogens directly as a result of contact with wastewater or indirectly through bioaerosols and fomites.[2,5] Most plants supply their operators, utility crews, and plant maintenance crews with the necessary coveralls and personal protective equipment to ensure employees are minimally exposed. Published research on hospital textiles and uniforms has documented the contamination of fabrics with a wide range of indicator organisms and pathogens, such as *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus pyogenes*, *Bacillus cereus*, *Acinetobacter baumannii*, *Pseudomonas* spp, *Klebsiella* spp, *Salmonella typhimurium*, *Salmonella hadar*, enterococci, fecal coliform bacteria, hepatitis A, rotavirus, adenovirus, *Sarcoptes scabiei*, and *Microsporum canis*.[1,6-10] No similar studies exist in the wastewater industry. The risk of infection from contaminated coveralls used in tasks involving raw, partially treated, or treated wastewater remains unknown.

At a WWTP, once-soiled coveralls could be laundered at the plant, sent out for laundering to specialized facilities, or laundered at home. The Centers for Disease Control and Prevention's guidelines for environmental infection control in health care facilities[11] contain many recommendations for laundering textiles in the health care environment. The guidelines highlight the importance of using commercial washing machines capable of 71°C and the use of bleach. Unfortunately, these recommendations are in direct conflict with "ASTM F1449-08 (2015): Standard guide for industrial laundering of flame, thermal, and arc resistant clothing,"[12] which is what industrial facilities often rely on for all coverall laundering. The ASTM standard suggests that industrial washing machine formulas are developed using

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detergents and wash temperatures (up to 60°C) adequate to thoroughly clean all contaminants from garments. The removal of pathogens has never been a consideration.

We conducted this study to address a few knowledge gaps in the occupational health literature as it relates to WWTP workers. We wanted to understand whether, similar to textiles in the health care industry, WWTP coveralls that come into contact with wastewater effluent can become contaminated with indicator organisms and pathogens that could be considered a potential source of occupational infections. If so, does laundering contaminated coveralls at current industry standards (60°C) effectively reduce the microbial burden? To address this, we enumerated heterotrophic plate counts (HPCs), total coliforms (TCs), *E coli*, *Pseudomonas aeruginosa*, *S aureus*, MRSA, *Clostridium difficile*, and *Acinetobacter* spp on coveralls that were experimentally contaminated with raw, partially treated, or fully treated wastewater at different stages of decontamination. Our hypothesis was that WWTP coveralls exposed to wastewater effluent would be contaminated with indicator organisms and pathogens that would require decontamination after usage, and that laundering would be an effective decontamination method.

METHODS

Wastewater treatment plant

The study was conducted at the Gold Bar WWTP in Edmonton, Alberta, Canada. The plant serves a population of approximately 820,000 residents. It treats 310 million liters of wastewater per day, with peak flows up to 910 million liters per day. The plant is a tertiary treatment plant that receives raw wastewater from the sewer system and performs the following treatment steps: (1) primary sedimentation in clarifiers, which produces a primary effluent; (2) secondary treatment by biological nutrient removal, which produces a secondary effluent; and (3) ultraviolet disinfection to inactivate a wide range of microbial pathogens before returning this final treated effluent to the North Saskatchewan River. The plant employs more than 150 employees who interact with different effluents to varying degrees, thereby soiling their coveralls and personal protective equipment.

The Gold Bar WWTP uses a Continental Girbau E-Series (model EH040) industrial washing machine (Girbau, Vic, Spain) at 60°C for 25 minutes as per ASTM Standard F1449-08.[12] Three chemicals are used during each laundry load and are automatically dosed into the washing machine: 6 oz of high-pH alkaline break, XLQ052, alkaline laundry booster, sodium hydroxide solution, product #B240 (Challenger Clean Systems, Edmonton, AB); 4 oz of laundry oil emulsifier, XLQ072, high-density solvent oil emulsifier, product #B256 (Challenger Clean Systems); and 2 oz of sour/soft, XLQ061, laundry softener and neutralizer, product #B243 (Challenger Clean Systems).

Coverall inoculation and swatch preparation

In July 2017, 4 clean coveralls were collected from utility workers at the plant. Three clean 12 × 8.5-cm swatches (total area = 102 cm^2) were removed from each coverall and labeled "clean." These were used to establish baseline levels of contamination prior to exposing the remainder of each coverall to 1 of 4 wastewater matrices: raw, primary, secondary, and final effluent. Then, each coverall was immersed in the selected wastewater matrix for 5 minutes (ie, 1 coverall per matrix). The coveralls were removed from the wastewater, placed in sterile bags, and allowed to sit overnight in the dark to simulate what would happen if coveralls were not washed immediately after a shift or upon coverall reuse.

The next day, each coverall was removed from its bag, and enough material was cut off to allow for the analysis of three 102 cm^2 swatches. These swatches were labeled "contaminated." The remainder of the coveralls were then placed in the washing machine and laundered at 60°C for 25 minutes as per ASTM Standard F1449-08.[12] These are the most common laundering conditions used in industrial settings, as they avoid coverall fading and shrinkage. After laundering, enough material was removed to allow for the analysis of three 102 cm^2 swatches labeled "laundered." After laundering, the coveralls were placed in an industrial tumble dryer programmed to standard industry settings to avoid shrinkage (low heat setting for 21 minutes). After the cycle was completed, enough material was removed to allow for the analysis of three 102 cm^2 swatches labeled "tumble dried." Remaining coveralls were stored in the lab for 2 weeks, and three 102 cm^2 swatches were analyzed and labeled "stored" to identify regrowth potential or contamination during storage.

Microbiological analyses

Each swatch was placed in a separate Ziploc bag (S.C. Johnson & Son, Racine, WI) with 10 mL of sterile phosphate buffered saline (PBS). Homogenization of samples was achieved by massaging the swatches manually for 15-20 seconds. The liquid inside each bag was used to enumerate the following organisms: HPCs, TCs, *E coli*, *P aeruginosa*, *S aureus*, MRSA, *C difficile*, and *Acinetobacter* spp. All analyses were run in duplicate. Appropriate ATCC positive and negative controls were used for each organism isolation and enumeration.

To enumerate HPCs, appropriate dilutions were prepared from the homogenized swatch liquid, and 100 μL was spread plated onto prepared R2A agar plates (EMD Millipore, Billerica, MA). The plates were incubated at 35°C ± 2°C for 48 hours under aerobic conditions, and colonies were counted at the end of the incubation period. Total coliforms and *E coli* were enumerated by defined substrate analysis using Colilert (IDEXX, Markham, ON). Two milliliters of homogenized liquid was poured into 100-mL Colilert sample vessels. The vessels were topped off with sterile water and poured into Quanti-Trays (IDEXX), and then enumerated for TCs and *E coli* after incubation at 35°C for 24 hours. *E coli* and *Acinetobacter* spp identifications were confirmed by plating purified colonies onto tryptic soy agar, preparing pure colony suspensions in 5 mL of 0.85% saline and homogenizing. The homogenized suspension was used to inoculate API 20E strips (Biomerieux, Saint-Laurent, QC) as per the manufacturer's instructions.

P aeruginosa was enumerated by Pseudalert (IDEXX) in a manner similar to Colilert and incubated at 38°C for 24 hours. *C difficile* was enumerated by isolation on *C difficile* selective agar. One hundred microliters of homogenized liquid was spread plated onto BBL *C difficile* selective agar (CDSA; BD, Sparks, MD) and incubated at 35°C for 48-72 hours inside a GasPak EZ anaerobic system (BD) as per the manufacturer's instructions. The plates were removed and examined for growth and fluorescence. Target colonies were pale to bright yellow and exhibited fluorescence. *S aureus* was enumerated by BBL CHROMagar (BD). One hundred microliters of homogenized liquid was spread plated onto CHROMagar and incubated at 35°C for 24 hours under aerobic conditions. Plates were removed and examined for growth after incubation. Target colonies were mauve or orange-mauve in color. MRSA identification was performed using antibiotic susceptibility testing as per Fernandes et al.[13] Briefly, we performed a disk diffusion method on ISO-Sensitest agar with cefoxitin (Oxoid, Nepean, ON). The cefoxitin minimum inhibitory concentration for methicillin-susceptible strains was ≤4 mg/L.

Statistical analyses

To compare and visualize means of pathogen and indicator organism concentrations, the average concentrations were calculated by decontamination stage (clean, contaminated, laundered, dried, stored) and wastewater treatment level (raw, primary, secondary,

and final effluent). Since 3 swatches were used and analyses were run in duplicate, the reported means and SDs were run on 3 sets of duplicates (n = 3). We were unable to pool our concentration data by decontamination stage because the microorganism concentrations were not normally distributed (normality test, $P < .05$) and did not have equal variances across different wastewater treatment levels (Bartlett test for both, $P < .05$) and sampling was not random. Nonparametric techniques were therefore used.

To identify significant differences between decontamination stages and interactions between variables, we constructed a fit least squares means (LSM) model and ran an analysis of variance (ANOVA) on the results. In the LSM model, pathogen or indicator organism concentrations were used as the response variable, whereas 3 variables were used as effect predictors: decontamination stage of overalls, wastewater treatment level, and the interactions between both variables (n = 120). We ran an ANOVA on the same LSM model to evaluate the effects of laundering and drying separately.

To calculate log reductions in microorganism concentrations to represent decontamination efficacy, we subtracted the log of one decontamination stage from the log of the next stage of interest. The same previously described LSM ANOVA was used to compare the log reductions between various organisms. For all tests, a 2-tailed P value <.05 was considered indicative of statistical significance. JMP 13 (SAS Institute, Cary, NC) was used for all statistical analyses.

RESULTS

Effects of decontamination stages on indicator organism and pathogen concentrations

The mean concentrations of indicator organisms and pathogens on swatches and the effectiveness of decontamination varied depending on wastewater treatment levels (Table 1, Fig. 1 and 2). In the case of most organisms, contamination with raw water resulted in coveralls carrying the highest microbial loads; the exceptions were *E coli*, *P aeruginosa*, and *Acinetobacter* spp. In addition, in most cases, organisms experienced a sharp decrease in concentration as a result of laundering, the only exceptions being *P aeruginosa* recovered from swatches contaminated with raw or final effluent and *Acinetobacter* contaminated with any wastewater matrix. Despite these trends, ANOVA results highlighted the finding that decontamination was effective in all cases ($P < .05$). Laundering resulted in a statistically significant decrease in concentration of organisms from contaminated swatches in all cases, except that of HPCs. This effect was significant but much weaker in the case of *P aeruginosa*, *C difficile*, and *Acinetobacter* spp. Interestingly, the tumble-drying resulted in only a statistically significant decrease in organism concentrations after laundering for HPCs, *P aeruginosa*, and *Acinetobacter*. The overall results show that coveralls of WWTP workers can be contaminated with high concentrations of indicator organisms and pathogens as a result of exposure to different wastewater effluents, thereby making coveralls potential sources of contamination and infection. The results also highlight the finding that decontamination of coveralls exposed to different wastewater matrices by laundering and tumble-drying is effective at reducing microbial loads and can protect occupational health.

Efficacy of laundering contaminated coveralls

To evaluate the efficacy of laundering and drying in reducing microbial contaminant loads on coveralls of WWTP workers, we calculated the log reductions in microbial concentrations by pathogen for each decontamination stage and wastewater treatment level. The results are presented in Table 2. Negative numbers represent increases in organism concentrations. In some cases, such as *C difficile*, the numbers were too low to give reliable results, and the symbol "-" was used to denote log (0).

When coverall swatches were contaminated with raw and primary effluent, laundering and drying had the highest log reductions for HPCs and TCs, followed by *E coli*, *P aeruginosa*, *S aureus*, MRSA, *C difficile*, and *Acinetobacter*, respectively. When coverall swatches were contaminated with secondary effluent, laundering and drying had the highest log reductions for HPCs, TCs, *P aeruginosa*, and *E coli*, followed by *S aureus*, MRSA, and *Acinetobacter*, respectively. Finally, when coverall swatches were contaminated with final effluent, laundering and drying had the highest log reductions for *E coli*, HPCs, *P aeruginosa*, and TCs, followed by *S aureus* and MRSA, respectively. It is also worth noting that in the case of raw wastewater, tumble-drying was more effective than laundering at decreasing HPC and *P aeruginosa* concentrations by a statistically significant amount. Although this relationship was also seen in primary, secondary, and final effluent, those decreases were not statistically significant. This highlights the finding that different organisms experience different log removals during different decontamination stages, and this is highly dependent on contaminating wastewater treatment level. Considering that wastewater is heavily contaminated by multiple organisms, and since worker coveralls are not usually separated by contaminating matrix during laundering, identifying the decontamination method that will result in the highest log reductions across all contaminants is the best management strategy.

CONCLUSIONS

There is a growing body of evidence implicating uniforms and other textiles in the health care industry as potential reservoirs of pathogenic and multidrug-resistant organisms.[6,8,14,15] We conducted a study to determine if coveralls worn by WWTP workers performing tasks that require contact with raw, partially treated, or fully treated wastewater could potentially result in occupational infection risk comparable to those in health care settings. We enumerated some commonly detected indicator organisms and pathogens found on textiles in other settings (HPCs, TCs, *E coli*, *P aeruginosa*, *S aureus*, MRSA, *C difficile*, and *Acinetobacter* spp). We experimentally contaminated coveralls with raw, partially treated, or fully treated wastewater and examined swatches removed from these coveralls at different stages of decontamination. We detected a wide range of indicator organisms and pathogens on WWTP coveralls, some of which were only partially removed by laundering and more effectively removed by tumble-drying. These findings support the hypothesis that WWTP coveralls can be reservoirs of infectious agents, and that clearer industry decontamination guidelines would benefit occupational health.

Health care experts noted several decades ago that a considerable amount of attention is given to cleaning and disinfecting nonporous surfaces and fomites, but less emphasis is placed on effective reduction of pathogenic burden on porous surfaces and textiles.[15] Monitoring indicator organisms, such as HPCs and TCs, is an effective strategy for demonstrating the relative cleanliness of textiles in high-exposure environments.[8,10] Coliform bacteria are particularly useful because they imply fecal or environmental contamination, tend to remain viable on textiles for extended periods of time, and are easy to culture. Correlating with our results, Nordstrom et al[10] detected TCs, *S aureus*, and *E coli* on unwashed hospital scrubs, and TCs remained viable on home-laundered scrubs. However, Nordstrom et al[10] did not detect *C difficile* on any uniforms. This may be a function of concentrations of pathogens in the contaminating wastewater matrix. Colclasure et al[8] reported that TC survival on textiles is enhanced by dark and cool conditions—conditions that we replicated during coverall inoculation. The isolation of *S aureus* and MRSA from contaminated coveralls was expected because various studies have reported the presence of

Table 1
Mean concentration of pathogens and indicator organisms (HPCs, TCs, *P aeruginosa*, *S aureus*, MRSA, *C difficile*, and *Acinetobacter* spp ± SD) recovered from coverall swatches contaminated with raw, primary, secondary, and final effluent after various stages of the laundering process (n = 3)

Decontamination stage	Wastewater treatment level	HPC Mean ± SD	TC Mean ± SD	*E coli* Mean ± SD	*P aeruginosa* Mean± SD	*S aureus* Mean ± SD	MRSA Mean ± SD	*C difficile* Mean ± SD	*Acinetobacter* Mean ± SD
Clean	Raw	4 ± 2	0	0	0	0 ± 1	0	0	3 ± 5
	Primary effluent	4 ± 2	0	0	0	0	0	0	0 ± 0
	Secondary effluent	1 ± 1	0	0	0	1 ± 1	0	0	1 ±1
	Final effluent	11 ± 14	52 ± 89	0	8 ± 14	1 ± 1	1 ± 1	0	3 ±1
Contaminated	Raw	$1.3 \times 10^8 \pm 4.2 \times 10^7$	$1.1 \times 10^6 \pm 9.2 \times 10^5$	5.1 ± 0	$4.5 \times 10^3 \pm 7.7 \times 10^3$	$3.5 \times 10^3 \pm 3.7 \times 10^3$	253 ± 238	40 ± 34	10 ± 10
	Primary effluent	$6.6 \times 10^7 \pm 1.3 \times 10^7$	$6.2 \times 10^4 \pm 1.1 \times 10^4$	5.1 ± 0	$4.0 \times 10^4 \pm 1.1 \times 10^4$	$1.3 \times 10^3 \pm 7.3 \times 10^2$	48 ± 31	0	14 ± 2
	Secondary effluent	$4.9 \times 10^6 \pm 6.1 \times 10^6$	$5.2 \times 10^4 \pm 4.3 \times 10^3$	$1.9 \times 10^4 \pm 4.8 \times 10^3$	$5.1 \times 10^4 \pm 0$	$1.4 \times 10^2 \pm 1.2 \times 10^2$	13 ± 6	0	6 ± 2
	Final effluent	$4.4 \times 10^4 \pm 3.1 \times 10^4$	$2.0 \times 10^3 \pm 2.0 \times 10^3$	$5.1 \times 10^4 \pm 0$	$6.7 \times 10^3 \pm 6.3 \times 10^3$	47	6 ± 5	0	1 ± 1
Laundered	Raw	$7.5 \times 10^7 \pm 1.8 \times 10^7$	$1.7 \times 10^3 \pm 1.5 \times 10^3$	0	$4.9 \times 10^4 \pm 5.0 \times 10^3$	$2.0 \times 10^2 \pm 1.5 \times 10^2$	2 ± 2	5 ± 2	15 ± 9
	Primary effluent	$2.7 \times 10^7 \pm 1.7 \times 10^7$	$1.9 \times 10^3 \pm 1.7 \times 10^3$	2	$7.6 \times 10^3 \pm 5.7 \times 10^3$	0	0	0	16 ± 8
	Secondary effluent	$5.1 \times 10^6 \pm 6.9 \times 10^6$	$1.4 \times 10^3 \pm 1.2 \times 10^3$	0	$1.1 \times 10^4 \pm 9.6 \times 10^3$	0	0	0	28 ± 19
	Final effluent	$4.2 \times 10^4 \pm 4.1 \times 10^4$	$1.6 \times 10^3 \pm 1.5 \times 10^3$	0	$1.1 \times 10^4 \pm 1.6 \times 10^4$	0	0	1 ± 2	8 ± 7
Tumble dried	Raw	0 ± 0	0	0	0	0	0	0	0
	Primary effluent	3 ± 4	0	0	0	0	0	0	0
	Secondary effluent	0 ± 1	0	0	0	0	0	0	0
	Final effluent	6 ± 9	0	0	0	0	0	0	0
Stored	Raw	23 ± 25	6 ± 8	0	3 ± 3	6 ± 7	0	0	3 ± 5
	Primary effluent	22 ± 6	3 ± 4	0	1 ± 1	0	0	0	0
	Secondary effluent	3 ± 1	2 ± 1	0	0	1 ± 1	0	0	1 ± 1
	Final effluent	$83 \pm 1.3 \times 10^2$	52 ± 89	0	8 ± 14	1	1	0	3 ± 1
Fit least squares (n = 120)	Whole model ANOVA *P* value	<.0001	.0001	<.0001	<.0001	<.0001	<.0001	.0019	.0075
Contaminated>> laundered		0.0581	0.0057	<0.0001	0.0282	<0.0001	<0.0001	0.0371	0.0136
Laundered>>dried		0.0371	0.9880	0.9999	0.0054	0.8412	0.9893	0.7073	<0.0001

ANOVA, analysis of variance; *C difficile*, *Clostridium difficile*; *HPCs*, heterotrophic plate counts; *MRSA*, methicillin-resistant *Staphylococcus aureus*; *P aeruginosa*, *Pseudomonas aeruginosa*; *S aureus*, *Staphylococcus aureus*; *TCs*, total coliforms.

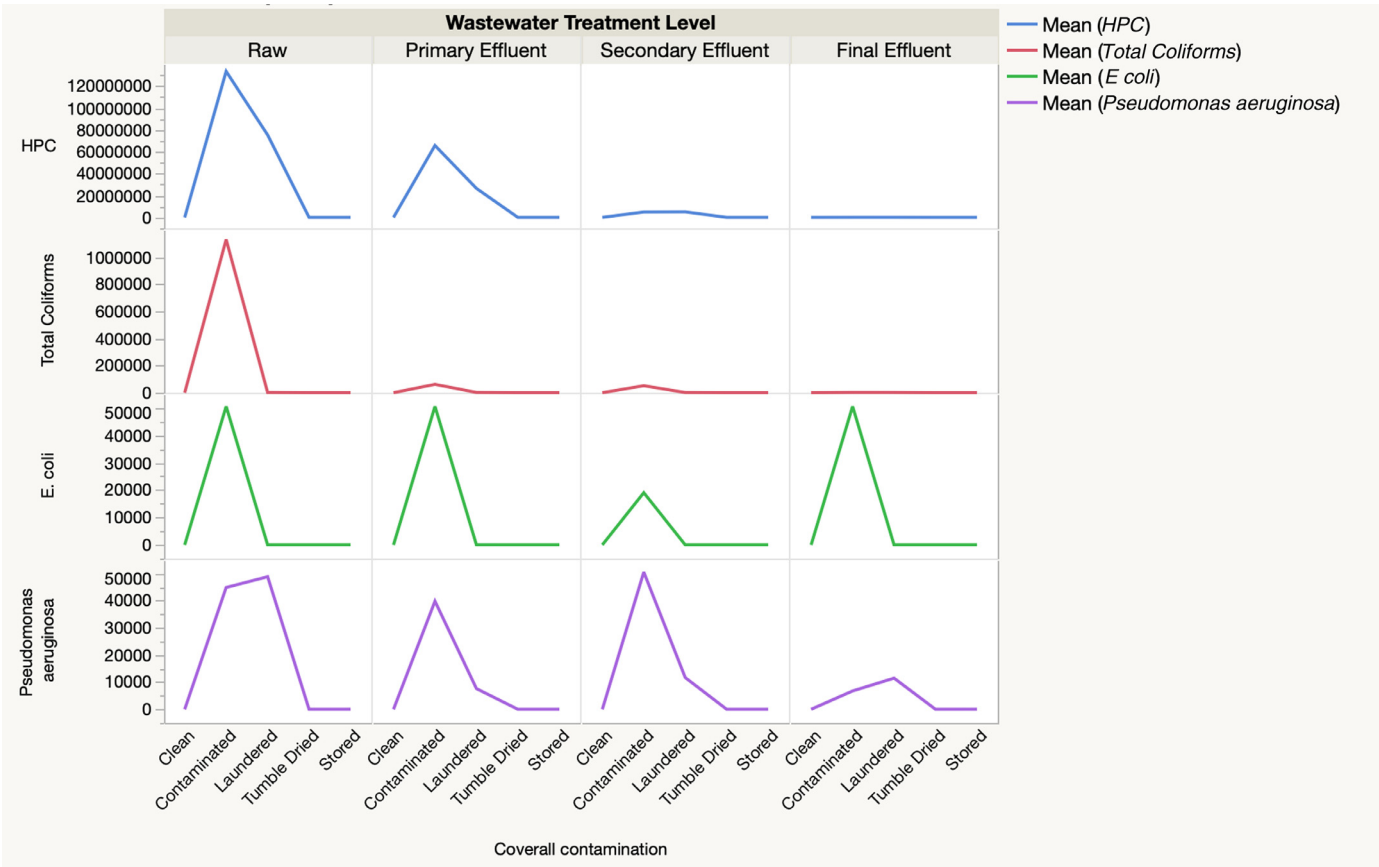

Fig 1. Mean concentrations of heterotrophic plate counts (HPCs), total coliforms, *Escherichia coli*, and *Pseudomonas aeruginosa* during decontamination of coveralls contaminated with wastewater at different treatment levels (n = 3).

MRSA in wastewater and activated sludge.[16] The elimination of *S aureus* by washing at 60°C correlates with the results of other authors.[17] The isolation of *Acinetobacter* spp from scrubs has been reported in the literature as well.[6] Munoz-Price et al[6] found that the majority of organisms isolated from scrubs were normal skin flora, but that potential pathogens, including *A baumannii*, *S aureus*, and enterococci, were identified. Thus, WWTP coveralls, much like uniforms, accumulate a complex microbial community during a shift as a result of both the wearer's natural biota and the interactions with the work environment that contains the potential pathogens.[18] Our results highlight the finding that there are many parallels between contamination of personal protective equipment in health care and other less explored occupational settings.

The Occupational Health and Safety Administration defines decontamination as a physical means to remove, inactivate, or destroy pathogens to the point where they are no longer capable of transmitting infectious particles.[19] In the wastewater industry, coveralls are decontaminated by laundering and drying, which, in combination, we found to be effective at reducing pathogen and indicator organism concentrations. Many variables affect the level of contamination detected on washed scrubs, including initial level of contamination, laundry procedure used, and storage conditions.[10] Interestingly, laundering resulted in increases in the detected number of pathogens in some cases due to cross-contamination, which correlates with the findings of other authors.[7] Similar to other studies, we found that storage influenced indicator and pathogen concentrations. Fijan and Turk[9] concluded that insufficient antimicrobial laundry procedures result in the spreading of microorganisms throughout previously clean areas of laundry facilities. The authors recommended workers receive regular training and education on disinfecting procedures for all laundry areas to prevent the recontamination of laundered textiles during postlaundry handling processes such as sorting, ironing, folding, and storing.[9] The effectiveness of thermal disinfection and mechanical agitation resulting from tumble-drying has also been reported in the health care literature. Wilson et al[14] highlighted the effectiveness of tumble-drying, indicating reductions in pathogens of up to 10^9, with a greater effect on gram-negative than gram-positive microorganisms. Nordstrom et al[10] found similar results and suggested that all hospital attire be dried completely in a dryer and then stored in a manner that would ensure continued cleanliness and minimize fungal growth.

In adherence to ASTM Standard F1449, industrial clothing manufacturers and commercial laundering machine suppliers regularly emphasize that (1) industrial detergents and wash temperatures of 60°C are adequate to thoroughly clean all contaminants from garments in industrial settings, and (2) sodium hypochlorite and other chlorine-based bleaches should not be used for laundering.[12] The main goal of these manufacturers and suppliers is removal of stains, grease, and fats, while maintaining the protective qualities of the garments and reducing coverall fading and staining; the removal of pathogens is not intended. The laundering machine used in this investigation certainly meets industry standards.

Clearer guidelines are needed to ensure WWTP workers are also protected from horizontal transmission of pathogens present in their occupational environment. For example, there may be benefits to following Centers for Disease Control and Prevention recommendations[11] and laundering coveralls at 71°C for at least 3

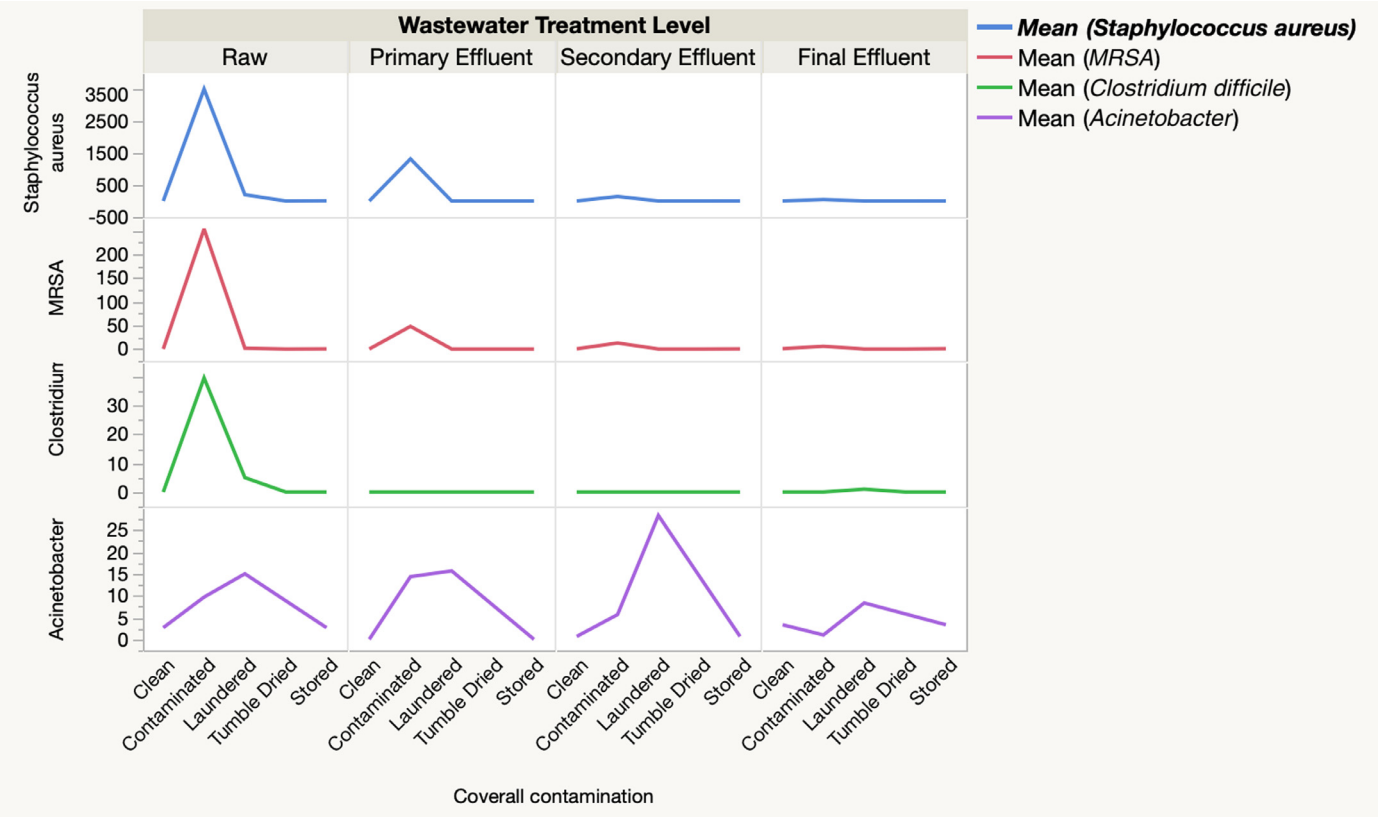

Fig 2. Mean concentrations of *Staphylococcus aureus, methicillin-resistant Staphylococcus aureus* (MRSA), *Clostridium difficile*, and *Acinetobacter* spp during decontamination of coveralls contaminated with wastewater at different treatment levels (n = 3).

minutes or at 65°C for 10 minutes to achieve effective thermal disinfection during laundering.[9,15,17] If maintaining the cycle water temperature at 65°C for 10 minutes is not possible, one of the many hydrogen peroxide systems with activators could be used. One example is the tetraacetylethylenediamine/sodium perborate activator system that has been shown to have kill power at 40°C equivalent to thermal disinfection at 71°C.[20] The addition of bleach is highly recommended if coveralls are not fire resistant

Table 2
Log reductions in microbial loads for HPCs, TCs, *P aeruginosa*, *S aureus*, MRSA, *C difficile*, and *Acinetobacter* spp for different contaminating wastewater effluents as a result of laundering, tumble-drying, and storing (n = 3)

		Log reductions in microbial contamination							
Wastewater treatment level	Coverall contamination	HPC	TC	*E coli*	*P aeruginosa*	*S aureus*	MRSA	*C difficile*	*Acinetobacter*
Raw	Clean>>contaminated	**−7.56**	**−6.05**	−4.70	−4.65	**−4.02**	**−2.40**	**−1.60**	−0.56
	Contaminated>>laundered	0.25	**2.83**	4.70	−0.04	**1.25**	**2.18**	**0.90**	−0.19
	Laundered>>dried	**7.88**	3.22	-*	**4.69**	2.29	0.22	0.70	1.18
	Dried >>stored	−1.37	−0.80	-	−0.48	−0.75	-	-	−0.43
	Contaminated>>dried	8.12	6.05	4.70	4.65	3.54	2.40	1.60	0.99
Primary effluent	Clean>>contaminated	−7.22	−4.79	−4.70	−4.60	−3.12	−1.68	-	−1.16
	Contaminated>>laundered	0.39	1.51	4.40	0.72	3.12	1.68	-	−0.04
	Laundered>>dried	6.95	3.29	0.30	3.88	0.00	-	-	1.19
	Dried >>stored	−0.87	−0.52	-	0.18	0.00	-	-	0.00
	Contaminated>>dried	7.34	4.79	4.70	4.60	3.12	1.68	-	1.16
Secondary effluent	Clean>>contaminated	**−6.69**	−4.71	**−4.28**	−4.70	**−2.45**	**−1.10**	-	−0.93
	Contaminated>>laundered	−0.01	1.57	**4.28**	0.64	**2.15**	**1.10**	-	**−0.70**
	Laundered>>dried	7.18	3.14	-	4.07	-	-	-	1.45
	Dried >>stored	−0.95	−0.30	-	-	0.18	-	-	0.18
	Contaminated>> dried	7.17	4.71	4.28	4.70	2.15	1.10	-	0.75
Final effluent	Clean>>contaminated	−3.62	−1.58	−4.70	−2.93	−1.75	−0.93	-	0.52
	Contaminated>>laundered	0.02	0.08	4.70	−0.23	1.67	0.75	-	−0.92
	Laundered>>dried	3.87	3.21	-	4.06	-	-	-	0.92
	Dried >>stored	−1.17	−1.72	-	−0.92	0.18	0.18	-	−0.52
	Contaminated>> dried	3.89	3.29	4.70	3.83	1.67	0.75	-	0.00

NOTE. Bolded numbers indicate statistically significant changes ($P < .05$).
C difficile, *Clostridium difficile*; *E coli*, Escherichia coli; *HPCs*, heterotrophic plate counts; *MRSA*, methicillin-resistant *Staphylococcus aureus*; *P aeruginosa*, *Pseudomonas aeruginosa*; *S aureus*, *Staphylococcus aureus*; *spp*, species; *TCs*, total coliforms.
*Symbol "-" represents log (0) and cannot be calculated.

or retardant.[7] When purchasing new coveralls, the possibility of the addition of bleach should be taken into account, as should the possibility of purchasing engineered fabrics that exhibit fluid repellency and antibacterial qualities. Even laundering schedules have an impact on decontamination effectiveness. Wiener-Well et al[21] found higher contamination of uniforms when washing was performed every 2 days rather than daily. If WWTP workers are performing tasks involving highly contaminated matrices, such as raw or primary effluent, coveralls should be changed after every shift, even if they appear clean, and washed the same day. Coveralls should not be worn outside the WWTP, even if they appear clean, so as to avoid the potential for transmission to the public. Storage conditions of clean coveralls should be closely examined, and inanimate surfaces that come into contact with coveralls should be wiped down periodically to ensure no residual pathogenic burden remains. Finally, home laundering of contaminated coveralls should be discouraged to avoid cross-contamination with regular street clothing.

This study has several limitations. The sample size is very small, but using the LSM model for statistical analyses increased statistical power. The microbiological quality of wastewater experiences high variability, and the results could have been quite different had multiple samples been taken from each treatment stage or had samples been collected on different dates. In the case of *Acinetobacter*, it is possible that other sources of contamination were missed, as the trends were very unusual. Determination of pathogen concentrations was based on a standard textile area of 102 cm^2, a technique common in these types of studies. However, differences in textile characteristics (composition, hydrophilicity, etc) and effects of dilution (ie, water to fabric ratio) during laundering were not taken into consideration. Finally, we soaked the coveralls in effluents. No task performed at Gold Bar WWTP would result in that level of direct exposure, and most high-exposure tasks require the donning of water-resistant Tyvek suits (DuPont, Wilmington, DE). Despite these limitations, our results imply that WWTP workers could be exposed to pathogens as a result of wearing contaminated coveralls, and that decontamination of coveralls is essential. More studies evaluating contamination of different coveralls in high-exposure industries based on number of days of wear, water temperature for laundering, and other decontamination strategies should be encouraged to further protect occupational and public health.

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