Major article

Comparison of bacteria on new, disposable, laundered, and unlaundered hospital scrubs

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Background: As a cost-saving measure, an increasing number of hospitals allow personnel to launder their uniforms, lab coats, and operating room scrubs at home. With rising nosocomial infection rates and increasing levels of multidrug-resistant bacteria in hospital settings, uniform contamination may be an environmental factor in the spread of infection.
Methods: We quantified the number and identity of bacteria found on swatches cut from unwashed operating room, hospital-laundered, home-laundered, new cloth, and new disposable scrubs.
Results: Of the 29 unwashed hospital operating room scrub swatches analyzed, 23 (79%) were positive for some type of gram-positive cocci, with 3 (10%) of those classified as *Staphylococcus aureus*, and 20 (69%) were positive for coliform bacteria, 3 of which were *Escherichia coli*. Home-laundered scrubs had a significantly higher total bacteria count than hospital-laundered scrubs ($P = .016$). There was no statistical difference in the bacteria counts between hospital-laundered scrubs and unused new and disposable scrubs. In the home-laundered scrubs 44% (18/41) were positive for coliform bacteria, but no isolates were *Escherichia coli*.
Conclusions: Significantly higher bacteria counts were isolated from home-laundered scrubs and unwashed scrubs than from new, hospital-laundered, and disposable scrubs.

How hospital scrubs are handled, laundered, and stored appears to influence the level of bacterial contamination on scrubs.[1] Laundry methods used at home and in hospitals have changed over the past several decades.[1-4] To reduce energy usage, some hospitals are washing at temperatures lower than the Centers for Disease Control and Prevention (CDC)-recommended temperature of 71.1°C.[5] Hospital laundry managers have adjusted chemical usage and increased wash cycle running time by 15%-20% to compensate for this reduction in wash water temperature, resulting in higher water consumption and a chemical cost increase of 8%-10%.[4]

A consequence of higher laundering costs is that an increasing number of hospitals are allowing personnel to launder their uniforms, lab coats, and even operating room (OR) scrubs at home. The frequency of home-laundering of uniforms is unknown,

although several surveys have found that 21%-26% of nursing staff wash their uniforms at home.[1,6] Home washing conditions (ie, water-to-fabric ratio, temperature, detergent formulation) differ greatly from industrial laundering practices.[7,8] In the home setting, lower wash temperatures are used, and bleach is often not added to wash loads, given the increased number of brightly colored and permanent press fabrics in use. A study of staff who home-laundered their scrubs found that 70% (35/50) used a warm-water cycle, 73% (37/50) washed the scrubs separate from other clothing, and all completely dried their scrubs in a dryer; however, these conditions did not meet commercial laundry guidelines. Most commercial hospital laundries follow CDC guidelines[5] to provide thermal disinfection in the wash cycle by washing for ≥25 minutes and holding the temperature at 71°C for 3 minutes, or using chemicals suitable for low-temperature washing if laundry cycles of ≤70°C are used.[9]

Previous studies have documented the contamination of hospital scrubs with microbial pathogens, including methicillin-resistant *S aureus* (MRSA), vancomycin-resistant enterococci (VRE), and *Clostridium difficile*. Perry et al[10] found that 39% (22/57) of home-laundered nurse uniforms were positive for 1 or more of

these organisms before the start of the shift. Scrub contamination of at least 1 of the test organisms increased to 54% (31/57) at the end of shifts. VRE was found on 31% (22/57) of uniforms, *C difficile* on 19% (11/57), and MRSA on 15% (8/57). Similarly, Pilonetto et al[11] isolated pathogens from 48% (15/31) of hospital gowns. A significant ($P = .027$) increase in total bacteria from the beginning to the end of a work shift was found, with average counts increasing from 2.2 CFU/cm^2 to 4.9 CFU/cm^2.

Contamination of scrubs also may be seen after commercial laundering. Fijan et al[12] identified specific organisms on surfaces from a hospital laundry clean area, as well as on ironed and folded textiles processed at the laundry. Normal skin *Micrococcus* and *Staphylococcus* spp were the most commonly found bacteria. *Bacillus* and *Corynebacterium* spp were also frequently detected, even after surface disinfection measures were implemented.

Few studies have compared the microbial flora or decontamination effectiveness of hospital-laundered and home-laundered attire.[13] Previous research has focused mainly on enumeration rather than identification of specific biota. The present study aimed to identify and quantify types of heterotrophic (HPC) bacteria found on unwashed OR, hospital-laundered, home-laundered, new cloth, and new disposable scrubs.

MATERIALS AND METHODS

Preliminary testing using a standard dilution/plating method for HPC bacteria was performed to identify the scrub locations most likely to elicit the highest numbers. Scrub tops, pants, and jackets worn for a shift were taken from a local hospital OR laundry collection bin at the end of the shift. Each clothing piece was placed separately into a clean plastic bag, sealed, and refrigerated until analysis. Fabric sections (swatches) with known areas were cut from necks, sleeves, pockets, front pants, front shirts, front jackets, crotches, and under arms using sterile scissors and placed separately into sterile plastic bags with 50 mL of buffered peptone solution. Each sample was pummeled for 4 minutes on high speed in a Seward stomacher (Seward, London, UK) to recover the bacteria into the peptone liquid. The liquid was pipetted off, and for each swatch, 0.1 mL and 0.01 mL samples of the liquid were plated on R2A agar (Difco, Sparks, MD). The plates were incubated for 3-5 days at 37°C, after which colonies were counted. Because only total bacteria numbers were of interest, in the preliminary study, bacterial identification was not performed.

Based on results from the preliminary study, subsequent sampling locations were narrowed down to the neck and front pocket area of scrub tops, and the central front area of scrub pants. Further testing on OR jackets was not conducted because of the low numbers of HPC bacteria found. The experimental procedures are summarized in Figure 1.

Scrubs laundered at home or in the hospital came from a variety of manufacturers and were constructed of either 100% cotton or a polyester/cotton blend. Hospital-laundered scrubs were all processed within the company-owned industrial laundry facility. Ten steps were used during the 61-minute wash/mechanical action cycle for heavily soiled laundry items, such as OR scrubs. Chemicals were added during 5 of these steps, consisting of a phosphate-free detergent, a product to restore the water- and soil-repellent finish, a laundry rinse additive, a germicide, and a pH reducer (sour) to eliminate yellowing. Water temperature reached 71.1°C for at least 3 minutes, in accordance with CDC recommendations.[5] No information on specific laundering practices or type of nursing assignment was sought from donors of the home-laundered scrubs, but all scrubs were obtained from nurses who had contact with patients in a hospital setting. Two different styles of new disposable scrubs were purchased and tested (from Molnlycke Healthcare,
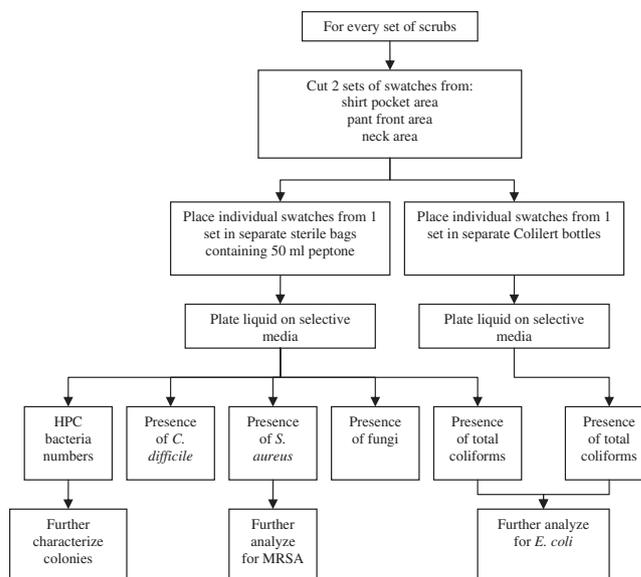

Fig 1. Flowchart of the scrub experiments.

Anderson, SC and Lakeland Industries, Ronkonkoma, NY). Two different colors of a single style of new cloth scrubs (65% polyester/35% cotton, model B101; Natural Uniforms, Commerce, CA) were used.

HPC plate counts

HPC testing was performed using the dilution and plating method. Peptone collected after pummeling each swatch in the Seward stomacher, as described previously, was serially diluted and assayed on R2A agar. The plates were incubated for 3-5 days at 37°C and then counted. Unique-appearing colonies that could be harvested without contamination from other colonies were plated on Difco tryptic soy agar (BD Diagnostics, Sparks, MD) and MacConkey agar (BD Diagnostics). Plates were incubated for 24 hours at 37°C.

Further identification of isolated colonies was performed. Freshly grown lactose-forming colonies on MacConkey agar were Gram-stained (BD Gram crystal violet stain; BD Diagnostics), then observed under a microscope (model HFX-II; Nikon, Meliville, NY) to identify Gram-negative cocci and rods. Further characterization of Gram-negative cocci and rods was done using API-20E test strips (bioMérieux, St Louis, MO). Similarly, freshly grown non−lactose-forming colonies on MacConkey agar were stained with BD Gram crystal violet stain and then observed under a Nikon HFX-II microscope to identify Gram-positive cocci and rods. Further characterization was done using the Biolog GP2 microplate identification system (Biolog, Hayward, CA).

Presence/absence of *C difficile*

For each swatch, 10 mL of peptone was placed into a test tube and capped. The sample was heat-shocked for 10 minutes at 70°C, then filtered through a 0.45-μm, 47-mm membrane (Millipore, Billerica, MA). The intact membrane was placed on cycloserine-cefoxifin fructose agar (Hardy Diagnostics, Santa Maria, CA), placed in an anaerobic chamber, and incubated for 24 hours at 37°C. After incubation, colonies were counted. Because no *C difficile* was found during the first 30 sets of scrubs tested, including several sets of unwashed scrubs, it was not performed in later testing.

Presence/absence of Staphylococcus aureus/*methicillin-resistant* S aureus

Peptone collected after pummeling of each swatch in the Seward stomacher was serially diluted and plated on tryptic soy agar amended with 5% sheep's blood (Hardy Diagnostics), 0.015 g/L of nalidixic acid (Sigma-Aldrich, St Louis, MO), and 0.01 g/L of colistin (Sigma-Aldrich), then incubated at 37°C for 48 hours. Creamy-white and yellow-colored colonies demonstrating β-hemolysis were further subjected to a series of biochemical tests to confirm *S aureus*. Biochemical tests included slide coagulase (BD Diagnostics), tube coagulase, catalase, and polymixin-B (BD Diagnostics) sensitivity. Confirmed *S aureus* isolates were plated on 2 different types of MRSA agar, a differential CHROMagar medium containing cefoxitin (BD Diagnostics) and a selective MRSA screening plate containing oxacillin (Hardy Diagnostics). Full growth occurring on both types of media confirmed isolates as MRSA. If no growth or only residual growth occurred, the strains were considered methicillin-susceptible *S aureus*.

Presence/absence of total coliforms and Escherichia coli

For each swatch, 1 mL of peptone was added to tubes containing Colilert (IDEXX, Westbrook, ME), and the tubes were incubated for 24 hours at 37°C. A yellow tube indicated the presence of coliforms, and yellow tubes that fluoresced under UV light were preliminarily deemed positive for *Escherichia coli (E coli)*. Liquid (0.01 mL) from tubes that had fluoresced were plated onto MacConkey agar for further confirmation testing using API-20E test strips. In addition to the Colilert test, entire 6" square sections of scrubs were added to 100-mL bottles containing Colilert media. The contents were shaken for 60 seconds and then incubated for 24 hours at 37°C. This step was performed to determine whether the 2 methods would achieve identical results, given that Coliert media is not routinely used for fomite testing.

Presence/absence of fungi

Peptone was collected after pummeling each swatch in the Seward stomacher as described previously, then serially diluted and plated on Sabouraud dextrose agar with chloramphenicol (Hardy Diagnostics) at 37°C for 48 hours. Over the subsequent 21 days, plates were checked for growth every 2 days, any growth was noted, and positive plates were immediately discarded.

Statistical analysis

Welch's *t* test, assuming unequal variances, was used to identify significant differences ($P < .05$) in HPC bacteria counts for the different categories of scrubs. Welch's *t* test comparisons, as well as average, geometric mean, and standard deviation values, were obtained using Excel (Microsoft, Redmond, WA).

RESULTS

HPC bacteria counts for the different classifications of scrubs are presented in Table 1. Unwashed hospital OR scrubs had the highest HPC counts (geometric mean, 85 CFU/cm^2; range, 5-473 CFU/cm^2), followed by home-laundered scrubs (geometric mean, 16 CFU/cm^2; range, 1-848 CFU/cm^2), new cloth scrubs (geometric mean, 5 CFU/cm^2; range, 1-145 CFU/cm^2), new disposable scrubs (geometric mean, 5 CFU/cm^2; range, 1-118 CFU/cm^2), and hospital-laundered scrubs (geometric mean, 2 CFU/cm^2; range, 1-27 CFU/cm^2). Hospital-laundered scrubs had significantly fewer HPC bacteria than home-laundered scrubs ($P = .016$). There was no statistical difference in the total number of bacteria on hospital-laundered scrubs and on unused new and disposable scrubs.

C difficile was not isolated from any of the scrubs tested, although positive controls for *C difficile* were obtained. Of the 29 unwashed hospital operating room scrub swatches analyzed, 23 (79%) were positive for some type of gram-positive cocci, with 3 (10%) of those classified as *Staphylococcus aureus.* None were confirmed as MRSA. No *S aureus* was isolated from any of the home-laundered (0/41), hospital-laundered (0/36), new disposable (0/48), or new cloth (0/15) scrubs; however, many other potentially pathogenic gram-positive rods and cocci were identified.

Twenty of the 29 (69%) swatches of unwashed hospital OR scrubs tested positive for coliform bacteria, 3 of which were confirmed as *E coli*. Eighteen swatches (18/41, 44%) of home-laundered scrubs tested positive for coliforms. None of these were identified as *E coli*. No coliforms or *E coli* were found on hospital-laundered, new disposable, or new cloth scrubs. Identical results were achieved regardless of whether the samples were run using the Colilert tubes or the 100-mL bottles containing Colilert media.

Fungi were found on 10% (2/21) of hospital-laundered swatches, 93% (28/30) of home-laundered swatches, 36% (13/36) of new disposable swatches, 22% (4/18) of unwashed hospital swatches and 27% (4/15) of new cloth swatches (Table 2).

Gram-positive cocci of potential health significance were identified from unwashed hospital OR scrubs (*S aureus*, *Staphylococcus saprophyticus*, *Aerococcus viridans*, *Staphylococcus caprae*, *Staphylococcus kloosii*, and *Enterococcus flavescens*) and home-laundered scrubs (*Staphylococcus lugdunensis*) using the Biolog MicroLog system, version 4.20. In all cases, similarity rates exceeded 0.5, indicating an acceptable species identification result for each isolate. Results are shown in Table 3.

No gram-positive rods were identified from unwashed hospital OR scrubs; however, several different gram-positive rods were isolated from home-laundered scrubs, none of which was a potential health concern. Gram-positive rods isolated from new cloth scrubs (eg, *Tsukamurella inchonensis*, *Microbacterium* spp) have been associated with infections.

Enteric gram-negative rods of potential health significance (*E coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*) were isolated from unwashed hospital OR scrubs using API-20E test strips. Gram-negative rods (*K pneumoniae*, *E cloacae*, *Klebsiella oxytoca*, *Raoultella terrigena*, and *Serratia rubidaea*) also were isolated from home-laundered scrub swatches. Test results were compared with the bioMérieux apiweb system database, and identification was carried out using apiweb version 4.1. The identification of significant taxa exceeded 90% (indicating "good" identification) for all except *S rubidaea* at 87.7% ("very good" identification). The results are presented in Table 3.

DISCUSSION

In this study, HPC bacteria were chosen to demonstrate the relative cleanliness of scrubs, based on similar studies.[6,10,11] Significantly fewer bacteria were detected on hospital-laundered scrubs than on home-laundered scrubs (average, 4 CFU/cm^2 [range, 1-27 CFU/cm^2] vs 143 CFU/cm^2 [1-848 CFU/cm^2]; $P = .016$). Most of this difference is attributable to 2 sets of home-laundered scrubs with average HPC bacteria counts of 848 CFU/cm^2 and 627 CFU/cm^2, and the difference is less pronounced when geometric mean values are compared (geometric mean, 2 CFU/cm^2 vs 16 CFU/cm^2). Both average and geometric mean HPC values were calculated because average values take into account worst-case scenarios and can be used in the development of a quantitative risk assessment, whereas geometric means are used for comparison of this data with data from similar studies. The highest home-laundered HPC counts

Table 1
Summary of HPC counts for different classifications of scrubs

Type of scrub	Number of garments	Arithmetic mean, CFU/cm^2	Geometric mean, CFU/cm^2	Range, CFU/cm^2	SD
Unwashed hospital	18	180	85	5-473	178
Home-laundered	26	143	16	1-848	274
Hospital-laundered	20	4	2	1-27	8
New disposable	16	16	5	1-118	29
New cloth	10	35	5	1-145	62

Table 2
Swatches testing positive for selected microorganisms, % (n/N)

Type of scrub	Fungi	Gram-positive bacteria	S aureus	Coliform	E coli
Unwashed hospital	22 (4/18)	79 (23/29)	10 (3/29)	69 (20/29)	10 (3/29)
Home-laundered	93 (28/30)	12 (5/41)	0 (0/41)	44 (18/41)	0 (0/41)
Hospital-laundered	10 (2/21)	0 (0/36)	0 (0/36)	0 (0/36)	0 (0/36)
New disposable	36 (13/36)	4 (2/48)	0 (0/48)	0 (0/48)	0 (0/48)
New cloth	27 (4/15)	7 (1/15)	0 (0/15)	0 (0/15)	0 (0/15)

Table 3
Identification of gram-positive and coliform bacteria isolated from hospital scrubs

Type of scrub	Organism*	Illness; occurrence
Unwashed hospital	Staphylococcus aureus	Common cause of nosocomial infections
Unwashed hospital	Staphylococcus saprophyticus	Bladder and urinary tract infections
Unwashed hospital	Aerococcus viridans	Common cause of nosocomical infection; antibiotic-resistant infections; enterococcus
Unwashed hospital	Staphylococcus caprae	Bacterimia in neonates; meningitis
Unwashed hospital	Staphylococcus kloosii	Infections in patients with colon cancer
Unwashed hospital	Enterococcus flavescens	Antibiotic-resistant nosocomial infections
Unwashed hospital	Escherichia coli 1	Indicator of fecal contamination; virulent strains can cause diarrhea.
Unwashed hospital	Enterobacter cloacae	Antibiotic-resistant nosocomial infections
Unwashed hospital	Klebsiella pneumoniae	Antibiotic-resistant nosocomial infections, particularly respiratory infections
Unwashed hospital	Pseudomonas aeruginosa	Common cause of nosocomial infections
Home-laundered	Staphylococcus lugdunensis	Osteomyelitis infections; infections after cardiac surgery; 24% mortality
Home-laundered	Raoultella terrigena	Environmental isolate; cause of waterborne diseases
Home-laundered	Enterobacter cloacae	Antibiotic-resistant nosocomial infections
Home-laundered	Klebsiella pneumoniae	Common cause of nosocomial infections, particularly respiratory infections
Home-laundered	Klebsiella oxytoca	Antibiotic-resistant nosocomial infections, particularly respiratory infections
Home-laundered	Serratia rubidaea	Nosocomial infections, mostly in neonates and infants
New cloth	Tsukamurella inchonensis	Lung infections
New cloth	Microbacterium spp (CDC A-4)	Infections in cancer patients and transplant recipients

*Gram-positive bacteria were identified using Biolog MicroLog version 4.20. Coliform bacteria were identified using API-20E test strips.

were greater than any seen in unwashed hospital OR scrubs (436 CFU/cm^2 and 473 CFU/cm^2). There was no statistical difference in the number of bacteria on hospital-laundered scrubs and on unused new and disposable scrubs. For this study, detailed laundering practices used by the hospital-owned and operated laundry were known, but home-laundering treatments were not. Each set of home-laundered scrubs tested (n = 10) came from a different owner. A Seward stomacher was used to recover bacteria from scrub fabric. In our earlier laboratory studies using this method, an estimated 8.2% of organisms were recovered, so the actual HPC counts present on the scrubs likely are much higher.

Many variables will affect the level of contamination detected on washed scrubs, including initial level of contamination, laundry procedures used, and storage conditions. The average HPC levels on home-laundered scrubs were 80% of the counts recovered from unwashed hospital OR scrubs (average, 143 CFU/cm^2 vs 180 CFU/cm^2). In previous studies, Callaghan[6] and Perry et al[10] found that bacteria counts varied little between scrubs worn at the beginning of a shift and those tested in the middle and at the end of shifts.

The level of postduty contamination of hospital uniforms also varies between ward areas.[10] In the study of Perry et al,[10] only 7.7% (4 of 57) of surgical staff uniforms tested positive for MRSA, C difficile, and/or VRE, whereas 92% (52 of 57) of general medicine ward area staff uniforms, 83% (47 of 57) of renal medicine staff uniforms, and 62% (35 of 57) of renal transplant staff uniforms tested positive for at least 1 of these pathogens. Compared with

surgical staff, ward staff have direct contact with many more patients during a single shift, which may account for the differences in contamination between ward and OR apparel. In this study, no attempt was made to track specific activities and exposure levels of persons wearing the scrubs, which could have biased some of the relative comparisons of scrub contamination levels.

C difficile was not isolated from any scrub tested, washed or unwashed. C difficile is a common cause of hospital infections and has been isolated from worn hospital clothing.[10] On the other hand, some type of gram-positive cocci were isolated from 79% (23/29) of unwashed hospital OR scrub swatches, with 10% (3/29) of those classified as S aureus. This is not surprising, given that nearly 32% of people carry S aureus on their skin and/or nares at any time.[14]

Only 10% (3 of 29) of the coliform bacteria isolated from worn hospital scrubs and home-laundered scrubs tested positive for E coli. However, many of the other gram-negative bacteria identified (eg, E cloacace, K pneumoniae, P aeruginosa, K oxytoca, S rubidaea) are capable of causing serious nosocomial infections, including pneumonia, meningitis, and septicemia.[15] A number of gram-positive organisms identified (S aureus, A viridans, E flavscens) are also capable of causing infections in high-risk individuals, such as those with underlying diseases.

The high frequency of fungi found on scrubs was unexpected, given that samples were collected from areas with low relative humidity (Tucson and Phoenix, Arizona) during a typically dry part of the year (December). Storage conditions (humidity and

temperature) for the home-laundered scrubs, as well as time since last washing, were not known for the home-laundered scrubs; however, neither visible fungi nor noticeable moldy smell was detected on any of the scrubs before testing. Still, 93% (28 of 30) of swatches from home-laundered scrubs tested positive for fungi. Identification of specific fungal species was beyond the scope of this project, and so the health hazards from the fungi found are uncertain. Some species of *Aspergillus*, known to grow well on clothing, are opportunistic pathogens.[16]

Whether microbes on clothing are transferred to patients was not assessed during this study, however Hedin[17] found that 3 of 5 patients were colonized with an *S epidermis* strain identical to one found on staff clothing. Indirect contact with staff clothing has been identified as a route for cross-infection in a clinical setting.[17-19]

The relative contribution of contaminated scrubs in the spread of nosocomial infections is not known. Given that rates of MRSA have decreased in some hospitals despite continued home laundering, suggests that other methods of infection control are more important, such as patient surveillance and contact precautions.[20] Recent studies have concluded that existing evidence does not support uniforms as a vehicle for the transmission of infections,[1,21,22] and the CDC's 2002 Guidelines for Laundry in Health Care Facilities states that "uniforms laundered at home have shown no link with an increase in infection rates and no pathogens have been recovered from either home- or hospital-laundered scrubs."[5] There are conflicting opinions regarding whether to allow staff to home-launder hospital attire. Hospital administrators and infection control teams must weigh the risk of potential infection transmission against the cost savings realized by the company if staff purchase and launder their own scrubs. Either way, health care providers should be made aware of the potential for uniforms to become contaminated and steps they can take to minimize transmission of microorganisms within the hospital setting and when washing scrubs at home. Providing written guidelines for staff to follow when home-laundering scrubs may help reduce the risk of nosocomial infection. Laundering scrubs at 71°C, as recommended by the CDC, is not feasible in a home setting. Washing at 60°C or above using any laundry product or washing at 40°C using a bleach-containing laundry product should be sufficient to kill most microorganisms, including *C difficile* spores.[23] Bleach should be used when possible, and always on grossly contaminated garments. All hospital attire should be dried completely in a drier, and then stored in a manner to ensure continued cleanliness and minimize fungal growth.

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