Evaluation of the efficacy of disinfectant footbaths as used in veterinary hospitals

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Objective—To evaluate efficacy of 2 disinfectants as used in footbaths in veterinary hospitals for reducing bacterial contamination of footwear.

Design—Prospective study.

Sample Population—Bacteria collected from the soles of rubber boots after experimental contamination and exposure to disinfectant solutions or control conditions.

Procedures—Investigators contaminated boots by walking through soiled straw animal bedding. Swab samples were collected from the sole of 1 boot (right or left) without treatment. The other boot was briefly immersed in a disinfectant solution (either a quaternary ammonium compound [QAC] or a peroxygen compound) or water, and samples were collected after 7 minutes. Differences associated with the experimental treatments were analyzed statistically.

Vernery teaching hospitals (VTHs) in the United States and Canada were contacted to obtain information about the use of footbaths.

Results—Mean bacterial concentrations from peroxygen-treated boots were 67% to 78% lower, compared with samples taken from untreated boots. In contrast, there were no statistically detectable differences in mean bacterial concentrations in samples taken from QAC- or water-treated boots, compared with control boots. Disinfectant footbaths were reportedly used in 30 of 31 VTHs.

Conclusions and Clinical Relevance—Disinfectant solution containing peroxygen applied in a footbath reduced bacterial concentrations on rubber boots under conditions representative of those found in VTHs. Footbaths are commonly used as a method to control infectious diseases in veterinary hospitals. Disinfectant footbaths should not be expected to sterilize footwear, but they may help in reducing the risk for nosocomial infection when used with effective disinfectants. (J Am Vet Med Assoc 2005;226:2053–2058)

Infection control is an integral aspect of providing quality care at medical facilities. One vital component of an integrated infection control or biosecurity program is to minimize trafficking and distribution of potential pathogens by movement of personnel through the facility. This is often achieved by segregation of patients, limiting access, use of separate footwear or shoe covers, and use of disinfectant footbaths. Our experiences suggest that footbaths are one of the most common control measures used to reduce trafficking of pathogenic microorganisms in veterinary hospitals and livestock operations. Results of several surveys of food animal producers indicate that control measures related to footwear hygiene are among the most commonly applied biosecurity practices, especially on large, intensive operations. However, there are no previously published reports regarding footbath use in veterinary hospitals.

Footbaths are generally used because of the belief that disinfection of footwear will provide a clinically relevant degree of decontamination, reducing the risk of transmitting important disease agents. However, few objective investigations have evaluated the efficacy of footbaths for reducing bacterial concentrations on footwear, and even fewer have evaluated their use as applied in veterinary hospitals. A few studies have evaluated risk reduction related to bacterial infections in large poultry houses, but there are no similar published studies evaluating the potential reduction in the risk of nosocomial infections in veterinary hospitals.

Other studies have examined the efficacy of footbaths for reducing bacterial concentrations on footwear in experiments that mimic conditions of intensive swine and cattle production environments or have revealed that pathogens can be recovered from footwear worn in animal production environments. However, those studies are not perfectly relevant to veterinary hospital environments because of differences in footwear cleanliness (ie, the amount of feces and organic material) that are typically expected in veterinary hospitals, compared with intensive livestock production facilities. The purpose of the study reported here was to evaluate the efficacy of 2 disinfectants as used in footbaths in veterinary hospitals for reducing bacterial contamination of footwear. The prevalence of disinfectant footbath use in veterinary teaching hospitals (VTHs) in the United States and Canada was also investigated.

Materials and Methods

Study overview—Rubber boots were contaminated for use in the study by contact with used animal bedding as per a standardized procedure. One boot in a pair remained untreated, and the other was treated in a footbath with a disinfectant or water. The disinfectant efficacy of 2 disinfectants was compared with water treatment and no treatment; numbers of culturable aerobic bacteria recovered from boots...
briefly treated with disinfectant solutions or water were compared with numbers of bacteria recovered from untreated boots. The prevalence of disinfectant footbath use was investigated by making personal inquiries with representatives from all VTHs in the United States and Canada.

**Boots**—Thirty pairs of rubber overboots were purchased for this study. Prior to initial use, a template was used to paint around the outside of 4 sampling zones (20 × 1 cm) on the sole of every boot (Figure 1). Soles of these boots are generally smooth with minimal tread. The exterior of boots were then scrubbed with a detergent solution, thoroughly rinsed with water, and disinfected by soaking for 5 minutes in a 70% ethanol solution. After air-drying, pairs of boots were stored in new plastic bags until used.

**Contamination process**—The goal for this aspect of the study was to obtain uniform bacterial contamination that could be encountered in large animal hospitals. A mature bull with an orthopedic injury was housed for 5 days in a straw-bedded stall in the James L. Voss VTH (JLV-VTH) at Colorado State University. Fecal material and soiled bedding was not removed from the stall during this stabling period, but additional straw bedding was added daily. Experimental contamination of rubber boots was achieved by walking through the contaminated stall in a serpentine pattern for 2 minutes. Generally, after this process, soles of boots were moist and small amounts of small straw particles and minute amounts of fecal material were present, but large amounts of feces were never present.

**Footbaths**—New wide-mouth plastic tubs were purchased (capacity, 7 gallons [26.5 L]). Prior to use, tubs were thoroughly rinsed with water, and filled with 4 gallons (15.1 L) of water. The 2 disinfectants used in this experiment included a product that contained a mixture of quaternary ammonium compounds (QAC) and a peroxygen disinfectant that contained potassium peroxymonosulfate. These disinfectants were selected for evaluation because they had been used in footbaths at the JLV-VTH prior to this study. Tap water was used as a control treatment for comparison. Fresh disinfectant solutions were prepared according to manufacturers’ instructions just prior to the study (1:32 aqueous dilution of the QAC concentrate and 1% aqueous solution of the peroxygen disinfectant). Footbaths were filled with 4 gallons (15.1 L) of solution to create a solution depth of approximately 6 inches (15.2 cm). The same solutions were used to process all boots.

**Boot disinfection**—Protocols for boot disinfection were rigorously standardized and timed, and all trials were conducted at ambient environmental temperature (approx 20°C [68°F]). Briefly, after the contamination process, 1 boot (right or left) was randomly selected by use of a coin toss to remain as an untreated control and the other boot was treated in a footbath. The untreated boot was handed to another investigator for sampling, and then the investigator stepped with the other boot into the footbath for 2 seconds and then stepped out. The treated boot was removed, placed on its side, and sampled after 7 minutes. The order for experimental application of disinfectant solutions or water was determined by use of a random numbers table. Repeat number, boot identification (right or left), treatment (quaternary ammonium, peroxygen, water control, or untreated control), and sample zone (1 to 8; Figure 1) were recorded for each sample. Vigorous scrubbing and extended exposure to disinfectant solutions would most likely have greater disinfectant efficacy, compared with the brief exposures used in this study, but this is not consistent with footwear hygiene practices often used in veterinary hospitals.

**Sampling process**—Sterile cotton swabs were premoistened with Dey-Engley (neutralizing) broth, which contained neutralizers for common disinfectants. A different swab was then used to vigorously sample each of the premeasured sampling zones on every boot; a single investigator collected all samples to minimize variability. After collection, swabs were placed in 10 mL of neutralizing broth and immediately transported at ambient temperature to the laboratory for processing.

**Laboratory processing**—Samples were processed in the laboratory within 3 hours of sampling. Tubes that contained swab specimens and neutralizing broth were vortexed prior to making six 10-fold dilutions in buffered peptone water. The original sample and diluted specimens were plated on trypticase soy agar with 5% sheep RBCs (blood agar [BA]) and MacConkey agar (MAC). Plates were incubated aerobically for 48 hours at 37°C. Bacteria were enumerated at 24 hours and 48 hours on the plates that yielded 20 to 200 colony forming units/10 μL of inocula. The corresponding dilution factor and surface area that was originally swabbed were used to estimate the colony forming units per square centimeter for each sample. It was assumed that culturing on BA allowed quantification of both aerobic gram-positive and aerobic gram-negative bacteria, whereas culture on MAC primarily allowed quantification of enteric gram-negative bacteria. It was not possible to use culture methods that would allow recovery of every type of potentially pathogenic bacteria because many require special culture enrichments, which make enumeration difficult (eg, Salmonella spp), or require different environmental conditions for growth (eg, anaerobic and microaerophilic species). It was assumed that evaluating numbers of bacteria recovered by use of these aerobic culture techniques would provide efficacy data that could be generalized to a broad variety of potential bacterial pathogens.

**Survey of footbath use at VTHs**—Representatives of all 31 VTHs in the United States and Canada were contacted by e-mail or telephone and asked about routine footbath use in...
their facilities. Individuals were chosen for contact because of their responsibility for infection control efforts at a facility or because they were responsible for hospital administration. Contacts were asked to describe footbath use and to list all types of disinfectants used in the footbaths.

**Statistical analysis**—Bacterial concentrations were transformed to $\log_{10}$ values to allow parametric analyses. Generalized linear modeling was used to analyze differences in bacterial concentrations, with $\log_{10}$ bacterial concentrations as the dependent variable and treatment (QAC, peroxygen, water, or untreated control) as the independent variable of interest. Separate analyses were used on BA or MAC to evaluate bacterial concentrations estimated at 24 or 48 hours. Statistical analyses used generalized estimating equations to control for the hierarchical and repeated nature of the data; boot identification (right or left) was nested within replicate identification (1 to 30). Boot identification (right or left) was also forced into models as a fixed effect. Although contamination was not considered likely to vary between right and left boots or among different sampling zones on boots, these variables were controlled in models to ensure that unforeseen differences were accounted for. Least square means for $\log_{10}$ bacterial concentrations and variance estimates were determined from these models and used to compare differences associated with the experimental treatments.

**Results**

**Footbath efficacy**—After the experimental contamination process, moisture and small amounts of bedding were frequently found on the soles of boots, but heavy fecal contamination was not found. In general, adjusted mean bacterial concentrations were highest in samples from water-treated boots and lowest in peroxygen-treated boots (Table 1). As expected, mean bacterial concentrations were approximately 2 to 3 $\log_{10}$ lower on MAC, compared with BA, and 0.3 to 0.5 $\log_{10}$ lower on plates read after 24 hours of incubation, compared with those read at 48 hours, but patterns related to treatment differences generally were the same at both time points. Least square mean bacterial concentrations were 67% to 78% lower in samples taken from peroxygen-treated boots, compared with samples taken from control boots ($P < 0.001$). In contrast, there were no statistically detectable differences in least square mean bacterial concentrations in samples taken from QAC-treated boots, compared with control boots ($P \geq 0.13$). Mean bacterial concentrations in samples taken from water-treated boots were consistently higher than in samples taken from control boots, although this difference was not always significant ($P = 0.01$ to 0.89). Although a variable for boots was forced into models, there were no significant differences between samples taken from right and left boots ($P > 0.52$).

**Footbath use at VTHs**—Information regarding footwear disinfection was obtained from all 31 VTHs in the United States and Canada. Disinfectant footbaths were reportedly used at 30 of 31 VTHs. Among those that used disinfectant footbaths, 68% used a single disinfectant and 32% reported using more than 1 type of disinfectant (not mixed together). The disinfectants most commonly reported as being used in footbaths were QACs (13/31 [42%]) and phenolics (12/31 [39%]), followed by hypochlorite solutions (7/31 [23%]) and peroxynitrates (6/31 [19%]). Other disinfectants that were reportedly used in footbaths were povidone iodine, chlorhexidine, and ammonia (each was used at 1/31 VTHs). In addition to footbaths, 8 (26%) hospitals reported also using disinfectant footmats in some locations. Although not specifically asked, some contacts reported that use of footmats improved compliance and willingness to disinfect footwear, compared with use of footbaths. Most of the VTHs reported using footbaths or footmats outside of stalls housing patients with known or suspected infectious conditions (eg, salmonellosis) or outside entrances to isolations.

**Table 1**—Mean aerobic bacterial concentrations obtained from soles of rubber boots after standardized contamination and exposure to disinfectants in footbaths.

<table>
<thead>
<tr>
<th>Culture variable</th>
<th>Treatment</th>
<th>n</th>
<th>LS mean (SEM) of $\log_{10}$ CFU/cm²</th>
<th>Reduction in $\log_{10}$ CFU/cm²</th>
<th>Percentage reduction*</th>
<th>Statistical difference†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood agar (24 h)</td>
<td>Control</td>
<td>120</td>
<td>5.58 (0.07)</td>
<td>Reference</td>
<td>ab</td>
<td>ab</td>
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<tr>
<td></td>
<td>Water</td>
<td>40</td>
<td>5.85 (0.14)</td>
<td>–0.27</td>
<td>–88%</td>
<td>a</td>
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<tr>
<td></td>
<td>QAC</td>
<td>40</td>
<td>5.72 (0.06)</td>
<td>–0.14</td>
<td>–38%</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>Peroxygen</td>
<td>40</td>
<td>5.10 (0.07)</td>
<td>0.48</td>
<td>67%</td>
<td>c</td>
</tr>
<tr>
<td>Blood agar (48 h)</td>
<td>Control</td>
<td>120</td>
<td>6.02 (0.07)</td>
<td>Reference</td>
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<td>a</td>
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<tr>
<td></td>
<td>Water</td>
<td>40</td>
<td>6.13 (0.09)</td>
<td>–0.10</td>
<td>–27%</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>QAC</td>
<td>40</td>
<td>6.01 (0.07)</td>
<td>0.01</td>
<td>3%</td>
<td>a</td>
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<tr>
<td></td>
<td>Peroxygen</td>
<td>40</td>
<td>5.36 (0.08)</td>
<td>0.66</td>
<td>78%</td>
<td>b</td>
</tr>
<tr>
<td>MacConkey agar (24 h)</td>
<td>Control</td>
<td>120</td>
<td>3.73 (0.08)</td>
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<td>a</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>40</td>
<td>4.06 (0.11)</td>
<td>–0.33</td>
<td>–112%</td>
<td>a</td>
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<tr>
<td></td>
<td>QAC</td>
<td>40</td>
<td>3.93 (0.07)</td>
<td>–0.20</td>
<td>–58%</td>
<td>b</td>
</tr>
<tr>
<td></td>
<td>Peroxygen</td>
<td>40</td>
<td>3.10 (0.10)</td>
<td>0.63</td>
<td>77%</td>
<td>c</td>
</tr>
<tr>
<td>MacConkey agar (48 h)</td>
<td>Control</td>
<td>120</td>
<td>3.97 (0.09)</td>
<td>Reference</td>
<td>a</td>
<td>a</td>
</tr>
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<td>–0.41</td>
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<td>c</td>
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<tr>
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<td>–32%</td>
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</tr>
<tr>
<td></td>
<td>Peroxygen</td>
<td>40</td>
<td>3.40 (0.12)</td>
<td>0.57</td>
<td>73%</td>
<td>b</td>
</tr>
</tbody>
</table>

*Percentage difference between LS mean CFU for treatment and control boots; positive values represent reduced growth after treatment, negative values represent higher mean concentrations. $1P < 0.002$ for overall treatment in all models.

†Least squares geometric mean.

‡Different letters among rows within culture variable groups indicate significant ($P < 0.05$) differences.

Discussion

Results of this study suggested that the peroxygen disinfectant had superior decontamination effects on rubber boots, compared with the QAC disinfectant, as applied in footbaths under conditions that can be encountered in large animal hospitals. Brief (2-second) treatment of boots with the peroxygen disinfectant under these experimental conditions, followed by a 7-minute contact period, resulted in approximately 75% reduction in adjusted mean bacterial concentrations. Although this obviously does not equate with sterilization, these results suggest that regular use of footbaths would typically decrease bacterial loads on footwear, which theoretically should decrease the probability of transmitting agents susceptible to the peroxygen disinfectant. This would seem to be especially true if footbaths were frequently used as personnel moved through hospital environments. Greater reductions may have been detected if growth had been evaluated after longer treatment or contact periods, but antimicrobial activity of the disinfectant should also theoretically continue after stepping out of footbaths unless the disinfectant solution was rinsed from surfaces. Results of this study also suggested that QAC disinfectants similar to the one evaluated in this study, which contained peroxymonosulfate as an active ingredient, have low toxicity, are biodegradable, and have broad-spectrum activity against a variety of hardy microbes. Hypochlorite (bleach) solutions are typically used in veterinary hospitals. Personnel in veterinary hospitals typically do not scrub boots or other footwear before using every footbath nor do they typically use a lengthy soaking process or consistently allow the frequently recommended 15-minute contact time before moving through the hospital. Thus, the expectations for efficacy of disinfectants are fairly rigorous when used in footbaths in typical animal husbandry environments, and results of this study should in this sense be considered reasonably conservative. It is also possible that holding samples at ambient temperature prior to culture may have allowed some replication to occur, which may have affected results. However, all samples were handled uniformly regardless of treatment, and the potential for bacterial replication would have been uniform for all samples, assuming that the neutralizing buffers were effective.

The 2 disinfectants chosen for evaluation in this study (a QAC and a peroxygen) were primarily selected because these were the disinfectants that were being used in footbaths at the JLV-VTH prior to this study. Results of the survey of VTHs indicated that disinfectants containing QAC and phenolic compounds were most commonly used in footbaths. The common use of phenolics is likely attributable to their broad spectrum of activity, and phenolics are thought to retain their activity fairly well for the presence of organic material. However, phenolic compounds are considered to have a greater potential to be toxic to humans and animals, compared with other commonly used types of disinfectants, and there are also concerns about environmental persistence of phenolic compounds. Quaternary ammonium compounds were also reported to be commonly used in footbaths in VTHs, and although high-concentration solutions can be irritating, they are generally considered to be less reactive than phenolic compounds. However, QACs are generally more likely to be inactivated in the presence of organic materials and generally have less activity against hardy microbes such as nonenveloped viruses. Peroxygen compounds can be irritating to skin because of their inherent drying action, but the disinfectant used in this study, which contained peroxymonosulfate as an active ingredient, has low toxicity, is biodegradable, and has broad-spectrum activity against a variety of hardy microbes. Hypochlorite (bleach) solutions
were also reportedly used at a few VTHs. Hypochlorite solutions have activity against some of the more hardy microbes, such as bacterial spores, but organic material rapidly binds and inactivates chlorine in dilute solutions such as dilute hypochlorite solutions.

Because there is diversity among animal species in fecal flora and diversity among bacterial flora found in animal stalls, experimental contamination was carried out in a single patient's stall to help make the contamination process more uniform. Despite the use of a single stall, bacteria recovered from treated and control boots were undoubtedly quite diverse. It has been estimated that ruminant intestinal contents can contain up to $10^{12}$ viable bacterial cells/mL from as many as 200 species. Flora found in animal environments change over time because of competition and fermentation. A recent study found that total coliform concentrations in composted bovine manure and bedding were $10^{7.9}$ g of feces (dry weight) on day 1 but had decreased to $10^{7}$ by day 7. Only 20% to 40% of those species are expected to be culturable by use of current technology. Most bacteria in animal environments are not pathogenic, except under extreme conditions; in addition to these, there are smaller numbers of potentially pathogenic organisms including gram-positive bacteria, such as Enterococcus spp, Clostridium spp, Bacteroides spp, Bacillus spp, Streptococcus spp, and Lactobacillus spp, and gram-negative bacteria, such as Enterobacteriaceae (including Escherichia coli, Salmonella spp, and Citrobacter spp), Campylobacter spp, and pseudomonads. Results of the present study suggested that ≤10% of the aerobic bacteria in the stall were gram-negative. This is consistent with results of studies regarding intestinal flora of animals and data regarding quantification of bacteria in the JLV-VTH environment. Culture conditions used in this study undoubtedly did not allow for recovery of all bacteria found on boots and most notably did not account for bacteria that are adapted to anaerobic and microaerophilic conditions. Although culture and enumeration procedures were not absolutely sensitive, procedures for sampling and culture were uniform for all treatments and generally allowed for unbiased comparison of bacterial concentrations among treatments.

This study was not designed to evaluate the risk of transmitting specific pathogens, such as Salmonella spp, but treatment-associated reductions in bacteria that can be cultured under aerobic conditions on BA or MAC, such as E. coli or other coliforms, suggested that the same treatment would decrease numbers of related pathogenic bacteria such as Salmonella spp. This is consistent with in vitro susceptibility data indicating that important pathogens such as Salmonella spp and Staphylococcus aureus are susceptible to antimicrobial effects of the peroxxygen disinfectant.

These results suggest that footbath hygiene can be improved through appropriate use of disinfectant footbaths, but results of this and other studies also indicate that footbaths should not be relied on as the only method of controlling the trafficking of infectious agents in veterinary hospital environments. General cleanliness of footware should always be emphasized in hospital settings, and footbaths cannot be relied upon to be an absolute barrier against the spread of contagious bacteria. Because reductions in bacterial concentrations of $\geq 3 \times 10^{-5}$ were not achieved with either of the disinfectant treatments, it might be concluded that footbaths are not worth the labor required to appropriately maintain them and other measures targeted at optimizing footware hygiene may be more worthwhile (eg, disposable shoe covers or separate footware for different hospital areas). However, although use of separate footwear may be of added benefit, it is better not to rely on a single preventive measure; the potential benefit of 75% reduction in bacterial concentrations on footware when using footbaths containing peroxxygen or other effective disinfectants may provide an important barrier to the spread of infectious agents. Further research is needed to characterize the practical benefit and cost effectiveness of using disinfectant footbaths.

The risk of Campylobacter infections in commercial broiler flocks in Great Britain was significantly reduced by the application of effective hygiene barriers, including appropriate use of disinfectant boot dips. Similarly, results of an epidemiologic study of Campylobacter infection in broiler flocks in The Netherlands suggested there was a reduced risk of infection when separate boots were used for each broiler house and when disinfectant footbaths were used when entering the broiler houses. Although these data provide support for the idea that footbaths are effective in reducing the risk of bacterial infections in intensive food animal operations, there is more direct evidence about the effect that footbaths have on bacterial contamination of footware. One recent study evaluated foot contamination on dairy operations and found that Salmonella spp could be routinely cultured from the surface of rubber boots after wearing them in housing areas at a large California dairy. Amass et al evaluated the use of footbaths by immersing boots in pig manure and then immersing them in disinfectant for 2 minutes and concluded that there was no decrease in bacterial concentrations unless boots were scrubbed with peroxxygen disinfectant; no difference was detected when using other disinfectants with or without scrubbing. Kirk et al evaluated foot contamination and footbath efficacy with an ampicillin-resistant strain of E coli that was used to spike bovine manure slurry, which was then applied to rubber boots by immersion. They found that bacteria could be spread by walking up to 400 feet (122 m) on a plastic surface and up to 150 feet (48 m) on a concrete surface. Footbath efficacy was evaluated by immersing boots in water for 1 minute and then immersing in disinfectant solution for 1 minute. After examining a limited number of replicate treatments, the authors concluded that alcohol and QAC solutions appeared to be the most effective disinfectants, but no formal statistical analysis was performed. Although methods of experimental contamination used in those studies are relevant for some livestock operations, they are not analogous to most situations encountered in veterinary hospitals. Furthermore, those methods of disinfection are not similar to those typically used at veterinary hospitals, where footbaths are often placed at control points throughout a facility where per...
sonnel step into and out of the footbaths without soaking or scrubbing.

It is important to note that footwear characteristics likely have a great impact on the efficacy of disinfectant footbaths as they are applied in field situations. Boots used in this study were new, had minimal tread, had no seams, and were impervious to water. These features allow for less accumulation of dirt and organic material, and the boots' waterproof nature improves compliance with footbath protocols. If footwear with deep treads are worn, it is more likely that brushing will be necessary to achieve measurable decreases in bacterial concentrations, as was noted by Amass et al.\textsuperscript{10,11} In our experiences at the JLV-VTH, compliance with footbath-use protocols is substantially reduced when typical street shoes or nonimpervious work shoes are worn in the hospital; personnel do not consistently immerse their footwear characteristics allow for less accumulation of dirt and organic material, and were impervious to water. These features, and as a result, personnel are generally not very compliant with using typical footbaths, but these are easily torn with minimal walking, and as a result, personnel are generally not very compliant with using typical footbaths, which rely on immersion when wearing plastic boots.

Footbath use is somewhat improved with the use of disposable boots, but these are easily torn with minimal walking, and as a result, personnel are generally not very compliant with using typical footbaths, which rely on immersion when wearing plastic boots.

References