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Short report

Infection control implications of the laundering of ambulance staff uniforms and reusable mops

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SUMMARY

There is a lack of published studies on laundering in ambulance services. We performed bacterial culture on soiled and unsoiled uniforms and reusable mop heads artificially contaminated with *Escherichia coli*, *Staphylococcus aureus*, and *Clostridium difficile* spores. Current laundering processes used for routine cleans in the ambulances appears, from our simulations, to be effective at reducing vegetative pathogenic bacteria to undetectable levels, $<3.398\ \log_{10}$ colony-forming units (*S. aureus* and *E. coli*). Reduced levels of *C. difficile* were still detected after laundering but the risk this poses for infection is unknown, as background levels of these spores in the environment are unknown.

Introduction

Most patients arrive at hospital in an ambulance, yet little has been published on infection control in the pre-hospital setting. During 2014/2015 in Scotland there were 740,631 emergency and urgent incidents involving ambulances.[1] During 2013/2014, a total of 2.87 million calls to the ambulance service in England resulted in a response from an emergency vehicle, and in Victoria (Australia) there were 823,278 ambulance callouts during 2012/2013.[2,3] Patients and healthcare personnel may be exposed to pathogens while in ambulances and this may be a route for the spread of pathogens between healthcare facilities, yet little information is currently available in the literature.

The Scottish Ambulance Service has laundering facilities on site that are used by the majority of staff to wash uniforms and also central facilities to wash reusable mop heads used to clean ambulances. The uniforms of healthcare staff and the environments they work in may become contaminated with potential human pathogens.[4] However, there are few published, peer-reviewed scientific studies on the infection control risks of ambulance staff uniforms or the microbiological quality of

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their laundering. The results of a cross-sectional study on the microbiological contamination of ambulance staff uniforms has provided direct evidence that uniforms can become contaminated with potential human pathogens.[5] Evidence also exists for the contamination of ambulances with meticillin-resistant *Staphylococcus aureus* (MRSA).[6]

We examined the effectiveness of ambulance station laundering in reducing bacterial contamination of ambulance staff uniforms, and the effectiveness of laundering of reusable mop heads used to clean the ambulances.

Methods

Bacterial strains were supplied by the National Collection of Type Cultures (London, UK). *Escherichia coli* (NCTC 9001), *Staphylococcus aureus* (NCTC 8178) and *Clostridium difficile* (NCTC 11209) spores were used in this study. *E. coli* and *S. aureus* were cultivated overnight in broth culture. *C. difficile* was supplied as a spore stock by Blutest Laboratories Ltd, UK, and prepared after the method described in Fraise *et al.*[7] The Scottish Ambulance Service provided a clean, unused, plastic-wrapped ambulance staff uniform and reusable mop heads. Ninety-millimetre circular uniform swatches and reusable mop heads were sterilized by autoclaving at 121°C for 15 min. Swatches and mop strands (4 cm) were placed within sterile plastic 90 mm Petri dishes (Fisher Scientific, Loughborough, UK) in a class 1 biosafety cabinet (MDH Ltd, Andover, UK). The swatches and mop strands, in triplicate, were artificially contaminated with bacteria [4.685–7.304 $\log_{10}$ colony-forming units (cfu) for *E. coli*, 4.749–6.816 $\log_{10}$ cfu for *S. aureus*, and 6.426–7.495 $\log_{10}$ cfu for *C. difficile* spores] with soiling (defibrinated horse blood) or without soiling (phosphate-buffered saline) (100 μL), covered and allowed to air dry at room temperature for 90 min. Unwashed controls were also analysed to determine the inoculum cfu, and a control without bacterial challenge washed to seek evidence for transfer of bacteria within the wash. Swatches were identified by punching holes near the edge prior to sterilization – different numbers corresponding to different challenges. The swatches were washed together (Miele PW6055 machine/Cleanline biological washing powder) at 30°C (38 min cycle) or 60°C (49 min cycle) and reusable mop heads at 30°C (38 min cycle) or 95°C (62 min cycle).

All swatches and mop strands were processed at the same time as follows: placed separately in 50 mL of sterile buffered peptone water (Oxoid, Basingstoke, UK), and processed in a stomacher (Stomacher 400, Seward, Worthing, UK) (4 min) using the 'high' setting. The liquid was removed and analysed using the Miles and Misra plate counting method.[8] *E. coli* and *S. aureus* were assayed on CLED (cystine-, lactose-, electrolyte-deficient) plates incubated at 37°C for 24 h. *C. difficile* spores were assayed on *Clostridium difficile* agar (CDA) plates [*Clostridium difficile* agar base, D-cycloserine (250 mg/L), cefoxitin (8 mg/L)] (Oxoid, Basingstoke, UK) and 7% defibrinated horse blood (Fisher Scientific, Loughborough, UK). CDA plates were incubated at 37°C for 72 h under anaerobic conditions in gas jars (Becton, Dickinson & Co., Oxford, UK).

We compared the $\log_{10}$ cfu counts reported for the washed test materials to the unwashed controls ($\log_{10}$ reduction). Where the cfu counts for the washed test materials were below the lower limit of detection for the Miles and Misra assay the $\log_{10}$ reduction was estimated based on the distance between the $\log_{10}$ cfu value for the unwashed controls and the lower limit of detection of the assay (3.398 $\log_{10}$ cfu), and reported as 'greater than' values.

Results

E. coli were not recovered from any of the unsoiled or soiled swatches. *S. aureus* were only recovered from swatches washed at 30°C. *C. difficile* spores were recovered from all swatches washed at 30°C and 60°C (Table I). Neither *E. coli* or *S. aureus* were recovered from washed unchallenged swatches. However, *C. difficile* spores, washed at 30°C and 60°C, were recovered from unchallenged swatches at the lower limit of detection of the assay (3.398 $\log_{10}$ cfu).

E. coli were recovered from all mop strands washed at 30°C but not at 95°C. *S. aureus* were recovered from two of three unsoiled mop strands and all soiled mop strands washed at 30°C but none at 95°C. *C. difficile* spores were recovered from all mop strands washed at 30°C and one of three unsoiled mop strands washed at 95°C (Table II). Neither *E. coli*, *S. aureus* nor *C. difficile* spores, washed at 30°C and 95°C, were recovered from unchallenged mop strands.

Discussion

This study examined the reduction in bacterial counts following standard laundering practice for staff uniforms and the reduction in bacterial counts following standard laundering practice for reusable mop heads.

Higher temperatures were effective at reducing *E. coli* and *S. aureus* to undetectable levels on uniform swatches and mop strands. However, *C. difficile* spores were still detectable after washing at 60°C both on the deliberately contaminated (challenged) (Table I) and unchallenged uniform swatches, and one mop strand washed at 95°C but not the rest (Table II). These data suggest that a proportion of *C. difficile* spores survived the wash and remained within the washing machine or rinse water.

Evidence exists that healthcare workers' uniforms may become contaminated with bacteria during use, with a mixture of bacteria from the wearers' own flora (mostly non-pathogenic in nature) and potential pathogens from handling patients.[9] Nurse uniforms in hospitals have been shown to harbour MRSA, *C. difficile* and vancomycin-resistant enterococci both before and after shifts.[4] The ability of laundering to kill *Enterococcus faecalis* has been shown to be temperature dependent with a >4 $\log_{10}$ reduction at 65°C and >8 $\log_{10}$ reduction at 85°C (20 min contact time).[10] The $\log_{10}$ reduction was heavily influenced by the temperature of the wash for *S. aureus*, *E. coli*, and *C. difficile* spores.

Little information is available on the potential survival of *C. difficile* spores during laundering. Our data show that *C. difficile* spores could potentially survive laundering at temperatures as high as 60°C and perhaps as high as 95°C. These results are important and have implications in any environment where patients and staff are at risk of coming into contact with *C. difficile*. We also observed that *C. difficile* spores were transferred to the sterile unchallenged materials when uniform swatches were washed at 30 and 60°C, which suggests that there may be a risk of other garments becoming contaminated if washed along with materials contaminated