Major article

Spread of infectious microbes during emergency medical response

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Background: To our knowledge, no studies to date demonstrate potential spread of microbes during actual emergency medical service (EMS) activities. Our study introduces a novel approach to identification of contributors to EMS environment contamination and development of infection control strategies, using a bacteriophage surrogate for pathogenic organisms.

Methods: Bacteriophage ΦX174 was used to trace cross-contamination and evaluate current disinfection practices and a hydrogen peroxide (H_2O_2) wipe intervention within emergency response vehicles. Prior to EMS calls, 2 surfaces were seeded with ΦX174. On call completion, EMS vehicle and equipment surfaces were sampled before decontamination, after decontamination per current practices, and after implementation of the intervention.

Results: Current decontamination practices did not significantly reduce viral loads on surfaces ($P = .3113$), but H_2O_2 wipe intervention did ($P = .0065$). Bacteriophage spread to 56% (27/48) of sites and was reduced to 54% (26/48) and 40% (19/48) with current decontamination practices and intervention practices, respectively.

Conclusion: Results suggest firefighters' hands were the main vehicles of microbial transfer. Current practices were not consistently applied or standardized and minimally reduced prevalence and quantity of microbial contamination on EMS surfaces. Although use of a consistent protocol of H_2O_2 wipes significantly reduced percent prevalence and concentration of viruses, training and promotion of surface disinfection should be provided.

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During an emergency medical service (EMS) call, patient information is limited, and the infectious disease status of a patient is not always known. EMS responders are in frequent contact with high-risk populations, such as patients in hospitals or long-term care facilities, and engage in rapid interactions with patients in uncontrolled environments, which could lead to worker and patient safety issues.

Prior studies have identified that methicillin-resistant *Staphylococcus aureus* (MRSA) poses a significant health and occupational risk to firefighters and medical patients, with numerous cases reported across the nation resulting in severe or fatal outcomes.[1]

Previous U.S. studies show that 4.6% (13/280) of EMS personnel and 22.5% (9/40) of firefighters were colonized with MRSA, rates much higher than in the general population (0.8%).[2-5] Roberts et al[3] determined that 58% of nasal MRSA isolates obtained from fire station personnel were confirmed to be genetically related to environmental surface isolates, suggesting transmission between personnel and the surfaces. Although MRSA has been the primary focus of EMS responder infectious disease transmission studies, the information is relevant to exposure risks from other infectious agents. Fomites have been shown to play a role in transmitting an array of human pathogens either directly by surface-to-mouth or abraded skin contact or indirectly by contamination of fingers and subsequent hand-to-mouth, eyes, nose, and cut or abraded skin contact, suggesting potential infection risk for EMS responders and their patients.[2,6,7]

In many communities, such as the one in this study, fire services are the first line of response in emergency medical calls. Typically, in U.S. fire services, medic trucks are staffed by a firefighter and a firefighter paramedic and dispatched to scenes to provide medical assistance. The fire engines are staffed by a firefighter engineer,

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Table 1
Common EMS equipment and vehicle sites sampled for bacteriophage

Surfaces	Description	Surface area (cm²)
Seeded surfaces		
Clasp of EPCR*	EPCR: used to input patient information on arrival at scene	67.6
Handle of LIFEPAK†	Heart monitor: used on every EMS call	171.39
Additional sites sampled for cross-contamination		
EPCR touchscreen*	EPCR	65.52
EPCR keyboard*	EPCR	305.64
MDT keyboard*	Mobile data terminal: used as GPS and to receive preliminary information regarding the call from dispatcher	305.64
Portable radios*	Carried by each firefighter	50.84
Inside cab*	Steering wheel, grab bar, seatbelt buckles	477.38
Headphones*	In engine only	148.44
LIFEPAK keypad*	Heart monitor digital keypad	108.57
Jumpbag handle‡	EMS equipment bag	141.95
Glucometer*	Digital blood glucose meter	7.8

EMS, emergency medical service; EPCR, electronic patient care record; GPS, Global Positioning System; MDT, mobile data terminal.
*Nonporous surface.
†Part nonporous, part hard rubber, semiporous surface.
‡Hard rubber, semiporous surface.

firefighter captain, firefighter paramedic, and a firefighter and dispatched to more serious medical calls and fires. In the most serious emergency calls, both the medic truck and engine are dispatched to the scene. The medic trucks are able to transport patients; however, freestanding ambulatory services often assist the fire department in transporting patients from the scene of an emergency to hospitals. Fire engines and the medical equipment carried in them have not been previously evaluated as reservoirs of potential pathogens. Data on cross-contamination of both of these common EMS vehicles during emergency calls were not available, prior to this study. The current study used a bacteriophage tracer to characterize the cross-contamination within medic trucks, fire engines, and on EMS equipment used during calls to elucidate the exposure potential to EMS responders and patients, and the fomite-hand-fomite transmission relationship in an actual field setting. Additionally, current decontamination practices were evaluated and compared with an activated hydrogen peroxide wipe (Clorox Healthcare, Oakland, CA) intervention.

METHODS

A bacteriophage tracer ΦX174 was used to mimic fomite-to-fomite transmission of human viruses on EMS vehicles and equipment during real-time emergency calls. Bacteriophage ΦX174 is a coliphage DNA virus that only infects the K-12 strain of *Escherichia coli*.[8] The inability of the tracer to infect human cells is important because it was used during actual EMS calls and patients were of unknown immune status. The University of Arizona Office for Human Research Protections, which oversees the institutional review board, approved the study as exempt.

This study was conducted within a fire station of the Northwest Fire District in Tucson, Arizona, using both engines and rescue trucks. Researchers accompanied firefighters on EMS calls prior to design of the study and recorded behavioral observations to select high frequency touch surfaces for subsequent bacteriophage seeding and to select postcall surfaces to sample.

Selection of sites and measurement of surface areas

Based on observation of frequent use, 2 hard nonporous sites, a heart monitor-defibrillator (LIFEPAK, Physio-Control, Redmond, WA) handle and electronic patient care record (EPCR) clasp, were selected as the 2 sites to seed prior to EMS calls. Both were not handled by firefighters until arrival on a scene of the EMS call. Thirteen sites were selected for postcall sampling in the engine

vehicle, whereas 12 sites were selected from the medic truck (Table 1). Sites identified ranged from 7.8-477.38 cm².

Selection of disinfecting product for intervention

Manufacturer care information for frequently used EMS equipment was studied to determine chemicals approved for use on each piece of equipment. Use of chemicals not on the approved lists provided by manufacturers would cause voiding of equipment warranties. Hydrogen peroxide wipes were selected for use in the intervention because they were safe to use on all of the EMS vehicle surfaces. Activated H_2O_2 wipes were supplied by Clorox Healthcare; however, comparable hydrogen peroxide wipes are available.[9]

Bacteriophage propagation

The ΦX174 suspensions were prepared by growing host bacterium *E coli* (ATCC 700609) in 125 mL of Tryptic Soy Broth (BD Diagnostics, Sparks, MD) at 37°C, shaking at 150 rotations per minute for 4 hours, applying a double agar overlay technique (BD Diagnostics), and incubating at 37°C for 24 hours.[10] Overlays were allowed to set, and the plates were inverted and incubated at 37°C for 24 hours. The plates containing bacteriophage were soaked with 6 mL of Tryptic Soy Broth for 2 hours, and eluent was collected and centrifuged (3,600 × g for 10 minutes at 4°C). The supernatant was filter sterilized using a syringe fitted with a 0.2-μm filter. Tenfold serial dilution were performed using 8.5% saline, and the double-layer overlay technique previously described was repeated. The plaques on each plate were counted to determine the titer of ΦX174 in plaque forming units (PFU) per milliliter. The remaining bacteriophage was stored at 4°C until used at fire stations.

Surface seeding and sampling

The sampling was divided into 3 phases to characterize transfer and presence of bacteriophage. In phase 1 (August 31, 2012-September 20, 2012), samples were taken before any vehicle or equipment decontamination was performed. In phase 2 (September 20, 2012-October 4, 2012), samples were taken after decontamination per current practices. In phase 3 (October 4-8, 2012), samples were taken after the implementation of an activated hydrogen peroxide wipe intervention. During each phase, 58 surface samples were obtained from 5 EMS calls: 3 from engine surfaces and 2 from the medic truck. On arrival to the station, surfaces of the engine and rescue vehicles were disinfected using activated

hydrogen peroxide (H$_2$O$_2$) wipes (Clorox Healthcare). Two hard nonporous sites (LIFEPAK handle and EPCR clasp) were seeded with bacteriophage (concentration of approximately 10^8 PFU/mL) using cotton-tipped applicators (Puritan Medical, Guilford, ME) each hour, until the occurrence of an EMS call, to maintain viral loads. The surfaces selected for seeding were chosen because they were not handled by emergency responders until arrival on a scene and therefore represented contamination that may occur during a call. Samples were collected using swabs (3M, Forest City, IA) containing 1 mL of letheen broth to neutralize residual disinfecting chemicals on sampled surfaces. Before the start of the EMS calls, approximately half of each of the seeded surfaces were sampled using letheen broth swabs to obtain the starting concentration of the surfaces (n = 10 per phase). The additional nonseeded surfaces of interest were also sampled before the start of the EMS calls to ensure bacteriophage contamination of these sites did not occur prior to a call (n = 48 per phase).

On completion of the EMS call, the 2 seeded surfaces (n = 10 per phase) and the additional sites of interest were sampled for ΦX174 (n = 48 per phase). In phase 1, all surfaces were sampled prior to any decontamination activity; in phase 2, all surfaces were sampled after decontamination per current practices, which were not standardized, and technique and supplies used varied from employee to employee; and in phase 3, all surfaces were sampled after decontamination using the activated hydrogen peroxide wipe intervention (Fig 1). During the phase 3 intervention, the activated hydrogen peroxide wipes were stocked in EMS vehicles, and firefighters were instructed to wipe down all surfaces or items they recalled handling during the EMS call.

Quality control

Entire surface areas of targeted fomites were sampled when possible. The sample swabs were transported on ice to the laboratory for processing and were processed within 24 hours using the top agar overlay technique, described previously. Negative controls consisted of overlays with before samples taken from surfaces prior to the EMS call to confirm that no onsite contamination occurred before EMS calls. Further, unused letheen broth swab overlays were analyzed as laboratory blanks. Positive controls included sampling of half of the seeded surfaces to quantify the bacteriophage present at onset of each EMS call, with overlays done using stock bacteriophage to confirm laboratory handling and technique were not deactivating bacteriophage. Bacteriophage plaques were enumerated and recorded as PFU per site.[7,11,12]

Statistical analysis

Viral load values were log transformed for the purpose of statistical analysis, and nonparametric tests were applied because of highly variable and skewed values. Differences in viral loads between the 3 phases (predecontamination, current practices, activated H$_2$O$_2$ intervention) were analyzed using the Kruskal-Wallis test. In cases where the Kruskal-Wallis test was significant, a Wilcoxon signed-rank test was also applied for multiple pairwise comparisons. Using a preadjusted 2-tailed probability value of <.05, a Bonferroni adjustment was applied to control for type I error; therefore, $\alpha < .0167$ was chosen as the level of significance. All statistical analysis was performed using Stata version 12 (StataCorp, College Station, TX).

RESULTS

The mean bacteriophage load of seeded surfaces from phase 1 (predecontamination) was $(1.59 \pm 1.98) \times 10^6$ PFU before the start of EMS calls and $(1.89E \pm 2.81) \times 10^5$ PFU on completion of EMS
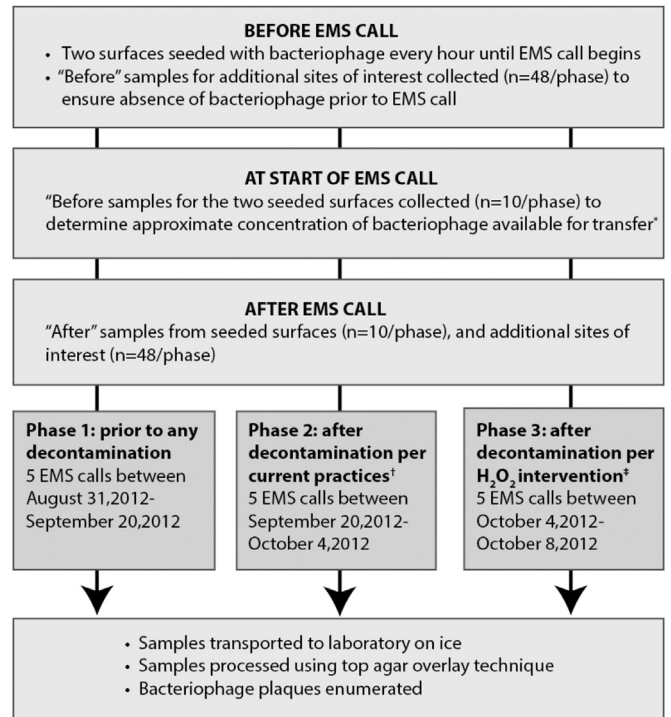

Fig 1. Phage tracer movement in emergency medical services (EMS) vehicles: summary of field sampling protocol. *Only half of the seeded surface were sampled to obtain the approximate starting concentration of bacteriophage available for transfer to avoid removal of all bacteriophage. †Current practices were not standardized and varied from sample to sample. ‡During the intervention the firefighters were given hydrogen peroxide wipes and asked to disinfect any surfaces they recalled handling during the EMS call.
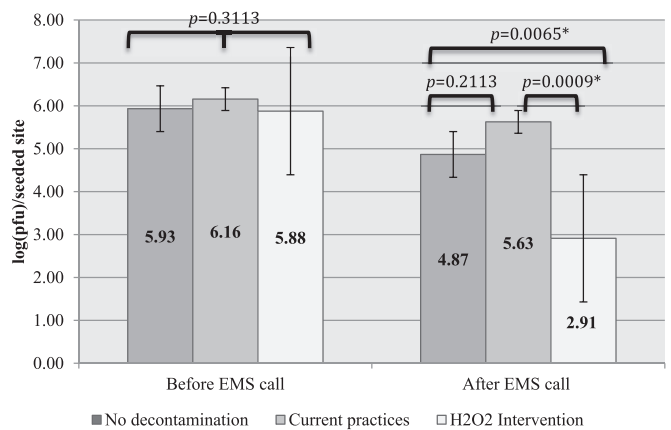

Fig 2. Mean viral load of seeded surfaces per phase, before and after EMS calls. *EMS*, emergency medical services; *pfu*, plaque forming units. *Statistical significance.

calls. During phase 2 (decontamination per current practices), the mean viral load of seeded surfaces was $(2.03E \pm 1.86) \times 10^6$ PFU prior to the start of EMS calls and $(8.37 \pm 7.43) \times 10^5$ PFU after decontamination by firefighters. The mean viral load of seeded surfaces during phase 3 (decontamination after H$_2$O$_2$ wipe intervention) was $(2.93E \pm 4.35) \times 10^6$ PFU before the start of EMS calls and $8.14 \times 10^4 \pm 2.26 \times 10^5$ PFU after EMS personnel performed decontamination using the activated H$_2$O$_2$ wipes.

Viral loads of seeded surfaces were not significantly different before the start of EMS calls between phases ($P = .3113$, Kruskal-Wallis), but they were significantly different after EMS calls

($P = .0005$, Kruskal-Wallis) (Fig 2). Decontamination per current practices did not significantly reduce viral loads of the seeded surfaces ($P = .2113$, Wilcoxon rank-sum). Intervention with activated H_2O_2 wipes significantly reduced the viral loads when compared with surfaces before any decontamination ($P = .0065$, Wilcoxon rank-sum) and when compared with decontamination per current practices ($P = .0009$, Wilcoxon rank-sum) (Fig 2).

Bacteriophage cross-contamination of EMS equipment surfaces was detected during all EMS calls (15/15), regardless of the phase. Cross-contamination occurred on a total of 56% (27/48), 54% (26/48), and 40% (19/48) of surfaces predecontamination, after disinfection per current practices, and after H_2O_2 wipe intervention, respectively. Table 2 describes the frequency of cross-contamination and quantitative results for each site within each phase of the study.

After decontamination per current practices and the hydrogen peroxide wipe intervention, the rubber jumpbag handle still had the greatest viral load of the cross-contaminated surfaces (226 ± 153 PFU, and 230 ± 315 PFU, respectively). Introduction of the activated hydrogen peroxide intervention decreased the total viral load on all cross-contaminated fomites combined from both disinfection per current practices and no decontamination; but the differences in total viral load of combined surfaces between phases was not significant ($P = .3703$, Kruskal-Wallace) (Fig 3).

DISCUSSION

Previous studies have confirmed the presence of certain bacteria in and on EMS vehicles and equipment, but this is the first study, to our knowledge, to characterize the transfer of microbes from surface to surface during actual EMS calls.[2,3,7,11,12] Coliphages, such as the one used in the current study (ΦX174), have long been used as a human virus surrogate in studies that examine the spread and inactivation of pathogens (eg, norovirus, poliovirus, influenza, rhinovirus) in laboratories, hospitals, day care facilities, and nursing homes.[8,13-17] Although ΦX174 is physiologically very similar to human viruses, its environmental stability is comparable with both pathogenic viruses and some bacteria. However, as a surrogate it may underestimate the persistence of some bacteria.[8] Human pathogenic viruses (eg, influenza, severe acute respiratory syndrome, norovirus) have been shown to survive 24 hours-12 days on nonporous surfaces, whereas human pathogenic bacteria (eg, MRSA, vancomycin-resistant *Enterococcus*, *Pseudomonas aeruginosa*, *Clostridium difficile* spores) have been shown to survive anywhere from 7 hours-5 months on nonporous surfaces.[18-25] Based on some of these values, as a surrogate, ΦX174 may underestimate the environmental persistence of some of the healthcare-associated infection—causing micro-organisms.

Consideration of microbe survival in addition to transfer efficiency and disinfectant resistance is necessary to more accurately estimate surrogate relationships to specific pathogens. A previous laboratory-based study by Lopez et al[26] reported average fomite-finger transfer efficiencies for poliovirus, *E coli*, and *S aureus* as 36.3%, 21.7%, and 4.3%, respectively. With ΦX174 being most physiologically similar to poliovirus, its transfer efficiencies from fomite to hand and vice versa are likely greater than that of bacteria, which may cause overestimation of rate of transfer for bacteria, such as MRSA, in this study. However, with bacteria likely being more apt to survive on environmental surfaces for much longer periods of time than ΦX174, it is likely that its greater persistence would eventually lead to greater transfer incidence than that measured in a single laboratory study.

The initial concentration of ΦX174 suspension used to seed the EPCR clasp and LIFEPAK handle was approximately 10^8 PFU/mL. Seeded surfaces were sampled prior to the start of all EMS calls, and the amount of PFU per surface recovered was

Table 2
Prevalence of cross-contamination and viral loads of sites after EMS calls

Sites	No decontamination		Current practices		H_2O_2 intervention	
EPCR keyboard	3/5	54.2 ± 91	1/5	5.8 ± 11	1/5	72 ± 158
EPCR touchscreen	1/5	67.4 ± 138	3/5	76.4 ± 84	2/5	90 ± 141
Glucometer	2/5	8.40 ± 7	2/5	10.4 ± 10	1/5	15 ± 26
Headphones	3/3	128 ± 188	1/3	7.33 ± 11	1/3	9 ± 14
Inside cab	3/5	390 ± 15	2/5	7.60 ± 9	5/5	96 ± 129
Jumpbag handle	4/5	40.2 ± 28	5/5	226 ± 153	3/5	230 ± 315
LIFEPAK keypad	3/5	39.4 ± 58	3/5	58.4 ± 70	1/5	33 ± 68
MDT keyboard	3/5	92.4 ± 173	5/5	33.0 ± 9	0/5	$<10 \pm 0$
MDT touchscreen	2/5	13.4 ± 14	1/5	10.4 ± 12	2/5	24 ± 35
Portable radios	3/5	10.4 ± 191	3/5	11.4 ± 10	3/5	20 ± 17

NOTE. Values are positive samples/total samples or mean $\pm$ SD.
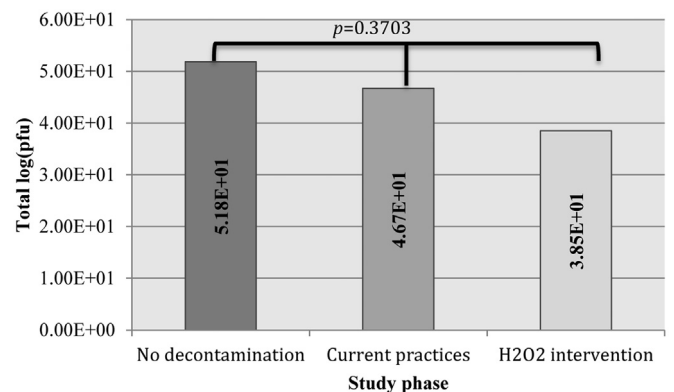*EPCR*, electronic patient care record; *MDT*, mobile data terminal.

Fig 3. Total viral load of combined cross-contaminated sites per phase. Total viral load of all sites sampled (n = 48 per phase) per each phase of the study.

consistently at approximately 10^6. This is within the range of what would realistically be detected on surfaces contacted by infected or colonized individuals.[27-30] Results were reported as PFU per site, and surface areas sampled were not equal, but instead they were based on likely surface areas contacted during EMS calls.[7,11,12] This method does not allow for a direct comparison of sites contaminated per unit surface area, but it is more reflective of exposure potential, which is the primary concern of this study.

Viral loads from seeded sites were not significantly reduced with cleaning and disinfection practices in place prior to the intervention, and the percent of cross-contaminated sites was only reduced by 2%. However, prior to intervention education and training, firefighters only disinfected surfaces when visibly soiled, which is likely why the reduction observed was not significant. In the only other existing study to assess efficacy of current cleaning procedures in emergency vehicles, the total percent reduction of bacteria-positive sites was greater than what was observed in this study at 25.6%, but attributed to researchers using paramedics to swab allocated areas of the vehicle, once before cleaning and once after cleaning, enabling EMS crews to know which sites were of interest.[12] This may have caused them to focus on those sites more rigorously for cleaning and disinfection, which would bias results. In the current study, the firefighters were blinded to which surfaces were being sampled to minimize the potential for information bias. Additionally, researchers accompanied firefighters on calls for several weeks prior to the start of the study in an attempt to lessen the Hawthorne effect, which is the tendency of subjects who know they are being observed to temporarily change their behavior.[31-34]

The viral loads of seeded surfaces were significantly reduced with the introduction of the H_2O_2 wipe intervention ($P = .0009$, Kruskal-Wallis), and the percent of cross-contaminated sites was reduced by 16% (from 27/48 to 19/48). During the intervention phase, firefighters were instructed to use the activated H_2O_2 wipes on surfaces they recalled handling during the EMS call. Often, sites targeted by researchers within the vehicle (eg, steering wheel, headphones, grab bars, portable radios) were not selected for disinfection by firefighters. Had firefighters been trained on what surfaces to clean and disinfect, the results may have been more pronounced. The amount of virus recovered from these surfaces prior to EMS calls was not significantly different between study days ($P = .3113$, Kruskal-Wallis), indicating that significant differences detected in viral loads after the EMS calls were not caused by variability in starting seeded virus concentrations.

The presence of bacteriophage on the additional surfaces of interest indicates transmission of the tracer virus, via direct contact, surface to surface, or by an airborne route. Although it is possible for viruses to be airborne on surface disruption, the physical separation of many of the cross-contaminated additional surfaces of interest from the seeded surfaces suggests that the firefighters' hands were the main vehicles for transfer of the virus. The cross-contamination of surfaces within the emergency response vehicles suggests that the firefighters were exposed to the virus during EMS calls. Although gloves were observed being used during most EMS calls, some of the cross-contaminated surfaces (eg, steering wheel, headphones, EPCR, seatbelt buckles, portable radios) are commonly handled with ungloved hands immediately after the call. The aforementioned sites handled with ungloved hands were contaminated 46.4% (35/69) of the time after EMS calls, during all 3 phases of the study, highlighting the importance of the fomite-hand-fomite transmission route.

High-touch surfaces, such as the ones sampled in this study, should be targeted for regular disinfection to decrease exposure to EMS responders and their patients. Future studies should identify procedures and products to disinfect soft and porous surfaces within the emergency response vehicles and EMS facilities because the porous jumpbag handle had the greatest viral load, even after implementation of the activated H_2O_2 wipe intervention. EMS vehicle seats and other soft surfaces in the vehicles should be targeted for pathogen sampling, and if necessary, development of sanitizing procedures for soft surfaces in EMS vehicles should be investigated.

Characterization of microbe transfer during real-time EMS calls provides information on how to prioritize best practices for environmental decontamination in EMS settings. Further development, enforcement, and evaluation of an infection control program, with greater attention to decontamination of vehicle surfaces, will result in reduced infectious microbe exposures for both EMS responders and patients.

CONCLUSIONS

Our study demonstrated that 2 contaminated sites caused cross-contamination of emergency vehicle sites in 100% (15/15) of EMS calls. Cross-contamination was detected on a total of 56% (27/48), 54% (26/48), and 40% (19/48) of surfaces predecontamination, after current decontamination practices, and after introduction of the activated H_2O_2 wipe intervention, respectively. Although gloves were used during EMS calls, the aforementioned sites were sometimes handled with ungloved hands after EMS calls, suggesting microbial exposure to firefighters. This study identified a lack of standardized surface decontamination protocols, and results suggest that current practices only minimally reduce prevalence

and quantity of microbial contamination on EMS surfaces. Concentrated effort to develop and evaluate more effective infection control practices in EMS settings should be promoted to reduce potential risk to the health and safety of EMS responders and their patients.

Acknowledgment

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