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Major article

Environment surface sampling in 33 Washington State fire stations for methicillin-resistant and methicillin-susceptible *Staphylococcus aureus*

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Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-susceptible *S aureus* (MSSA) were isolated from environment surfaces sampled from 33 Washington State fire stations.
Methods: Samples were collected by fire personnel using commercial testing swabs. One to 6 surfaces were sampled per swab with 20 swabs per station. Biochemical tests were used to confirm MRSA and MSSA isolates. A short survey designed to collect information on cleaning procedures in the stations was included in the kits.
Results: MRSA was isolated from 8.0% and MSSA from 18.5% of the 653 samples. Nineteen fire stations (58.0%) were MRSA positive, 27 stations (82.0%) were MSSA positive, and 14 stations (42.4%) were positive for both MSSA and MRSA. Three stations (9.0%) were negative for MSSA and MRSA. Twelve fire stations (37.5%) reported fire service professionals with MRSA needing medical care. Positive controls were detected at levels of $>10^2$ CFU/mL and negative controls were negative.
Conclusions: The kit system allowed sampling of >2,000 surfaces from fire stations across Washington State. This is the first time an estimate of the level of MRSA-infected fire personnel has been determined from multiple districts within a single state. Further work is needed to determine if these data can be extrapolated to other career-based fire stations across the country.

Staphylococcus aureus is part of the normal human flora and routinely isolated from the anterior nares, skin, axilla, perineum, and pharynx. In healthy humans from the community, 25%-35% carry methicillin-susceptible *S aureus* (MSSA) in their anterior nares.[1] Carriage varies by age with ~60% of the population intermittently colonized with *S aureus*.[2] During the past decade community-acquired methicillin-resistant *S aureus* (MRSA) has emerged as a major cause of disease in the general population with no health care exposure or known classic risk factors.[3] Approximately 60% of hospital patients colonized with MRSA developed a MRSA infection, whereas 25% of colonized patients develop an infection within 12 months of returning home from a hospital stay.[4] About one-third of patients newly identified as MRSA-positive develop subsequent MRSA infections regardless of whether or not they were colonized or previously infected.[5] People colonized or infected with MRSA and/or MSSA shed these bacteria into their environments, contaminating surfaces and fomites at concentrations sufficient for survival for extended periods of time. This allows for transfer to skin, clothing, and other fomites.[6] Hospitalized patients and patients in nursing homes may have MRSA colonization rates reaching 60% and these are the people commonly served by fire personnel and other first responders.

Previously, we sampled 9 different areas in the garages and living quarters at 2 fire stations in 2 districts within western Washington State.[7] In that study,[7] the Replicate Organism Detection and Counting (RODAC) plates detected 10%-40% of seeded bacteria and Sanicult transport swabs (Starplex Scientific, Etobicoke, Ontario, Canada) detected 10-100 CFU/mL under laboratory conditions. The Sanicult swabs identified 82% and the RODAC plates identified 18% of the 44 MRSA-positive samples.[7] Only 1 other study has reported both MRSA- and MSSA-contaminated surfaces in fire-related facilities.[8] In both studies, trained laboratory personnel did the environment sampling and in general 1 surface was collected per swab. In our first Washington State fire study,[7]

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nasal cultures were done on personnel from 1 fire district resulting in 22.5% MRSA-positive samples. One other study has looked at nasal colonization among emergency personnel from 2 fire departments in a small mid-Atlantic state where 6.4% had positive tests for MRSA.[9]

We determined that multiple surfaces ($\geq$2) could be sampled with a single swab. This reduced sampling and processing time for the 33 fire stations and >2,000 surfaces were sampled. Kits were assembled and sent to the fire stations where environment sampling was done by fire personnel. Samples were processed in a laboratory to determine if the percentage of MRSA- and MSSA-positive samples were similar to those obtained in our previous Washington State fire station study.[7] Positive MRSA controls were included in 6 kits to determine quality of the laboratory's detection limits. In addition, a short self-administered survey was developed to collect information on MRSA infections in fire personnel as well as cleaning and disinfection protocol information for individual stations.

MATERIALS AND METHODS

Composite surface sampling

We have previous experience with using Sanicult transport swabs with 1 mL solution for sampling fire station surfaces.[7] In that previous study[7] a single surface was sampled/swabbed and a substantial number of people were required to collect the samples and process the samples. One way to streamline the collection and processing was to sample multiple surfaces within a single area using a single swab. This allows for an increased number of surfaces to be sampled while reducing the number of swabs, processing time, and costs. To test this, a characterized environmental MRSA strain 9-48 was grown overnight to approximately 10^8 CFU/mL and then 100 μL fluid containing 10^1, 10^2, 10^3, 10^4, or 10^5 CFU were plated on cleaned sterilized gurney straps provided by a fire station. Multiple spots with 100 μL fluid were spread out to a 5 cm^2 area. The seeded strap was left in the biosafety cabinet for 1 hour to dry and then any remaining liquid was spread out and allowed to completely dry for an additional 1-2.5 hours. Both seeded and sterile areas were included on each strap. Four spots were sampled with each Sanicult swab (by moving the swab back and forth to cover the entire 5 cm^2 area) and then placed back into the tube. Swabbing was repeated with the other 3 areas. In each experiment, the first or last surface was seeded with MRSA the other 3 were not. After all 4 areas were swabbed, the swab was placed back in the tube and 1.5 mL Bacto m *Staphylococcus* broth (1.5×; Difco Laboratories, Sparks, MD) supplemented with a final concentration of 75 μg/mL polymyxin B and 0.01% potassium tellurite (Sigma-Aldrich, St Louis, MO) was added to the Sanicult tube and incubated in 5% carbon dioxide at 36°C as previously described.[7] Tubes were examined for growth at 24 and 48 hours and labeled positive if the liquid was turbid and there was black precipitate present. The experiments were repeated 3 times on separate days. Limited testing with soft surfaces indicated that recovery would be lower (10-100 times) than hard surfaces because the inoculum soaked into the surface rather than remaining on top.

Survival of MRSA on swabs

The MRSA strain 9-48 was grown overnight and 1 mL 10^1-10^5 CFU were added to the Sanicult swab containing 1 mL buffer and stored at room temperature (~22°C). Six tubes of each dilution for each test were set up and the experiment was repeated 3 times. Each day the swabs were vortexed and 0.1 mL samples were removed and placed into fresh tubes with supplemented Bacto m *Staphylococcus* broth and tested for ability to grow after incubation in 5% carbon dioxide at 36.5°C for 24-48 hours. Viability was defined as the ability of the strain to grow by 48 hours. Samples were shipped during the winter months and thus the potential for kits being exposed to 0°C was possible. To determine the effect of cold temperature ($\leq$0°C) on collected surface samples, we tested 2 additional sets of inoculated swabs incubated at 0°C from 6-48 hours before supplemented growth media was added to determine if exposure to low temperatures would reduce recovery of MRSA from positive samples.

Kits

The kits contained all supplies needed to conduct sampling, a survey, and a prepaid US Postal Service Priority Mail envelope for returning samples. The kit included directions and pictures on how to collect samples from various surfaces. Each kit had a WarmMark Indicator (37°C) and a ColdMark Indicator (0°C) (LabelMaster, Chicago, IL) that changed color if the temperature of the kit was above 37°C or below 0°C, a sheet with a list of surfaces to be sampled, and sample number tube labels. Each tube containing a sample swab was wrapped in parafilm and placed into a zippered plastic bag to prevent leakage. The kit was sent for processing via the US Postal Service in the prepaid mailer. A chain of custody form was also included in each kit. The stations were listed by number and the field blanks were randomly labeled in each kit but were not counted in the final results because all were negative and were specifically for quality control. All kits were returned to the Departmental Field Group where the kits were logged and the survey responses entered into a database. The kits were then transferred by Field Group personnel to the laboratory and processed within 2 hours of being received. Positive control samples were added to the kits before they were transported to the laboratory. Samples were processed the day of arrival or stored at room temperature and processed within 18 hours if they arrived late in the afternoon.

Environment surfaces sampled

Fire station recruiting was done in partnership with the Washington Fire Chief Association. Outreach activities to increase awareness of the project were done by attending firefighter association conferences, regional meetings, and workshops. Letters from personnel authorizing participation in the study were required before sending the kits out to individual stations. The study sampled 13.8% of stations (n = 33) from a total of 240 career-based fire stations in 28 different fire districts. Samples were collected November 2011-May 2012 from 6 eastern Washington State and 27 western Washington State fire stations. The area to be swabbed was a circle of approximately 6-7 cm in diameter (28-38 cm^2). Nineteen swabs were used for composite sampling of assigned surfaces, whereas the 20th swab was used to sample a single surface that each station chose that was not previously sampled. Two extra swabs were included in the kit that were not opened by the stations and used as field blanks (ie, negative controls). The environment surface areas to be sampled were based on 2 previous fire station studies[7,8] that determined which surfaces are most often MRSA positive. In addition, surfaces that had the highest risk of bare skin contact and locations amenable to cleaning and disinfecting were added.

Nine areas were sampled with 19 swabs. Medic truck sample areas included seat belts on driver and passenger sides (2 surfaces); the top and inside of the handles of 2 medical bags recently used inside a person's home during a call (4 surfaces); gurney straps, metal buckle, and ceiling grab bar (4 surfaces); diaphragm of the