

RESEARCH BRIEF

Detecting Environmental Contamination of MRSA in Ambulances: A Novel and Efficient Sampling Methodology

Robert C. Orellana^{1,2}; Armando E. Hoet^{1,3,4}; Joany C. van Balen³; Bo Lu⁵; Christina Kelley⁴; Kurt B. Stevenson^{1,6}

¹Division of Epidemiology, College of Public Health, The Ohio State University, Columbus, OH

²Department of Clinical Epidemiology, The Ohio State University Medical Center, Columbus, OH

³Department of Veterinary Preventative Medicine, College of Veterinary Medicine, The Ohio State University, Columbus, OH

⁴Veterinary Public Health, College of Public Health, The Ohio State University, Columbus, OH

⁵Division of Biostatistics, College of Public Health, The Ohio State University, Columbus, OH

⁶Department of Internal Medicine, Division of Infectious Diseases, College of Medicine, The Ohio State University, Columbus, OH

Corresponding Author: Armando E. Hoet, 1920 Coffey Road, A100Q Sisson Hall, Columbus, OH 43210, (614) 292-4142, hoet.1@osu.edu

Submitted June 15, 2020 Accepted September 13, 2020

ABSTRACT

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) can be found in emergency medical services (EMS) ambulances. This poses an occupational risk and patient safety hazard. Screening for environmental contamination is often not performed due to limited resources and logistical challenges. This study's objective was to compare traditional screening of individual surfaces versus "pooled sampling" to efficiently identify contamination.

Methods: A cross-sectional study, conducted among 145 Ohio EMS ambulances from 84 agencies, tested a novel pooled sampling methodology to detect MRSA contaminated ambulances. For ambulances screened using pooled sampling, 3 samples were collected within each ambulance. Pool One included cabinets, doorways, and ceiling bar. Pool Two included cot, seats, and backboard. Pool Three included steering wheel, kits, and clipboard. For ambulances screened with the traditional detection technique, each of the 9 aforementioned surfaces were sampled individually. Descriptive statistics and unadjusted and adjusted odds ratios (OR) were calculated to compare the 2 methods.

Results: Forty-seven of 145 ambulances (32.4%) had at least 1 of the 9 locations contaminated with MRSA. When comparing the 2 screening methodologies, no significant difference was observed regarding the overall detection of MRSA contaminated ambulances (24/60 [40%] versus 23/85 [27.6%], P value: 0.1000). This indicates that pooled sampling appears as an efficient method for identification of MRSA contaminated ambulances.

Conclusion: One-third of Ohio ambulances had MRSA contamination in this study. Therefore, an efficient methodology to identify contaminated ambulances with hazardous pathogens is incredibly valuable. Pooling can help save resources and simplify sampling logistics, all which could positively impact infection control practices in emergency medical services.

Keywords: Methicillin-resistant *Staphylococcus aureus*; MRSA; Emergency medical services; Infection control; Environmental sampling

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a Gram-positive bacteria capable of causing various infections which are difficult to treat with several groups of antibiotics.¹ Methicillin-resistant *Staphylococcus aureus* can be found in 7% to 49% of

ambulances.²⁻⁴ In these studies, MRSA contamination was found in patient care and non-patient care areas. A major concern for MRSA found in any health care environment is that it is an occupational and patient safety hazard for those who come in contact with contaminated surfaces.⁵



Higher MRSA environmental contamination within the health care setting have correlated with increased human carriage.⁶ These findings likely explain why MRSA rates in emergency medical services (EMS) providers have been reported higher than the general population.⁷ High MRSA contamination rates within ambulances and MRSA carriage among EMS providers suggest a threat to infection control.^{6,7} Reducing MRSA from the environment may reduce MRSA carriage in health care providers or in the patients utilizing EMS services.

Unfortunately, no well-established protocols exist to efficiently screen for MRSA environmental contamination in the EMS setting. A method to use electrostatic wipes has been suggested as a more efficient environmental surface sampling approach.⁸ The use of electrostatic wipes for environmental MRSA contamination remains uncommon and no known studies have been performed in ambulances. Furthermore, most of the screening methods reported are heavily focused on individual surface sampling, which significantly increase the logistics and cost of screening ambulances to determine their contamination status and the need for deep cleaning and disinfection.²⁻⁴

The study's objective was to compare 2 environmental sampling methodologies to identify MRSA contaminated ambulances. The first was to sample each surface individually (traditional method). The second approach sampled several surfaces using a single electrostatic wipe, hereafter referred to as "pooled sampling." Pooled sampling may reduce the burden of sample collection resources (ie, time, personnel, and cost). Knowledge gained from this study could aid future infection control practices and guidelines by facilitating the screening of emergency vehicles.

METHODS

Setting

Between March 2009 and March 2010, Ohio had 1 338 EMS agencies registered in 10 EMS regions. The dates as to when these data were collected is irrelevant since the purpose of the study was to compare the sampling techniques. Agencies were located throughout Ohio in both rural and urban settings. For the study duration, every agency had at least 1 functioning ambulance.

Design

This was a cross-sectional sample of ambulances from 84 randomly selected Ohio EMS departments. To obtain state-wide representation, agencies were sampled from each of Ohio's 10 EMS regions and from urban and rural locations within each region. Within these selected agencies, up to 2 ambulances were screened for MRSA.

Participants

Samples were collected from 145 different ambulances. Ambulance types eligible for environmental surface sampling included small ad hoc vehicles, vans, custom made heavy trucks, and heavy trucks. No ambulances were excluded after enrollment. For each sampled ambulance, data regarding agency, personnel, and vehicle

characteristics were collected. Model year refers to the year that the vehicle was manufactured. Agency setting refers to whether or not the ambulance was housed at a rural or urban agency.

Procedures

Because the ambulance screening was performed in conjunction with human sample collection,⁷ approval for the use of human subjects was granted from the Office of Responsible Research Practices Institutional Review Board at The Ohio State University. Multiple samples from within ambulances were collected via an electrostatic cloth (Swiffer®). In ambulances with surfaces individually sampled, 9 separate samples were collected from the ambulance cot, bench seats, cabinet doors/handles, doorways, backboard, steering wheel, ceiling bar, kit handles/straps, and clipboard/Mobile Data Terminal (MDT). For the ambulances with pooled sampling, 3 pooled samples were collected. Pool One included the cabinet doors/handles, doorways, and ceiling bar. Pool Two included ambulance cot, bench seats, and long backboard. Pool Three included steering wheel, kit handles, and clipboard/MDT. The researchers selected the 3 pooled sites based on suspected population hazard: paramedic and patient hazard (Pool One), primarily patient hazard (Pool Two), and primarily paramedic hazard (Pool Three).

Measures

All samples were initially pre-enriched for 24 hours in buffered peptone water media followed by culturing and selection on mannitol salt agar plates supplemented with 2 µg/mL of oxacillin. After incubation, 3 suspected MRSA colonies were plated on blood agar and confirmatory testing was completed according to standard protocols as previously published.⁷ Final MRSA phenotypic confirmation was performed on oxacillin screen agar plates supplemented with 4% sodium chloride and oxacillin (6 µg/mL) incubated at 35 °C for 24 hours. A surface or pooled sample was considered contaminated with MRSA if there was at least one MRSA colony identified.

Statistical Analysis

Summary statistics to describe ambulance characteristics are reported and the MRSA contamination frequency for all ambulances was measured. Individual and pooled samples were compared using Student *t* test and Pearson's chi-square or Fisher exact test. Odds ratios (OR) were calculated to compare pooled versus individually sampled surfaces for MRSA contamination. All statistical procedures were performed in SAS (version 9.3; SAS Institute, Inc. Cary NC). Values were determined statistically significant if the *P* value was <0.05.

RESULTS

Data regarding ambulance and agency characteristics for the 2 populations of ambulances (individual versus pooled) were similarly distributed (Table 1). In this study, custom made heavy trucks were the most commonly contaminated ambulance type



overall (78/118, 66.1%), as well as within individually sampled ambulances (29/41, 70.7%) and pooled sampled ambulances (49/77, 63.6%) (χ^2 *P* value: 0.5729). Mean model year was 2002 (SD: 4.4 years) for ambulances individually sampled, and was 2003 (SD: 4.3 years) for pooled sampled ambulances (*t* test *P* value: 0.4088). A higher proportion of ambulances serving urban areas was seen among both the individually sampled group (43/60, 71.7%) and pooled sample ambulances (51/85, 60.0%) (*P* value: 0.1735). Finally, the mean number of staff at agencies with ambulances that had surfaces individually sampled was 30.1 (SD: 11.7) and agencies with ambulances that had pooled surface sampling was 35.1 (SD: 22.4) (*P* value: 0.1232). No significant differences were seen in the baseline characteristics between individually sampled and pooled ambulances which allowed us to compare the 2 groups.

Thirty-two percent (47/145) of all ambulances had at least 1 MRSA contaminated surface (Table 1). When comparing the 2 sampling methods, no significant difference was observed regarding the overall MRSA contamination in individually sampled ambulances (24/60, 40%) versus the pooled ambulances (23/85, 27.6%) (*P* value: 0.1000). Examining by surface location, Pool Two (primarily patient contact surfaces) had the highest MRSA con-

tamination prevalence in both the individually sampled (17/60, 28.3%) and pooled groups (17/85, 20.0%). Pool Three (primarily paramedic contact surfaces) had a MRSA prevalence of 18.3% (11/60) and 15.3% (13/85) for individually sampled and pooled sampled ambulances, respectively (*P* value: 0.6277).

Only Pool One had a MRSA contamination rate that was significantly different for individually sampled ambulances (11/60, 18.3%), and for pooled sampled ambulances (5/85, 5.9%) (*P* value: 0.0184) (Table 1). However, after adjusting for relevant ambulance and agency characteristics, the odds ratio of MRSA detection is not significantly different for Pool One (Table 2). Consistent with the unadjusted findings, the odds ratios of MRSA detection were not significantly different between those ambulances that had surfaces pooled and those that did not overall or for Pools Two and Three (Table 2).

DISCUSSION

The study's objective was to compare 2 methods for detecting MRSA contamination within an emergency health care setting. Although individually sampled surfaces provided more positive MRSA results, the overall ambulance contamination rate (ie, an ambulance tests MRSA positive at any location) was not statistical-

Table 1. Prescreening Comparison of Ambulance and Agency Characteristics and MRSA Positive Sites Stratified by Sampling Methodology

	Ambulances with surfaces individually sampled (n=60)	Ambulances with pooled surfaces sampled (n=85)	Total (N=145)	<i>P</i> value
Ambulance characteristics				
Vehicle type, frequency [†] (%)				0.5729
Small <i>ad hoc</i> vehicles	1 (2.4)	3 (3.9)	4 (3.4)	
Van	0 (0.0)	3 (3.9)	3 (2.5)	
Custom made heavy truck	29 (70.7)	49 (63.6)	78 (66.1)	
Heavy truck	11 (26.8)	22 (28.6)	33 (28.0)	
Model year, mean (SD)	2002 (4.4)	2003 (4.3)	2002 (4.3)	0.4088
Urban, frequency (%)	43 (71.7)	51 (60.0)	94 (64.8)	0.1735
Total personnel, mean (SD)	30.1 (11.7)	35.1 (22.4)	33.0 (18.9)	0.1232
MRSA frequency (%)				
MRSA contaminated ambulances	24 (40.0)	23 (27.1)	47 (32.4)	0.1000
Pool One, frequency (%)	11 (18.3)*	5 (5.9)	16 (11.0)	0.0184
Cabinet doors/handles	3 (5.5)	---	---	
Doorways	8 (14.6)	---	---	
Ceiling bar	3 (5.5)	---	---	
Pool Two, frequency (%)	17 (28.3)*	17 (20.0)	34 (23.5)	0.2434
Ambulance cot	9 (16.4)	---	---	
Bench seats	11 (20.0)	---	---	
Long backboard	3 (5.6)	---	---	
Pool Three, frequency (%)	11 (18.3)*	13 (15.3)	24 (16.6)	0.6277
Steering wheel	4 (7.3)	---	---	
Kit handles/straps	7 (12.7)	---	---	
Clipboard/MDT	0 (0.0)	---	---	

Abbreviations: MDT, Mobile Data Terminal; MRSA, methicillin-resistant *Staphylococcus aureus*; %, percentage of sample;

[†]Frequency values do not add to total number of ambulance sampled due to missing data

*Artificial pooling of the 3 individual locations


Table 2. Odds Ratios for MRSA Contamination Comparing Pooled Versus Individually Sampled Surfaces

	Unadjusted analysis OR (95% CI)	Adjusted analysis* OR (95% CI)
MRSA contaminated ambulances	0.56 (0.28 – 1.13)	0.18 (0.03 – 1.03)
Pool One	0.28 (0.09 – 0.85)	0.36 (0.02 – 6.85)
Pool Two	0.63 (0.29 – 1.37)	0.18 (0.03 – 1.21)
Pool Three	0.80 (0.33 – 1.94)	0.17 (0.03 – 1.14)

Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; OR, odds ratio; CI, confidence interval

*Multivariable analysis adjusted for model year, agency setting (urban vs. rural), and number of agency staff using ambulance

ly different from ambulances that were screened using a pooled sampling approach. While these findings are from a small study, pooled sampling as here described poses a more efficient method for pathogen detection and identification of contaminated ambulances. Methods that requires less resources and less logistics (ie, requires less than half of the time) needed for collection and testing can efficiently identify contaminated ambulances. Regardless of when these data were collected, the findings remain relevant.

Previous studies sampled a relatively small and homogeneous fleet of ambulances which may not be generalizable. However, this study found that surfaces associated with high touch areas for patients have a contamination rate of 23.5% which falls into the range of those studies.^{2-4,9,10} This study and others show that MRSA was found on surfaces that patients were more likely to have direct contact.^{4,11,12} Infected individuals are known to directly contaminate their surrounding environment,^{13,14} and non-infected EMS patients are at risk of MRSA acquisition.⁶ These results continue to emphasize that sampling and decontamination efforts should prioritize surfaces that patients most frequently contact in contrast to surfaces only accessible to EMS staff.

Currently, there are no required active surveillance recommendations for MRSA environmental contamination in the EMS setting.¹⁵ Ideally, active surveillance would initially allow for baseline measurement and then be used to follow trends over time. Pooled sampling may make statewide surveillance achievable at one-third of the cost. However, a more pragmatic approach might be for agencies to bear the onus of routine environmental surveillance that may elicit a Hawthorne effect of EMS personnel cleaning habits. An alternate use for the pooled technique could be to provide guidance for which ambulances would benefit from lengthy terminal disinfection techniques like ultraviolet germicidal irradiation (UVGI).¹⁶ Methods like UVGI require upwards of 16 hours when the ambulance must be decommissioned for cleaning.¹⁶ Performing UVGI on all ambulances is not currently feasible but sampling ambulances efficiently using the pooling technique could identify a subset of contaminated ambulances that could benefit from UVGI.

There are several limitations in this study. First, no statewide registry of ambulances exist so there is no method to confirm that

the ambulances measured in this study are representative of the entire state of Ohio. Furthermore, we did not collect location of ambulance routes or time of sampling. Agencies enrolled in this study, however, were randomly selected and representative of the state. Ambulances selected from those agencies were not determined by agency staff to reduce selection bias. Future research may determine how ambulance routes or timing of sampling may impact MRSA sample collection. Second, using the same electrostatic cloth to collect pathogens from a larger surface area might decrease the sensitivity to detect MRSA. Future studies should carefully consider the maximum surface area allowable for a single electrostatic cloth to work reliably. Finally, the pooled method may not be generalizable for the environmental sampling of other pathogens. Depending on the microbiology properties of other organisms, other collection techniques may be required. However, the detection of MRSA is frequently used as a marker of environmental contamination.

Data outlined in this brief report strongly suggest the need for EMS infection prevention programs that focus on environmental cleaning of ambulances. The number of contaminated surfaces was high but not uncommon and presented a threat to infection control. Culture-based screening methods represent the most accurate and reliable method of determining the adequacy of cleaning. Culture-based methods, however, that require extended time for sampling and large number of samples will likely not be employed beyond research purposes. Limiting the number of required samples by pooling may be appealing for routine environmental sampling and ambulance screening. Furthermore, microbiologic cultures expend agency resources so limiting the total number of cultures needed is also highly favorable. Therefore, for circumstances requiring targeted environmental surveillance (ie, outbreak investigation or quality improvement), pooled sampling provides an efficient method to detect MRSA contaminated ambulances. Performing environmental screening will also help to determine the locations within ambulances that are consistently contaminated and will guide effective decontamination processes ultimately reducing MRSA acquisition for both patients and paramedics.



PUBLIC HEALTH IMPLICATIONS

A gap in environmental infection control of MRSA in ambulances for over a decade suggests that little progress has been made to protect both patients they serve and the providers who service them. Emergency medical services agencies have historically not conducted active surveillance as a part of infection control.¹⁵ Failure to perform active surveillance has been attributed to time and resource limitations.⁷ The overall findings of this report support the use of a novel pooled sampling methodology to detect MRSA contaminated ambulances that is efficient and may be cost-effective compared to traditional methods. This methodology could also be used to implement active or routine surveillance for infectious agents like MRSA. Thorough disinfection techniques, like UVGI, often require ambulances to be decommissioned for extended periods.¹⁶ Pooled sampling to detect heavily contaminated ambulances may be a practical alternative to identify which ambulances within a fleet require more rigorous cleaning and disinfection.^{16,17}

ACKNOWLEDGMENTS

Acknowledgements Christopher Bell

The study was funded by a grant from the Ohio Department of Public Safety. All authors report no conflicts of interest relevant to this article.

REFERENCES

- Lee AS, de Lencastre H, Garau J, et al. Methicillin-resistant Staphylococcus aureus. *Nat Rev Dis Prim*. 2018;4(1):18033. <https://doi.org/10.1038/nrdp.2018.33>
- Roline CE, Crumpecker C, Dunn TM. Can methicillin-resistant Staphylococcus aureus be found in an ambulance fleet? *Prehosp Emerg Care*. 2007;11(2):241-244. <https://doi.org/10.1080/10903120701205125>
- Brown R, Minnon J, Schneider S, Vaughn J. Prevalence of methicillin-resistant Staphylococcus aureus in ambulances in southern Maine. *Prehosp Emerg Care*. 2010;14(2):176-181. <https://doi.org/10.3109/10903120903564480>
- Rago J V., Buhs LK, Makarovaite V, Patel E, Pomeroy M, Yasmine C. Detection and analysis of Staphylococcus aureus isolates found in ambulances in the Chicago metropolitan area. *Am J Infect Control*. 2012;40(3):201-205. <https://doi.org/10.1016/j.ajic.2011.08.021>
- Lin D, Ou Q, Lin J, Peng Y, Yao Z. A meta-analysis of the rates of Staphylococcus aureus and methicillin-resistant S aureus contamination on the surfaces of environmental objects that health care workers frequently touch. *Am J Infect Control*. 2017;45(4):421-429. <https://doi.org/10.1016/j.ajic.2016.11.004>
- Wang J, Wang M, Huang Y, et al. Colonization pressure adjusted by degree of environmental contamination: a better indicator for predicting methicillin-resistant Staphylococcus aureus acquisition. *Am J Infect Control*. 2011;39(9):763-769. <https://doi.org/10.1016/j.ajic.2010.11.012>
- Orellana RC, Hoet AE, Bell C, et al. Methicillin-resistant Staphylococcus aureus in Ohio EMS Providers: A Statewide Cross-sectional Study. *Prehosp Emerg Care*. 2016;20(2):184-190. <https://doi.org/10.3109/10903127.2015.1076098>
- Lutz JK, Crawford J, Hoet AE, Wilkins JR, Lee J. Comparative performance of contact plates, electrostatic wipes, swabs and a novel sampling device for the detection of Staphylococcus aureus on environmental surfaces. *J Appl Microbiol*. 2013;115(1):171-178. <https://doi.org/10.1111/jam.12230>
- Vikke HS, Giebner M, Kolmos HJ. Prehospital infection control and prevention in Denmark: a cross-sectional study on guideline adherence and microbial contamination of surfaces. *Scand J Trauma Resusc Emerg Med*. 2018;26(1):71. <https://doi.org/10.1186/s13049-018-0541-y>
- Fattorini M, Quercioli C, Messina G, Nante N. MRSA contamination in ambulances: a systematic review. *Eur J Public Health*. 2019;29 (Supplement_4). <https://doi.org/10.1093/eurpub/ckz186.500>
- Eibicht SJ, Vogel U. Methicillin-resistant Staphylococcus aureus (MRSA) contamination of ambulance cars after short term transport of MRSA-colonised patients is restricted to the stretcher. *J Hosp Infect*. 2011;78 (3):221-225. <https://doi.org/10.1016/j.jhin.2011.01.015>
- El-Mokhtar MA, Hetta H. Ambulance vehicles as a source of multidrug-resistant infections: a multicenter study in Assiut City, Egypt. *Infect Drug Resist*. 2018;Volume 11:587-594. <https://doi.org/10.2147/IDR.S151783>
- Lowy F. Staphylococcus aureus Infections. *N Engl J Med*. 1998;339:520-532. <https://doi.org/10.1056/NEJM199808203390806>
- Wertheim HFL, Melles DC, Vos MC, et al. The role of nasal carriage in Staphylococcus aureus infections. *Lancet Infect Dis*. 2005;5(12):751-762. [https://doi.org/10.1016/S1473-3099\(05\)70295-4](https://doi.org/10.1016/S1473-3099(05)70295-4)
- Woodside J. *Guide to Infection Prevention in Emergency Medical Services*; 2013. https://www.ems.gov/pdf/workforce/Guide_Infection_Prevention_EMS.pdf
- Lindsley WG, McClelland TL, Neu DT, et al. Ambulance disinfection using Ultraviolet Germicidal Irradiation (UVGI): Effects of fixture location and surface reflectivity. *J Occup Environ Hyg*. 2018;15(1):1-12. <https://doi.org/10.1080/15459624.2017.1376067>
- Lowe JJ, Hewlett AL, Iwen PC, Smith PW, Gibbs SG. Evaluation of Ambulance Decontamination Using Gaseous Chlorine Dioxide. *Prehospital Emerg Care*. 2013;17(3):401-408. <https://doi.org/10.3109/10903127.2013.792889>