The Emergency Medical Service Microbiome

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ABSTRACT Emergency medical services (EMS) personnel are an integral component of the health care framework and function to transport patients from various locations to and between care facilities. In addition to physical injury, EMS personnel are expected to be at high risk to acquire and transmit health care-associated infections (HAIs) in the workplace. However, currently, little is known about EMS biosafety risk factors and the epidemiological contribution of EMS to pathogen transmission within and outside the health care sector. Health care facility microbiomes contain diverse bacterial, fungal, and viral pathogens that cause over 1.7 million HAIs each year in the United States alone. While hospital microbiomes have been relatively well studied, there is scant information about EMS infrastructure and equipment microbiomes or the role(s) they play in HAI transmission between health care facilities. We review recent literature investigating the microbiome of ambulances and other EMS service facilities which consistently identify antibiotic-resistant pathogens causing HAIs, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus*, and *Klebsiella pneumoniae*. Our review provides evidence that EMS microbiomes are dynamic and important pathogen reservoirs, and it underscores the need for more widespread and in-depth microbiome studies to elucidate patterns of pathogen transmission. We discuss emerging DNA sequencing technologies and other methods that can be applied to characterize and mitigate EMS biosafety risks in the future. Understanding the complex interplay between EMS and hospital microbiomes will provide key insights into pathogen transmission mechanisms and identify strategies to minimize HAIs and community infection.

KEYWORDS DNA sequencing, emergency medical services (EMS), *Staphylococcus aureus*, health care-associated infection (HAI), microbiome, pathogens, public health

Emergency medical service (EMS) personnel are often considered to be the “front lines” of the health care system and serve to transport millions of critically injured and ill patients to and between hospitals and other health care facilities. While performing their duties, EMS personnel often experience a wide variety of dangerous and unpredictable situations that jeopardize their safety and the safety of their patients. In fact, EMS personnel are up to seven times more likely to sustain a physical or mental injury in the workplace than national averages (1, 2). In addition to these risks, EMS personnel are likely routinely subjected to more inconspicuous biohazard risks posed by infectious disease-causing microorganisms (pathogens) via contact with infected patients and their bodily fluids, as well as from the diverse environments visited during shifts (e.g., homes, workplaces, and hospitals).

Health care-associated infections (HAIs) are infections acquired within a health care setting that affect approximately 7% of hospitalized patients in developed countries and up to 19% of patients in developing countries, translating to millions of HAI events each year (3). HAIs may be caused by a variety of bacterial, viral, or fungal pathogens; however, particular attention has been given to pathogens that are highly infectious, display high virulence (capacity to cause disease), or are resistant to common antibiotics. The American Centers for Disease Control and Prevention (CDC) and the Public

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Health Agency of Canada (PHAC) have deemed that bacteria such as *Clostridium difficile*, vancomycin-resistant *Enterococcus*, and methicillin-resistant *Staphylococcus aureus* (MRSA) are of particularly high concern due to their ability to cause serious and difficult-to-treat HAIs (4, 5).

Despite the perceived biohazard risks posed by the EMS environment, many questions regarding EMS biosafety remain largely unaddressed. What is the potential for EMS workers and patients to acquire infection in the workplace? What is the epidemiological contribution of EMS vehicles, equipment, and personnel to the transmittance of HAIs? How can EMS biosafety risks best be mitigated? In this review, we integrate available data on the topic of pathogen presence in EMS vehicles, equipment, and personnel to shed light on the potential for HAI transmission within the EMS environment and between health care facilities. We discuss the efficacy of cleaning practices for mitigating pathogen spread as well as future pathogen detection and monitoring strategies. Our review highlights a need for a more rigorous investigation of pathogen biosafety risks in the EMS sector worldwide, the development of more effective pathogen detection systems, and the implementation of evidence-based industry standards to protect EMS personnel, patients, and the public from pathogen transmission.

EMS pathogen monitoring studies. (i) Study designs and methodologies. To date, approximately 25 published studies have investigated pathogen presence in the EMS sector from 1986 to 2016 within the United States (6–20), Australia (21), the United Kingdom (22), Germany (23–25), Denmark (26, 27), South Korea (28, 29), Saudi Arabia (30), and Thailand (31, 32) (Fig. 1A). Of these studies, most have investigated pathogens within ground ambulances (6, 8–10, 14, 23–25, 29, 30, 32), although several studies examined air ambulances (7, 21), EMS stations (17–19), EMS medical devices (12, 28), uniforms (26), or EMS personnel (11, 13, 15, 16, 18, 20) for pathogen colonization (Fig. 1B).

Studies vary significantly in their scope and sampling strategies in terms of number (and type) of vehicles examined, the location of sampling areas, the total number of samples collected, and the frequency of sample collection (Tables 1 and 2). For example, some studies examined pathogen persistence at a single time point (8) or over one or more weeks (7), months (21), or an entire year (22). Similarly, some studies had relatively large sample sizes (>50 ambulances and 30 sampling sites), and statistical significance could be determined confidently (10, 14, 24). Meanwhile, other studies examined only one or a few locations (6, 9, 12) or had comparatively small sample sizes (<10 ambulances) (9). These inconsistencies are noteworthy and in some cases make a comparison of results and conclusions challenging.

Culturing techniques were employed in all studies to detect bacterial or fungal contamination within EMS environments. Sample collection is typically performed by swiping predicted fomites (objects carrying infectious organisms) using sterile cotton or rayon swabs moistened with sterile saline solution (8, 22, 27, 30). In one study, air samples were collected from ambulance cabins before and during patient runs and filtered prior to culturing (32). Within 24 h, collected samples are streaked on growth medium (e.g., blood agar) and incubated for 24 to 96 h, and the resulting colonies are counted and observed for morphological or biochemical features to determine their identity (8, 10, 16). Notably, depending on the growth media used and the nutritional requirements of the microbes present, only some microbes (including pathogens) can be cultivated (33). While some studies used rich growth media that support the growth of a variety of microbes (22, 24, 32), others employed selective media (e.g., to enrich for MRSA), limiting the scope of detectable microbes (8, 10, 12, 23).

Genomics and epidemiological studies have revealed that pathogen isolates may display substantial genetic diversity, with different strains possessing distinctive genetic profiles that may include additional antimicrobial resistance and/or virulence genes (34). For example, two genetically distinct hospital-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) strains have been identified, with some CA-MRSA isolates (e.g., USA300) possessing additional toxins and immunomodulating

FIG 1 Global studies investigating pathogen prevalence within the EMS sector. (A) The geographic locations of EMS pathogen studies are indicated, with expanded views of the United States (US) and western Europe. (B) Proportion and number of studies investigating pathogen presence for various portions of the EMS sector. (C) Contamination rates for ambulances, EMS facilities, and personnel are indicated, with the frequencies of MRSA and MSSA represented by red bars and gray bars, respectively.

factors that may produce more severe disease (34). Thus, in some studies, PCR (11, 27), pulsed-field gel electrophoresis (13, 18, 20), and/or mass spectrometry techniques (27) were also employed to provide additional strain information and/or determine antibiotic resistance status.

(ii) Pathogen prevalence by geographical location. Regardless of geographical location and selected sampling areas, all studies identified similar environmental (non-pathogenic) bacterial flora (Table 1). Numerous clinically important opportunistic bacterial pathogens were also frequently identified and included *S. aureus* (MRSA and methicillin-sensitive *S. aureus* [MSSA]) (8, 10, 16, 23), *Enterococcus* spp. (26, 27), *Klebsiella pneumoniae*

TABLE 1 Summary of studies examining microbes in EMS environments

EMS environment	Study location	Sample collection	Organism(s) detected ^a	Frequency of contamination ^b	Yr	Reference
Ground ambulance	Virginia (USA)	30 ambulances (humidifier bottles)	<i>Pseudomonas aeruginosa</i> , <i>Klebsiella pneumoniae</i> , <i>Staphylococcus epidermidis</i>	9/30 ambulances (30%), various bacteria	1986	6
	Western USA	21 ambulances (5 locations)	MRSA	10/21 ambulances (47.6%), MRSA	2007	8
	Maryland (USA)	4 ambulances (5 locations)	<i>S. epidermidis</i> , <i>Bacillus</i> sp., <i>Pseudomonas</i> sp.	3/4 ambulances (75%), various bacteria	2008	9
	New Jersey (USA)	50 stethoscopes	MRSA	16/50 stethoscopes (32%), MRSA	2009	12
	Southern Maine (USA)	51 ambulances (16 locations)	MRSA	25/51 ambulances (49%), MRSA	2010	10
	Illinois (USA)	71 ambulances (26 locations)	MRSA	49/71 ambulances (69%), <i>S. aureus</i> ; 12 MRSA isolates (of 100 <i>S. aureus</i>)	2012	14
	Wales (UK)	12 ambulances (7 locations)	<i>Bacillus</i> sp., coliforms, <i>Corynebacterium</i> sp., <i>Micrococcus</i> sp., <i>Pseudomonas</i> sp., <i>Staphylococcus aureus</i> , <i>S. epidermidis</i> , <i>Streptococcus viridans</i>	1.4%, <i>S. aureus</i> ; 2.7%, <i>S. viridans</i> ; 61%, various bacteria	2003	22
	Germany	89 transportation events	MRSA	8/89 transportation events (9%), MRSA	2010	23
	Germany	30 ambulances	MRSA	3/30 ambulances (10%), MRSA	2015	25
	Germany	150 ambulances (28 locations)	MRSA , <i>Corynebacterium</i> sp., <i>Micrococcus</i> sp., <i>Pseudomonas</i> sp., <i>Aspergillus</i> sp.	11/150 ambulance vehicles (7%), MRSA	2015	24
Air ambulance	Denmark	39 ambulances (1 location)	<i>S. aureus</i> (MSSA), <i>Enterococcus</i> sp.	10%, MSSA; 2%, <i>Enterococcus</i> sp.	2016	27
	South Korea	13 ambulances (33 locations)	MRSA , <i>K. pneumoniae</i> , various bacteria	0.4%, MRSA; 0.4%, <i>K. pneumoniae</i> ; 49.9%, various bacteria	2011	28
	South Korea	30 ambulances (33 locations)	<i>S. aureus</i> , <i>Pseudomonas</i> sp., <i>Serratia</i> sp., <i>Legionella</i>	16%, various bacteria	2012	29
	Saudi Arabia	10 ambulances (3 locations)	<i>Bacillus</i> sp., CoNS	1/10 ambulances (10%), CoNS	2014	30
	Thailand	30 ambulances (318 air samples)	<i>S. aureus</i> , <i>Aspergillus</i> sp., <i>Penicillium</i> sp., <i>Fusarium</i> sp.	47/91 collected bacterial colonies (51.6%), <i>S. aureus</i>	2015	32
	Mississippi (USA)	1 helicopter (7 areas)	<i>Staphylococcus</i> sp., <i>Pseudomonas</i> sp., <i>Escherichia coli</i> , <i>Aspergillus</i> sp., <i>Bacillus</i> sp.	6/7 samples (86%), <i>Staphylococcus</i> sp.; 2/7 samples (29%), <i>Pseudomonas</i> sp.	2006	7
	Australia	2 helicopters (5 areas)	MSSA, <i>S. epidermidis</i>	19/60 samples (32%), MSSA (no MRSA)	2016	21
	Arizona (USA)	9 EMS/fire stations (160 locations)	MRSA	11/160 locations (6.8%), MRSA	2010	17
	Washington (USA)	2 EMS/fire stations (1,064 samples)	MRSA	44/1,064 samples (4.1%), MRSA	2011	18
	Washington (USA)	33 EMS/fire stations (653 samples)	MRSA	52/653 samples (8%), MRSA	2014	19

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TABLE 1 (Continued)

EMS environment	Study location	Sample collection	Organism(s) detected ^a	Frequency of contamination ^b	Yr	Reference
EMS personnel	Kansas (USA)	109 EMS personnel, nasal swabs	MRSA	6/109 EMS personnel (10.2%), MRSA	2002	20
	New Jersey (USA)	52 EMS personnel, nasal swabs	MRSA	1/52 EMS personnel (1.9%), MRSA; 30/52 (57.7%), <i>S. aureus</i>	2010	13
	Washington (USA)	40 EMS personnel, nasal swabs	MRSA	9/40 EMS personnel (22%), MRSA	2011	18
	Mid-Atlantic USA	110 EMS personnel, nasal swabs	MRSA	7/110 EMS personnel (6.4%), MRSA	2013	11
	Indiana (USA)	134 EMS personnel and 152 EMT students	MRSA	6/134 EMS personnel (4.5%) and 8/152 EMT students (5.3%)	2013	15
	Ohio (USA)	280 EMS personnel, nasal swabs	MRSA	13/280 EMS personnel (4.6%), MRSA	2016	16
	Denmark	30 EMS uniforms	<i>S. aureus</i>, <i>Bacillus cereus</i>, <i>Enterococcus</i> sp.	11/45 samples (24%), <i>S. aureus</i> ; 7/45 samples (16%), <i>B. cereus</i> ; 2/35 samples (4%), <i>Enterococcus</i> sp.	2015	26

^aMRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-sensitive *S. aureus*; CoNS, coagulase-negative *Staphylococcus*. Potential pathogens or opportunistic pathogens are in bold.

^bNumber of vehicles or samples testing positive for contamination/total number of samples tested (%), or % of total samples.

TABLE 2 Locations examined and potential pathogen contamination identified in EMS vehicles

Sampling environment	Sampling location ^a	Potential pathogen(s) detected ^b	Reference(s)
Ambulance interior	Stretcher, mattress	MRSA (+++), <i>Staphylococcus</i>	7, 8, 22–24, 32
	Stretcher, handrail	MRSA (+++++), MSSA, CoNS, <i>Staphylococcus parasanguinis</i> , <i>Staphylococcus epidermidis</i> , <i>Sphingomonas paucimobilis</i> , <i>Micrococcus luteus</i>	8, 10, 14, 23, 24, 28–30
	Door handle	MRSA (+), CoNS, <i>Staphylococcus</i> sp., <i>Streptococcus viridans</i> , <i>Klebsiella</i> sp., <i>S. epidermidis</i> , α -hemolytic <i>Streptococcus</i>	7, 9, 10, 28–30
	Floor	MSSA, <i>Staphylococcus aureus</i> , <i>S. viridans</i> , <i>Staphylococcus</i> sp., <i>Pseudomonas</i> sp.	7, 14, 21, 22
	Preparation area	MRSA (++)	8, 10, 24
	Steering wheel	MRSA (++)	8, 10, 14, 22, 29
	Airway devices	<i>Pseudomonas putida</i> , <i>Neisseria sicca</i> , <i>Pseudomonas aeruginosa</i> , <i>S. epidermidis</i> , <i>Acinetobacter baumannii</i>	24, 28, 29, 32
	Walls	None	22, 23
	Bench seat	MSSA	9, 14
	Cabinets	None	9, 22
	Radio	<i>S. aureus</i> , MSSA	7, 14, 21
	Seatbelt buckle	<i>S. aureus</i> , MSSA	9, 14, 21
	Ceiling rail	MRSA (+)	10
	Stretcher, straps	MRSA (+)	10
	Pharmacists' cabinet handle	MRSA (+)	24
	Ventilating fan	CoNS, <i>M. luteus</i>	29
	Ceiling flap	None	24
Medical equipment	Oxygen flow control knob	MRSA (+), CoNS, <i>Bacillus</i> sp., <i>Staphylococcus saprophyticus</i>	9, 10, 24, 29, 30, 32
	Oxygen tank	MSSA, MRSA (+)	14
	Laryngoscope blade	CoNS, <i>S. paucimobilis</i> , <i>M. luteus</i>	29
	Laryngoscope handle	<i>Staphylococcus intermedius</i> , <i>Bacillus</i> sp.	29
	Stethoscope	MRSA (+), MSSA, <i>S. epidermidis</i>	10, 12, 14, 28, 29, 32
	Glucometer	None	14
	Blood pressure cuff	MRSA (++)	10, 14, 21, 24, 27
	Cardiac/oximeter monitor	MRSA (++)	7, 10, 14, 28
	Cardiac defibrillator	<i>S. aureus</i> , <i>Staphylococcus hominis</i> , <i>Bacillus</i> sp., <i>Staphylococcus lugdunensis</i> , <i>S. epidermidis</i>	21, 28, 29
	Bag valve mask, bag	<i>S. hominis</i> , <i>Staphylococcus capitis</i> , <i>S. epidermidis</i>	29
	Bag valve mask, mask	CoNS, <i>S. epidermidis</i>	29
	Entonox mask	<i>S. aureus</i>	22, 29
	Facial mask/nasal prongs	<i>S. intermedius</i> , CoNS, <i>Bacillus</i> sp., <i>M. luteus</i> , <i>Candida parapsilosis</i> , <i>S. aureus</i>	28, 29
	i.v. equipment	MRSA (++)	10, 14
	Oxygen generator	<i>S. epidermidis</i>	28, 29
	Suction bottle	None	22
	Tourniquet	None	24
	Yankauer suction tip	MRSA (+), <i>Stenotrophomonas maltophilia</i> , <i>S. paucimobilis</i> , <i>S. epidermidis</i> , <i>P. aeruginosa</i> , <i>Streptococcus mitis</i> / <i>S. oralis</i> , <i>Bacillus</i> sp., <i>S. aureus</i> , CoNS	8, 29
Miscellaneous equipment	Long spinal board	<i>S. hominis</i> , <i>S. warneri</i> , CoNS, <i>M. luteus</i>	28, 29, 32
	Microphone	MRSA (+)	9, 10
	Air conditioner	<i>S. warneri</i>	28, 29
	Oxygen humidifier, glass	CoNS, <i>S. paucimobilis</i> , <i>Burkholderia cepacia</i>	29
	Oxygen humidifier, water	<i>Bacillus</i> sp., <i>S. paucimobilis</i> , <i>S. epidermidis</i> , <i>P. aeruginosa</i> , <i>P. putida</i> , <i>Pantoea</i> sp.	29
	Clipboard	MRSA (+)	10
	Computer	MRSA (+)	10

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TABLE 2 (Continued)

Sampling environment	Sampling location ^a	Potential pathogen(s) detected ^b	Reference(s)
	Control switches	MRSA (+)	10
	Patient headset	<i>Staphylococcus</i> sp.	7
	Pilot controls	<i>Staphylococcus</i> sp.	7
	Carrying handles	MRSA (+)	24
	ECG cable	CoNS, MSSA, <i>S. saprophyticus</i> , <i>M. luteus</i>	14, 24, 29
	ECG control panel	MRSA (+)	24
	Jump bag handle	MSSA	14
	Endotracheal tube	<i>Pseudomonas</i> sp., <i>Bacillus</i> sp., <i>Serratia</i> sp., <i>Streptococcus</i> sp., <i>Staphylococcus</i> sp., <i>Sphingomonas</i>	29
	Laptop keypad	MSSA	14
	Suction water	<i>Bacillus</i> sp., <i>M. luteus</i> , <i>Legionella</i>	29

^aECG, electrocardiogram.^bNumbers of studies that detected MRSA contamination are indicated by plus signs in parentheses. MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-sensitive *S. aureus*; CoNS, coagulase-negative *Staphylococcus*.

(6, 29), *Bacillus cereus* (26), *Pseudomonas fluorescens* (29), *Serratia marcescens* (29), *Legionella* (29), and the fungal pathogen *Aspergillus* (7) (Table 1). *S. aureus* was detected in all studies, although the presence of many other pathogens could not be examined in all cases due to the use of selective culturing techniques that precluded their detection.

Interestingly, the frequency of MRSA contamination in ground ambulances correlates with the geographical location of studies (Fig. 1C). For example, U.S.-based studies found 12 to 49% of tested ambulances to have at least one MRSA-contaminated site (8, 10, 14), while German studies reported comparatively lower MRSA contamination rates of ambulances (7 to 9%); also, Danish, Saudi Arabian, and most Asian studies reported no incidences of MRSA (27, 28, 30, 32). Consistent with the study findings, the United States is predicted to have a greater burden of MRSA than some northern European countries (35). This is also in accordance with more stringent controls for the treatment and monitoring of MRSA-infected patients in German ambulances, such as access to MRSA infection status of patients, donning of additional personal protective equipment (e.g., face masks) while transporting MRSA-positive patients, and specialized cleaning of EMS vehicles and equipment posttransport of MRSA-infected patients (23).

Only two studies have specifically investigated air ambulances for pathogen contamination (7, 21). One U.S. study identified several opportunistic pathogens in a rotor wing air ambulance, including *Pseudomonas* sp., *Aspergillus*, and *E. coli* (7). *Staphylococcus* sp. was also identified (6 out of 7 samples); however, further testing was not performed to confirm the presence of MRSA (7). In contrast, a second Australian study of EMS helicopters detected only MSSA and nonpathogenic skin flora (e.g., *Staphylococcus epidermidis*) (21). The relatively small sample sizes of the two studies (1 and 2 ambulances tested) leave much room for further investigation; however, the studies indicate that pathogens may also be present within air ambulances. If true, this would be particularly concerning, because air ambulances service a wider geographical area than ground ambulances, and this could increase the range of HAI transmission.

EMS facilities may also be significant pathogen reservoirs. An Arizona study found that approximately 7% (11/160) of sampled sites at an EMS facility tested positive for MRSA (17), and two other studies conducted in Washington also showed similar MRSA contamination frequencies of 4.1% (44/1,060 samples) and 8% (52/653 samples) (18, 19). Strikingly, MRSA isolate typing in one study identified both HA-MRSA and CA-MRSA (USA300) (18). These findings indicate that pathogens may be readily transferred between EMS personnel and fomites, particularly in locations where the perceived risk of infection is lower (e.g., offices) and interventions to prevent pathogen spread (e.g., donning personal protective equipment [PPE] and routine hand washing) are not as frequently observed.

(iii) Pathogen prevalence by sampling location and patient exposure time. In all published reports, more than 50 locations have been examined for the presence of

pathogens within or on ambulance vehicles or equipment (Table 2). However, not all sampling locations were examined or available for testing in all studies, making it somewhat difficult to compare contamination frequencies between studies.

Pathogen presence is most often associated with areas of high patient and/or EMS personnel contact, such as stretchers (mattresses and handrails), door handles, EMS worker preparation areas, and steering wheels (Table 2). Commonly handled or touched areas on medical devices, such as blood pressure cuffs (10, 24), cardiac monitors (10), and intravenous (i.v.) equipment (10, 14), also showed elevated levels of MRSA. In one U.S. study, EMS personnel stethoscopes were found to have a high rate of MRSA contamination (32% [16/50]) (12), although other studies from the United States and outside reported lower contamination levels of MRSA (7% [5/71]) (14) or no detectable MRSA (10). Floor areas also showed high bacterial counts; however, no study specifically identified MRSA at this sampling location (Table 2). Ambulance interior walls, ceilings, and insides of cabinets had lower bacterial loads and were not found to host MRSA in any study (Table 2). Finally, one study examining EMS facilities showed high frequencies of MRSA contamination on couches (20% [4/20]) and student desks (10% [1/10]) (17).

The small and closed confines of the ambulance cabin also suggest the potential for airborne pathogen transmission between patient and EMS personnel and vice versa (36). In one study, 106 air samples and 452 surface swabs were taken from 30 ground ambulances in Thailand (32). While no significant differences were observed in the composition of the detected microbe community between the inside and outside of the ambulance, total bacterial and fungal counts increased slightly but significantly ($P = 0.005$ to 0.030) during patient transport events (32). The increase in total bacterial and fungal counts in air samples was also positively correlated with an increase in surface swab counts (32), suggesting that both ambulance air and surfaces may become contaminated during patient transport events. Relevantly, one German study that examined the relationship between patient occupancy time and MRSA contamination found that 8 out of 91 (9%; 90% confidence interval [CI], 4 to 14%) ambulance runs lasting 20 min or less had MRSA contamination after patient delivery (23). Longer ambulance runs (10 to 20 min) did not show significantly greater contamination than shorter runs (<10 min), indicating that pathogen contamination of EMS vehicles was immediate upon patient transfer (23).

(iv) Pathogen colonization of EMS personnel. Six U.S. studies have tested EMS personnel directly for MRSA nasal colonization (Fig. 1C). Four studies report remarkably similar frequencies of EMS personnel colonization by MRSA, at 4.5% (6 out of 134) (15), 4.6% (13/280) (16), 5.5% (6/109) (20), and 6.4% (7/110) (11). The remaining two studies reported comparably lower or higher MRSA colonization frequencies, at 1.9% (1/52) (13) and 22.5% (9/40) (19). Regardless of these differences, most studies report MRSA colonization frequencies for EMS personnel that are approximately three to four times higher than that reported for the general population (1.5%) (37), suggesting that EMS workers are at increased risk for colonization and/or infection by MRSA and possibly by other pathogens (36). Indeed, observational studies indicate that EMS personnel are at greater risk for infection during an epidemic (38) and that pathogen risk exposure may be exacerbated by improper workplace practices or patient handling (31). Moreover, Roberts et al. discovered that several EMS personnel were colonized by MRSA strains that were genetically related to samples collected from EMS facilities and included both HA-MRSA and CA-MRSA (USA300) isolates (18). While further studies are needed, these findings provide preliminary evidence that transmission of pathogens between EMS personnel and environmental fomites does occur and that this may contribute to HAIs (18). Additional studies that investigate EMS personnel colonization in areas outside the United States are needed to determine whether these findings are representative of EMS personnel in other countries.

Cleaning practices and pathogen mitigation strategies. EMS operations vary in their adopted cleaning practices for managing biological hazards but typically use a

combination of physical processes and chemical solutions to achieve suitable disinfection (7, 22). Initially, mops, towels, rags, and/or sanitary wipes are used to remove gross (visible) contamination, such as dirt, blood, and other bodily fluids, and this is expected to reduce microbial loads on contaminated surfaces (22, 27). Following this, cleaning solutions and disinfectants are employed to further decontaminate surfaces and may include 10% bleach, 80% ethanol, quaternary ammonium chloride, glutaraldehyde, and other chemical agents (7, 22). Occasionally, fumigation is used as a tertiary treatment for ambulance vehicle decontamination (30).

Despite the array of employed cleaning practices used in EMS operations, very few studies report on the efficacy of cleaning products and procedures for reducing pathogen risk in the EMS workplace. One U.S. study that investigated rotor wing air ambulances examined the effect of the cleaning products Staphene, KleenAseptic, and Virkon on microbial load for seven ambulance sampling locations (7). An initial set of swab samples was collected after removing loose material with a cloth and applying Staphene or KleenAseptic, and a second set was collected after subsequent application of Virkon (7). Initial swab samples (Staphene or KleenAseptic) produced moderate to heavy growth of *S. aureus* at most sampling locations and occasional light growth of *E. coli*, *Pseudomonas* spp., and Gram-negative *Bacillus* spp. (7). Samples taken after subsequent cleaning with Virkon showed only light growth for one sampling location (stretcher) (7). While the study demonstrates the efficacy of secondary cleaning for reducing microbial load, unfortunately, initial pathogen loads (before cleaning with Staphene or KleenAseptic) were not determined, and the individual contribution of each of the cleaning products cannot be evaluated.

Another study conducted in Saudi Arabia specifically examined the efficacy of 6% hydrogen peroxide fumigation on microbial contamination in ambulance vehicles (30). While not quantitative, the study reports a substantial reduction in viable bacterial contamination after fumigation, including no detectable *S. aureus* (30). Interestingly however, *Bacillus* sp. was detected after fumigation at several sampling sites, demonstrating that some bacteria (including possible pathogens, e.g., *B. cereus*) are resistant to this treatment (30).

Although many of the employed disinfectants are broad spectrum and/or recommended by government regulatory agencies, their efficacy may differ in actual practice. For example, a Welsh study by Nigam and Cutter reported only 8 to 45% reduction in total bacterial contamination following standard cleaning procedures (22). Strikingly, in the same study, some sites showed increased contamination by the opportunistic pathogens *S. aureus* and *Pseudomonas* sp. after cleaning, suggesting that the employed cleaning practices were insufficient for decontamination and/or were spreading contamination to new sites within EMS vehicles (22). The practice of effective decontamination procedures may also differ in different EMS operating modes. Brown et al. found that paid-per-call and volunteer services had higher rates of MRSA contamination (91% [10/11 ambulances]) than part-time (57% [4/7]) and full-time (32% [11/34]) services (10). The reason for the discrepancy could not be specifically determined; however, the authors note that full-time personnel were required to clean ambulance interiors at the end of each shift and perform weekly rigorous cleaning, which presumably lowered pathogen loads in ambulance vehicles (10).

Besides ambulance interiors and equipment, EMS worker sanitation is likely an important contributing factor to HAI transmission. Health care uniforms have been implicated as a fomite for HAI transmission in hospitals (39, 40); consequently, one Danish study investigated bacterial colonization rates of 30 EMS personnel uniforms before and after cleaning with detergents containing acetic peroxide (26). Initial prints before washing ($n = 90$) showed contamination with *Bacillus cereus* (27%), *Clostridium* and *Enterococcus* (2%), and *S. aureus* (21%); while postwashing, only *S. aureus* (4%) was detected, revealing a substantial decrease in microbial load (26). The hands of health care workers are also thought to be the most prominent mechanism for the transmission of HAIs (41). Consistent with this concept, Orellana et al. found that EMS workers who did not perform routine hand washing following glove use were approximately 10

times more likely to be colonized by MRSA (95% CI, 2.45 to 43.45 times; $P = 0.0012$) (16). Thus, as is true for other health care services, implementing mandatory hand washing practices for EMS personnel is likely a simple but effective measure to reduce HAI risks in the EMS environment.

EMS pathogen monitoring for the future. Currently, no study has reported on the prevalence of many other clinically important pathogens in the EMS environment, including high-priority pathogens (e.g., *C. difficile* and influenza virus). This situation is due in part to limitations of culture-based methods, which are the primary methods utilized for pathogen detection. Culture-based detection can demonstrate viability, antibiotic resistance, and hemolytic capability (indicating a virulent strain); however, culture conditions are biased and permit the detection of only one or a few organisms (16, 21, 23). Culturing methods also have long turnaround times for results (48 to 96 h), limiting the time frame in which microbial contamination may be detected and corrective action can be taken (e.g., decontamination). Finally, extensive biochemical testing, microscopy, and use of selective media for culturing may still misidentify microbes (42) or provide only coarse-grained (genus-level) identification that may include both pathogenic and innocuous species. The development of alternative pathogen detection methods that provide more rapid (<8 h) and comprehensive analysis of resident microbes is therefore needed to effectively identify and mitigate EMS workplace risks.

DNA sequencing provides several advantages over culturing techniques in terms of pathogen detection comprehensiveness. DNA sequencing is often performed on DNA extracted from pure colonies postculturing, followed by targeted (gene-specific) (43) or whole-genome (44) DNA amplification. The sequenced DNA is then matched to databases containing known DNA sequences to identify microbes and reveal additional strain or genotypic information, such as the presence of virulence genes. As DNA sequencing requires only a DNA input, the culturing step may be circumvented by sequencing DNA from environmental samples (45, 46). This removes the constraints of culture bias, enabling the simultaneous detection of a wider variety of bacterial, fungal, and viral pathogens (45–47). Moreover, the additional strain information from sequencing can be used to identify patterns of pathogen spread and/or reservoirs within and outside the health care framework (47). In this regard, high-throughput DNA sequencing technologies, such as Illumina sequencing, Ion Torrent sequencing, SOLiD sequencing, and pyrosequencing, are effective tools. Portable DNA sequencing technologies, such as the MinION device (Oxford Nanopore Technologies), are particularly interesting, as they can allow DNA sequencing to be performed on-site in EMS vehicles and/or facilities, with results being obtained in as little as 6 h (48, 49).

Nevertheless, DNA sequencing methods are not without limitations. Obtaining sufficient starting material from environmental samples is often challenging and typically requires a DNA amplification step to generate enough material for sequencing. PCR is the most widely used DNA amplification technique, which utilizes specific oligonucleotide primers to exponentially amplify one or more gene sequences of interest from a DNA sample (50). The ability of PCR-based methods to detect pathogens from environmental samples depends largely on the target gene(s) chosen for amplification. Sequencing of PCR-amplified 16S rRNA gene sequences is a frequently used strategy for examining bacterial communities (43, 51). However, depending on the PCR primers selected for 16S gene amplification (e.g., primer binding location) and natural variation in 16S gene sequences, only some bacterial sequences will be preferentially amplified, with low-abundance and atypical sequences being the most likely to be excluded (52–54). Nonbacterial microbes are also excluded by 16S rRNA gene sequencing, and their detection requires amplification of alternative genes (e.g., 18S rRNA for eukaryotic organisms) and/or organism-specific gene sequences. Some of the limitations of PCR can be overcome by other DNA amplification methods, such as multiple displacement amplification (MDA), in which randomized primers are used to amplify total DNA from a sample without any prior knowledge of sequence identity (55). MDA

enables a less sequence-biased approach to the examination of microbial communities, which can include a wide diversity of microbes (bacteria, protozoans, fungi, and viruses), but it usually requires more sequenced DNA fragments for organism identification and a more challenging downstream analysis of sequence data (55–57). While PCR and MDA can theoretically detect as little as one DNA molecule in a sample, successful amplification depends on multiple factors, including DNA sample quality, quantity, purity, and undesirable off-target amplification or preferential amplification of particular DNA sequences.

Downstream processing of environmental DNA is commonly done through amplicon sequencing, which is the direct analysis of the PCR-amplified DNA (i.e., 16S rRNA gene), providing taxonomical and phylogenetic insight into the diversity of the microbial community present and requiring minimal bioinformatic analysis. The information gained by amplicon sequencing is defined by the preceding PCR and therefore is subject to the limitations and biases described above. Alternatively, shotgun metagenomic sequencing involves the shearing of all collected DNA into small fragments, which are then independently sequenced (50). Therefore, metagenomic sequencing is frequently used to analyze samples where species-level identification, identification of novel genes, and the identification of metabolic pathways within the community are required. However, due to the volume and complexity of the data collected, the metagenomic analysis is computationally intensive, which limits its application in the field (50). In order for DNA sequencing-based pathogen detection to become more widespread, the difficulty of DNA sample collection, preparation, and data analysis needs to be addressed. Additionally, simplified and standardized workflows for sample processing and bioinformatic analysis are needed to make entirely lab-free DNA sequencing economically and practically viable (58).

Paper-based tests, such as lateral flow immunoassays (LFIA), are another alternative for on-site identification of pathogens. LFIA are simple and inexpensive strip tests (e.g., home pregnancy test) that use specific antibodies or nucleic acid probes to detect one or more molecules or organisms in a sample (59, 60). LFIA have been previously used to rapidly detect foodborne pathogens (61) and a variety of bacterial and viral pathogens (59, 60, 62); however, to our knowledge, they have not been utilized to monitor pathogens in EMS vehicles. If implemented, LFIA could enable rapid and inexpensive detection of pathogens in EMS environments with turnaround times as low as 15 to 30 min for as little as \$1.00 per test (60, 62, 63). LFIA may therefore be practically and feasibly employed for routine pathogen monitoring in EMS vehicles (e.g., between patients) or to evaluate cleaning practices by testing samples taken before and after cleaning.

DNA sequencing and paper-based detection approaches could be combined to create a powerful two-part pathogen detection system. DNA sequencing would first identify target pathogens and other microbes, which would then inform the design of specific and rapid LFIA. The two-step approach would enable customizable and site-specific monitoring of pathogens in the EMS workplace, with iterative cycles of DNA sequencing providing the opportunity to adapt LFIA in response to changing biosafety risks.

Conclusions. Although it has been over 30 years since the first published report of pathogens within ambulance vehicles, many questions remain regarding pathogen occupancy within or on EMS vehicles, medical equipment, and personnel. In contrast to several thousands of publications relating to hospital-borne HAIs (found in PubMed), only approximately 25 published studies have investigated pathogen prevalence and the efficacy of cleaning practices within the EMS framework, with many of these being self-reported as “preliminary” or “pilot” studies. Studies investigating pathogen presence in EMS vehicles or facilities have also been limited to developed countries within northern Europe and Asia, as well as in the United States and Australia. Currently, no data on pathogen prevalence in EMS exist from most other developed countries, including Canada and Mexico, whose geographical proximity to the United States may

suggest comparably high frequencies of MRSA. Moreover, no data exist on pathogen prevalence in developing countries, and because HAI prevalence may be up to three times higher than in developed countries (3), these should be considered high-priority areas of future investigation.

The issue of insufficient time or resources for adequate ambulance vehicle cleaning is a recurring theme (22, 30), indicating that the search for appropriate protocols, cleaning solutions, and guidelines that balance the needs of patient transport and care with vehicle decontamination is ongoing worldwide. No single disinfection method is expected to eliminate all biological hazards or is suitable for all types of surfaces, and consequently, EMS personnel must select a proper cleaning arsenal that meets the needs of individual EMS operations. Thus, achieving optimal decontamination of EMS vehicles and equipment depends on a complex combination of factors that include (i) the type and extent of pathogen loads present, (ii) access to cleaning materials, and (iii) adequate time to perform cleaning between service calls. Additional programs for EMS personnel that provide comprehensive education regarding important pathogens, their modes of transmissions, and suitable decontamination practices should help reduce biosafety risks in the EMS environment. Moreover, additional technologies that rapidly identify pathogens and quantitate biological loads are needed to establish evidence-based guidelines to reduce the frequency of HAIs within the EMS framework. The development of such technologies and widespread pathogen monitoring programs will help further elucidate biosafety risks in the EMS sector and increase preparedness for managing emerging biological threats, epidemics, and bioterrorism.

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