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Review

Microbial contamination on ambulance surfaces: a systematic literature review

A. Obenza*, P. Cruz, M. Buttner, D. Woodard

Department of Environmental and Occupational Health, School of Public Health, University of Nevada Las Vegas, NV, USA

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SUMMARY

Healthcare-associated infections (HAIs) are infections that patients acquire while receiving medical treatment in a healthcare facility. During ambulatory transport, the patient may be exposed to pathogens transmitted from emergency medical service (EMS) personnel or EMS surfaces.The aim of this study was to determine whether organisms commonly associated with HAIs have been detected on surfaces in the patient-care compartment of ambulances. Five electronic databases — PubMed, Scopus, Web of Science, Embase and Google Scholar were used to search for articles using inclusion and exclusion criteria following the PRISMA checklist. Inclusion criteria consisted of articles published in English, between 2009 and 2020, had positive samples collected from the patient-care compartment of a ground ambulance, and reported sample collection methods of either swab sampling and/or Replicate Organism Detection and Counting (RODAC) contact plates. Studies not meeting these criteria were excluded from this review. From a total of 1376 articles identified, 16 were included in the review. Organisms associated with HAIs were commonly detected in the patient-care compartment of ambulances across a variety of different surfaces, including blood pressure cuffs, oxygen apparatuses, and areas of patient stretchers. A high prevalence of pathogenic bacteria in ambulances suggests that standard protocols related to cleaning compliance may not be effective. The primary recommendation is that designated subject matter experts in infection prevention should be incorporated as liaisons in the pre-hospital setting, acting as a link between the pre-hospital (e.g., ambulance transport) and hospital environments.

Introduction

Although micro-organisms are ubiquitous in the environment, improved living conditions, advances in medicine, and access to healthcare have altered human morbidity and mortality from infectious diseases over the last two centuries [1]. Given the constant expansion in population, the use of healthcare facilities and emergency response transport vehicles will increase worldwide. In the USA, over 20 million patients receive pre-hospital care from emergency medical services each year [2]. Despite the advancements in public health, patients and medical staff continue to become sick from pathogens present in the healthcare setting.

Healthcare-associated infections (HAIs) are infections that are acquired in a healthcare setting while receiving or providing medical treatment [3,4]. Although HAIs are often

* Corresponding author. Address: School of Public Health, University of Nevada, Las Vegas, 4505 S. Maryland Parkway, MSM 102, Las Vegas, NV 89154-4009, USA. Tel.: +1 702 895 1417.
E-mail address: obenza@unlv.nevada.edu (A. Obenza).

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preventable, common sources of infections originate from indwelling (intravenous catheters) and invasive devices such as urinary Foley catheters and intubation (ventilators), as well as contaminated hands resulting from improper hand hygiene [5,6]. HAIs are frequently transmitted to patients from the hands of healthcare workers. For example, community-acquired and hospital-acquired strains of meticillin-resistant *Staphylococcus aureus* (MRSA) isolated from environmental fomites were found to be genetically similar to *S. aureus* and nasal MRSA isolates collected from emergency medical service (EMS) providers. This suggests that MRSA transmission between healthcare personnel and pre-hospital environmental surfaces is possible [7,8].

Although there is variability in estimating the total cost of HAIs in the USA, HAIs cause additional burden and cost to individual hospitals and healthcare systems [9]. The additional costs of HAIs are attributed to longer hospital length of stay, more diagnostic testing, treatments and post-discharge complications [9]. Schmier *et al.* [10] developed a spreadsheet model derived from published literature to estimate potential annual HAI cost savings to the US healthcare system with the implementation of healthcare antiseptics (e.g., handwashing, surgical hand scrubs, and patient pre-operative and pre-injection skin preparations) [10]. According to these data, estimates of the annual national economic burden of HAIs range from $1.42 billion to $14.1 billion for catheter-associated urinary tract infections, central line-associated bloodstream infections, gastrointestinal infections, surgical site infections, ventilator associated pneumonia and hospital-acquired (not ventilator associated) pneumonia. The use of antiseptics could reduce HAI costs by an estimated $142 million to $4.3 billion annually [10].

In addition to the financial burden of HAIs, the morbidity and mortality rates associated with HAIs are high. In developed countries such as the USA, at least 99,000 deaths per year occur from HAIs and approximately 7% of hospitalized patients in developed countries and 19% in developing countries are affected by HAIs [8]. Common micro-organisms responsible for HAIs, transmissible by contaminated hands, include *Staphylococcus aureus*, MRSA, and carbapenem-resistant Enterobacterales (CRE) such as *Klebsiella pneumoniae* [11,12].

Antimicrobial resistance is a global concern; it threatens effective treatment and prevention of various infections [13]. It occurs when micro-organisms are continuously subjected to antimicrobial agents and evolve to develop resistance to such agents. Once the antibiotics are no longer effective, the infections are able to persist in the host [13]. Although resistance can occur naturally over a long period of time, overuse of antimicrobial agents increases the rate at which micro-organisms resist antibiotic therapy. HAIs caused by antibiotic-resistant micro-organisms require more healthcare-related resources and patients are at an increased risk of worse clinical outcomes and death [13]. Specifically, those with MRSA are 64% more likely to die when compared to individuals with a susceptible strain [13]. Therefore, the prevention of HAIs is important for reducing morbidity and mortality at the global, community, and individual level. The purpose of this systematic literature review was to determine whether organisms commonly associated with healthcare-associated infections have been detected on surfaces in the patient-care compartment of ambulances.

An EMS environment may include ground ambulances, air ambulances, EMS facilities, or EMS personnel. Of these environments, the ground ambulance has been most researched for characterizing micro-organisms, and was the subject of this study.

Methods

The systematic review was completed in six steps (Figure 1): (1) identification of articles by using key search terms and removal of duplicate articles; (2) screening of titles and abstracts of relevant articles found by applying the inclusion and exclusion criteria; (3) acquisition of full text of eligible articles; (4) reading all eligible articles to determine whether the inclusion criteria were met; (5) reporting search results using a Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) diagram [14]; (6) synthesizing the methods and results of relevant articles.

Titles and abstracts were screened independently by two researchers using ProQuest RefWorks®. The screening results were compared in an effort to reduce selection bias. In the event of a selection discrepancy, a third researcher would have screened the article. However, no selection discrepancies occurred. Articles that appeared to meet the inclusion criteria were considered relevant. All other articles were excluded from this review.

Search strategy

This systematic review identified published articles from five electronic databases— PubMed®, Scopus, Web of Science, Embase® and Google Scholar. The search was conducted using the following key search terms: ambulance, emergency medical service, prehospital, contamination, infection, microbial, bacteria, pathogen, nosocomial, MRSA, meticillin-resistant *Staphylococcus aureus*, meticillin-resistant *S. aureus*, KPC, *Klebsiella pneumoniae* carbapenemase, carbapenem-resistant *K. pneumoniae*, and carbapenem-resistant Enterobacterales (CRE).

Inclusion and exclusion criteria

Articles considered eligible for review met the following inclusion criteria: (1) published in English; (2) published between 1st January 2009 and 31st December 2020; (3) had positive samples collected from the patient-care compartment (rear-interior) of a ground ambulance; and (4) reported sample collection methods consisting of either swab sampling and/or Replicate Organism Detection and Counting (RODAC) contact plates. We selected an 11-year range to capture the most relevant studies and evaluated the references of the selected articles in order to capture articles published prior to 2009. During selection of the inclusion and exclusion criteria for our study, research into reliable sampling methods for the detection of organisms on fomites was conducted. We concluded that in order to best capture studies targeting organisms commonly associated with HAIs such as MRSA and members of the Enterobacterales, it was appropriate to exclusively select swab sampling and RODAC plates as keywords for our review. Any analysis method used for identifying positive samples (e.g., polymerase chain reaction (PCR) or selective media, etc.) was also considered.

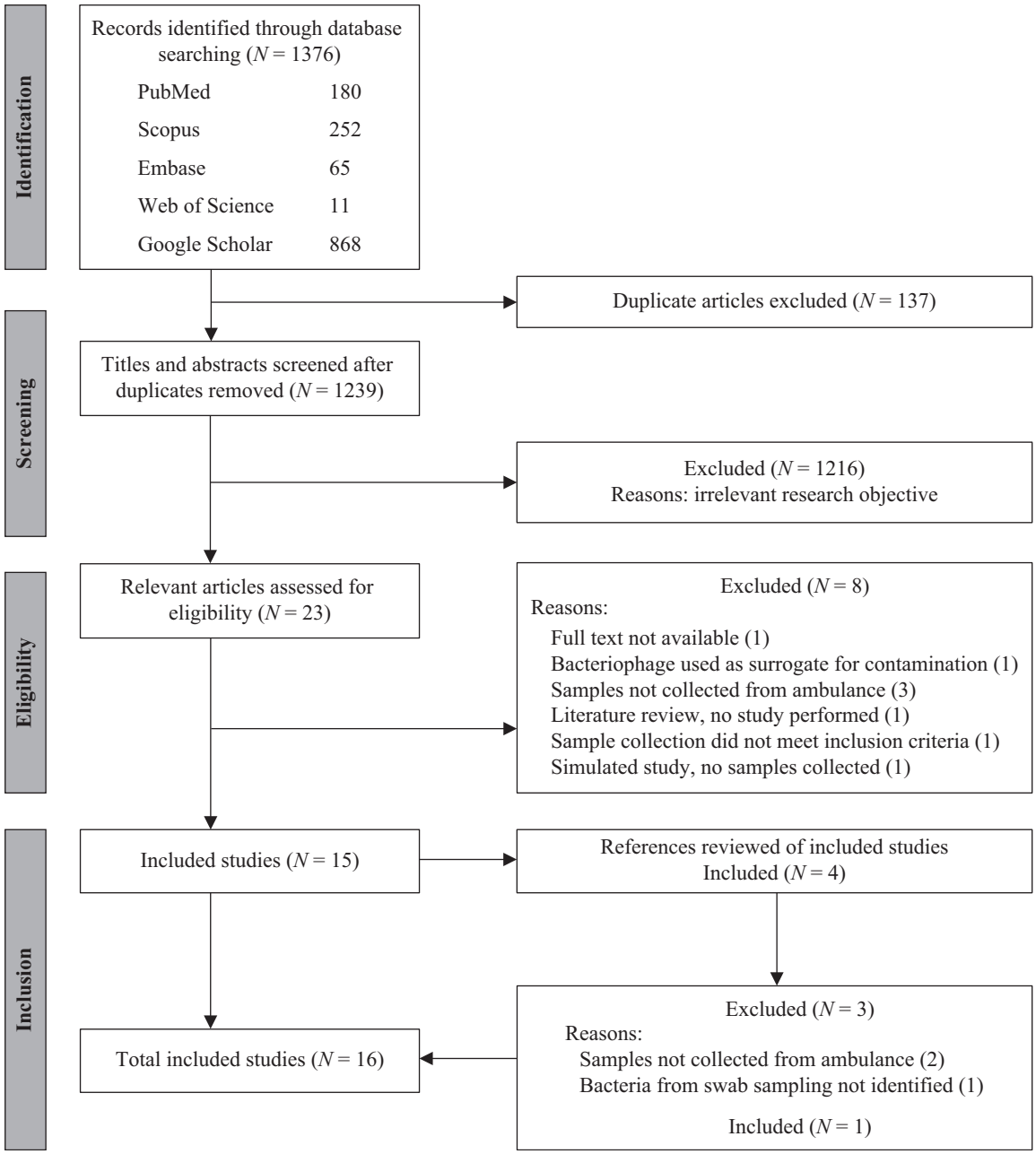

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram detailing the disposition of screened, included, and excluded records.

The exclusion criteria consisted of articles that: (1) were not published in English; (2) had studies not conducted in the patient-care compartment of an ambulance; (3) collected samples from emergency response vehicles other than a ground ambulance, such as a helicopter or a boat; and (4) utilized sample collection methods other than swabs or contact plates (e.g., air samples).

Results

Using the key terms defined above, a total of 1376 articles were identified across the five different electronic databases (Figure 1). After duplicate articles were removed and excluded, 1239 potential articles remained. Two researchers screened the titles and abstracts of the 1239 potential articles to determine if the inclusion criteria were met. After screening, 1216 articles were removed due to not meeting the inclusion criteria, resulting in 23 eligible articles. Eight of the 23 articles were excluded, resulting in 15 articles for review. The references from the remaining 15 articles were also screened and four additional full-text articles were reviewed; only one was included for meeting the inclusion criteria. A total of 16 studies were retained for review (Figure 1).

Description of studies

Sixteen studies of 1376 articles were reviewed. These studies were conducted in eight different countries (Denmark, Egypt, Germany, Iran, Saudi Arabia, South Korea, Spain, USA)

and assessed microbial contamination in the patient-care compartment of ambulances (Table I). While 10 studies included additional areas and surfaces outside of the patient-care compartment (e.g., fire station living quarters, EMS personnel, EMS clothing, and the driver's cabin), they were included as part of this review for having also researched the patient-care compartment of the ambulance [6,7,15–22]. Eight studies specifically targeted MRSA contamination [7,16,19–21,23–25] while others targeted *S. aureus* [15], general bacterial contamination [6,18,22,26,27], any organism associated with HAIs [28], and Gram-negative multi-drug-resistant organisms (MDROs) [17]. The majority of studies utilized swabs for sample collection [6,15–20,22–24,26,27], two used contact plates only [25,28], one used a combination of contact plates and swabs [7], and one used a combination of dip slides and swabs [21].

Microbial presence

Organisms commonly associated with healthcare-associated infections were detected on surfaces in the patient-care compartment of ambulances (Table I). Data are presented as a percentage of positive sampling based on the frequency of positive results from the number of ambulances or surfaces sampled. Only two studies [21,25] presented quantitative data (cfu/cm^2) on their published papers (data not shown). Every study, except two [15,17], detected *S. aureus* in the patient-care compartment. *Staphylococcus* spp. isolates detected were MRSA (Table I) [7,16,18–21,23,24,27,28], meticillin-susceptible *S. aureus* (MSSA) [6,19–21,23] and *S. aureus* [7,22,25,28]. Other organisms commonly associated with HAIs detected in the patient-care compartment of ambulances included: coagulase-negative staphylococci (CoNS) [6,7,15,26,28], meticillin-resistant CoNS (MRCoNS) [7,18,27], *Enterococcus* spp. [21,25], vancomycin-resistant enterococci (VRE) [21], *Klebsiella* spp. [15], *K. pneumoniae* [17,27], extended-spectrum β-lactamase (ESBL) -producing *K. pneumoniae* [18,27], ESBL-producing *Escherichia coli* [27], *Pseudomonas* spp. [15,17], and *P. aeruginosa* [18,22]. The most frequently detected organisms in the ambulance were *S. aureus* and MRSA. Other potentially pathogenic bacteria detected were *Legionella* and *Serratia marcescens* [22], *Citrobacter* spp. [27], *Proteus* spp. [15,27], *Acinetobacter* spp. [15], non-fermenting Gram-negative bacilli [6], and *E. vulneris* and *Pantoea agglomerans* [17]. Other organisms not commonly associated with HAIs were detected throughout the studies, but are not discussed in this review.

Sampled surfaces

Surfaces sampled in the ambulance were determined based on the scope of each study (e.g., one study focused on sampling blood pressure cuffs only while other studies sampled multiple surfaces (Table II)). Overall, the three most frequently sampled surfaces in descending order included the areas of the stretcher (i.e. handle, sidebar, headrest, etc.), patient-care compartment area (i.e. floor, walls, ceiling, etc. [6,7,15–24,26–28]), and the blood pressure cuff [7,15,16,19,20,24,27]. The stretcher handle and the stretcher sidebar were the most frequently sampled areas of the stretcher. One study [25], focused on sampling blood pressure cuffs only for the detection of HAI-causing bacteria. Various medical and non-medical devices were also sampled throughout the studies (Table II). Under the category of other medical devices, the electrocardiogram machine and defibrillation devices were the most frequently sampled. The number of surfaces that were sampled varied across the studies, ranging from one surface [25] to 33 surfaces [18,22]. Supplementary Table S1 shows a summary of surface designations and contamination frequency.

Contamination frequency

The frequency of contamination varied across the studies. Some studies reported their data by frequency of ambulance contamination as a whole [7,17], while the others reported frequency of contamination at the surface level. One study performed composite sampling where multiple surfaces were swabbed with a single swab [20] while other studies used individualized surface sample swabs or contact plates (Table I). In 13 of the studies, the authors reported surfaces that were of concern. These surfaces were noted for the following reasons: positive detection of MRSA, highest prevalence of pathogens, or most frequently contaminated. One study reported notable surfaces from the driver's cabin and suggested that cross-contamination from the patient area had occurred [6]. Although a few studies did not specifically report surfaces of concern in their article, they still found pathogenic bacteria in their study [17,20,25].

Some surfaces showed a higher percentage of contamination although the sampling frequency was lower (e.g., the oxygen apparatus was sampled less frequently, but showed a higher percentage of detections of organisms commonly associated with HAIs among studies (seven of eight total studies; 87%). This was also true for the stretcher headrest and the water in the suction device, which showed 100% contamination (three of three total studies, and two of two total studies, respectively (Table II)).

HAI-causing organisms

The literature examined demonstrates a high prevalence rate of HAI-causing organisms, particularly MRSA, in the patient-care compartment of ambulances [7,16,19,27]. MRCoNS and CoNS were also detected in several studies [15,18,22,26,27]. Similar to other bacteria endogenous to humans, CoNS are Gram-positive bacteria that represent a part of normal microbiota of the skin and mucous membranes [29]. *Staphylococcus epidermidis* is the most common species in CoNS infections and is also a common cause for healthcare-associated bacteraemia associated with indwelling procedures or implanted foreign bodies [29].

Antibiotic-resistant organisms that contain ESBL enzymes found in this review were *Klebsiella* spp. and *E. coli* [27]. ESBL enzymes allow resistance to beta-lactam antibiotics such as penicillin and its derivatives. The ESBL enzyme, coupled with known pathogenic organisms belonging to the Enterobacterales, play a significant role in HAIs and pose a major public health threat. Production of β-lactamases appears to be the most widespread cause of carbapenem resistance [30]. Often used as antibiotics of last resort, carbapenems provide the greatest potency against Gram-positive and Gram-negative bacteria [30]. However, with emerging MDROs, carbapenems are losing their effectiveness against pathogenic bacteria, as

Table I
Summary of findings

Organism(s) detected	Sample collection	Frequency of contamination	Reported notable surfaces	Country	Year	Reference no.
CoNS, *Pseudomonas* spp., *Klebsiella* spp., *Proteus* spp., *Acinetobacter* spp., *Corynebacterium diphtheria*	12 ambulances; 20 surfaces[a]	12/12 ambulances; 20/20 surfaces (100%) • CoNS[b] *Acinetobacter* spp. • 6 positive cultures *Pseudomonas* spp. • 10 positive cultures *Klebsiella* spp. • 8 positive cultures	Highest contamination site: oxygen tank	Iran	2020	[15]
MRSA	3 ambulances; 13 surfaces	3/3 ambulances; 5/39 surfaces • MRSA (13/39; 33%) ○ oxygen cylinders (9/9; 3 per ambulance) ○ compartment floor (3/3) ○ rear door handle (1/3)	MRSA-positive oxygen cylinders	USA	2019	[24]
Staphylococcus spp. MRSA & MRCoNS, *Klebsiella pneumoniae*, *Klebsiella* ESBL, *Escherichia coli*, *E. coli* ESBL, *Citrobacter* spp., *Proteus* spp. [c]	25 ambulances; 16 surfaces	25/25 ambulances; 400/400 surfaces • Staphylococci (184 isolates) ○ MRSA (35/184; 19%) ○ MRCoNS (22/184; 12%) • *K. pneumoniae* (49 isolates) ○ *K. pneumoniae* ESBL (18/49; 37%) • *E. coli* (40 isolates) • *E. coli* ESBL (11/40; 3%)	Highest pathogenic ratios: headboard of patient stretcher, suction devices, beds	Egypt	2018	[27]
S. aureus, MRSA, *Enterococcus*, VRE, Enterobacterales	80 ambulances; 6 surfaces[a]	49/480 ambulance surfaces • *S. aureus* (7%) • Enterobacterales (1%) 108 sites positive for pathogens[b] • MRSA (1/108) • VRE (2/108)	Highest prevalence of pathogens: blood pressure cuffs, medic bag handles, patient harness	Denmark	2018	[21]
CoNS, MSSA, Enterobacterales, non-fermenting Gram-negative bacilli, Enterococci, *Bacillus*	10 ambulances; 4 surfaces[a]	26/40 patient compartment surfaces • CoNS (15/44; 34%)[b] • Enterobacterales, non-fermenting Gram-negative bacilli, Enterococci, *Bacillus* (26/44; 59%)[c]	Highest bacterial growth: interior passenger door handle, steering wheel, left handle of stretcher	Spain	2017	[6]

S. aureus, Enterococcus	39 ambulances; 1 surface	6/50 blood pressure cuffs (11 duplicate cuffs) • *S. aureus* (5/50; 10%) • *Enterococcus* (1/50; 2%)	—	Denmark	2016	[25]
S. aureus, MRSA, CoNS[c]	150 ambulances; 28 surfaces	11/150 ambulances; 10/28 surfaces • 18 contact plates detected MRSA	Most frequently contaminated: carrying handles, oxygen saturation clip, cardiovascular bag handle, patient stretcher handle, BP cuff, pharmacist cabinet handle, patient stretcher headboard, ECG control panel, carrying chair	Germany	2015	[28]
CoNS, *Bacillus* spp., Enterococci[c]	10 ambulances; 3 surfaces	Contamination: (before/after fumigation) • Oxygen knob (9/1) • Stretcher handle (8/1) • Interior handle of rear door (10/4)	100% ambulance contamination rate: interior handle of rear door	Saudi Arabia	2014	[26]
MRSA, MSSA	33 Fire stations; 14 ambulance surfaces	653 composite samples (~2100 surfaces)[b] • MRSA (52/653; 8%) ○ Ambulance surfaces (12/52; 23%) • MSSA (119/653; 18%) ○ Ambulance surfaces (20/119; 17%)	—	USA	2014	[20]
Pseudomonas spp., *K. pneumoniae*, *Escherichia vulneris*, *Shigella flexneri*, *Pantoea agglomerans*	139 ambulances[d]	13/139 ambulances; 79/134 swabs yielded one or more Gram-negative colony forming units • *P. agglomerans* (34%) • *S. flexneri* (8%) • *E. vulneris* (6%) • *Pseudomonas* spp. (6%) • *K. pneumoniae* (6%)	—	USA	2013	[17]

(*continued on next page*)

Table I (*continued*)

Organism(s) detected	Sample collection	Frequency of contamination	Reported notable surfaces	Country	Year	Reference no.
S. aureus, *P. aeruginosa*, *Serratia marcescens*, *Legionella* spp.[c]	30 ambulances; 33 surfaces	30/30 ambulances positive ≥3 surfaces; 159/955 surfaces contaminated[b] 28 bacterial species isolated; 184 total isolates • Pathogenic bacteria (14/184; 8%) ○ *S. aureus* (7/184; 8%) • Endotracheal tube, suction tip ○ *P. aeruginosa* (4/184; 2%) • Suction tip, oropharyngeal airway, oxygen humidifier-water ○ *S. marcescens* (2/184; 1%) • Endotracheal tube ○ *Legionella* (1/184; 0.5%) • Suction water	Very high contamination rates: endotracheal tube (100%), oxygen humidifier-water (90%), water from suction reservoirs (80%)	South Korea	2012	[22]
MRSA, MSSA	71 ambulances; 26 surfaces	49/71 ambulances found ≥1 *S. aureus* isolate; 100/1125 isolates were *S. aureus* • 77% of isolates resistant to at least 1 antibiotic • 34% resistant to at least 2 antibiotics • 5/71 ambulances (MRSA) ○ 9/26 surfaces (≥1 MRSA isolate; 12 total isolates) • 49/71 ambulances (MSSA) 22/26 surfaces (≥1 MSSA isolate; 88 total isolates)	Positive for MRSA and MSSA most often: portable pulse oximetry finger sensors, portable pulse oximeter outer case, automatic pulse finger sensors, automatic blood pressure cuffs, workspace deck	USA	2012	[19]
MRSA, MSSA	89 MRSA transport events and 60 transport events without MRSA notification; 3 surfaces	MRSA (8/89; 9% transports with MRSA notification) • Headrest (5/8; 63%) • Stretcher handles (2/8; 3%) • Headrest & stretcher handles (1/8; 1%) MSSA (12/60; 20% transport events without MRSA notification) • Headrest (5/12; 42%) • Stretcher handles (6; 50%) • Cabin wall (1; <1%)	Most frequently contaminated: stretcher headrest	Germany	2011	[23]

MRSA, MRCoNS *K. pneumoniae* ESBL, *P. aeruginosa*[c]	13 ambulances; 33 surfaces[a]	13/13 ambulances showed microbial contamination; from 429 total surfaces, 396/624 (64%)[b] sample cultures positive for micro-organisms • *K. pneumoniae* ESBL (2) ○ Water in suction bottle ○ Bag-valve mask bag • MRSA (1) ○ Driver's side door handle • MRCoNS (1) ○ Stretcher car side bar • *P. aeruginosa* (1)	Pathogenic bacteria found: water of suction bottles, bag-valve mask bag, stretcher car side bars, driver's side door handle	South Korea	2011	[18]
MRSA, MRCoNS, CoNS, *S. aureus*	1064 total samples collected from surfaces across two fire stations[a]; 8 surfaces from each ambulance	First sampling event ($N = 600$); MRSA-positive samples (26/600; 4.3%)[b] • MRSA in ambulance (13/26; 50%) Second sampling event ($N = 464$) • MRSA-positive samples (18/464; 3.9%)[b] • MRSA in ambulance (3/18; 17%)	Of all sites sampled, the ambulance was the most common area for MRSA contamination	USA	2011	[7]
MRSA	51 ambulances; 16 surfaces	MRSA (25/51; 50% ambulances)[b] • Fire Dept. ambulances (25/51) ○ 14/25; 56% contaminated • Private nonprofit ambulances (19/5) ○ 9/19; 45% contaminated • Third-service municipal ambulances (2/51) ○ 1/2; 50% contaminated • Hospital-based ambulances (5/51) ○ 1/5; 20% contaminated	MRSA-contaminated areas most in contact with patients: work area adjacent to the patient, pulse oximeter, stretcher rail	USA	2010	[16]

BP, blood pressure; CoNS, coagulase-negative Staphylococci; ECG, electrocardiogram; ESBL, extended-spectrum β-lactamase; MRCoNS, meticillin-resistant coagulase-negative Staphylococci; MRSA, meticillin-resistant *Staphylococcus aureus*; MSSA, meticillin-sensitive *Staphylococcus aureus*; VRE, vancomycin-resistant Enterococci; —, not reported.

[a] Includes additional surfaces sampled outside the inclusion criteria.

[b] Includes positive results from surfaces outside the inclusion criteria.

[c] Other various non—healthcare-associated bacteria or fungal species detected.

[d] Study included air ambulance.

Table II
Sampled surfaces and detected healthcare-associated infection associated organism(s) per surface

Surface sampled	References[a]	References – HAI organism(s) detected
Blood pressure cuff	**7**, **15**, **16**, 19, 20, **21**[b], 22, 24, **25**, **27**, **28**	**7** - MRSA, **15** - CoNS, *Acinetobacter* spp., **16** - MRSA, **21**, **25** - *S. aureus*, *Enterococcus*, **27** - MRCoNS, **28** - MRSA
Adult blood pressure cuff	**16**	**15** - CoNS, *Acinetobacter* spp., **16** - MRSA
Automatic blood pressure cuff	19	
Child blood pressure cuff	**15**	
Manual blood pressure cuff	19	
Inside blood pressure cuff	20	
Jump bag	**7**, **15**, 19, 20, **21**[b], **28**	**7** - MRSA, **15** - CoNS, **21**, **28** - MRSA
Carrying handle	**15**, 21, **28**	**15** - CoNS, **28** - MRSA
Carrying handle of cardiovascular bag	**28**	**28** - MRSA
Carrying handle of pharmacist's cabinet	**28**	**28** - MRSA
Oxygen apparatus	**6**[c], **15**, **16**, 18, **19**, **22**, **24**, **26**, **27**, **28**	**6**, **15** - CoNS, *Pseudomonas* spp., *Klebsiella* spp., *Acinetobacter* spp., **16** - MRSA, **19** - MRSA, **22** - *P. aeruginosa*, **24** - MRSA, **26** - CoNS, **27** - MRSA, *Klebsiella* ESBL, **28** - MRSA
Oxygen knob	**26**	**26** - CoNS
Oxygen regulator	**16**	**16** - MRSA
Oxygen humidifier glass	22, **27**	**27** - MRSA, *Klebsiella* ESBL
Oxygen cylinders	**24**	**24** - MRSA
Oxygen gate connector	18	
Oxygen connector	18, 22	
Oxygen generator	18, 22	
Water in oxygen generator	18	
Oxygen tank/bag handle	**15**, **19**	**15** - CoNS, *Pseudomonas* spp., *Klebsiella* spp., *Acinetobacter* spp., **19** - MRSA
Oxygen switches	19	
Fixed oxygen delivery system flow meter	**6**[a]	**6**
Oxygen hose	28	
Oxygen saturation clip	**28**	**28** - MRSA
Oxygen humidifier-water	**22**	**22** - *P. aeruginosa*
Patient compartment area	**6**[c], **7**, **15**, **16**, 17, 18, **19**, 20, **21**[a], 22, 23, 24, **26**, **27**, 28	**6**, **7** - MRSA, **15** - CoNS, *Pseudomonas* spp., *Acinetobacter* spp., **16** - MRSA, **19** - MRSA, **21**, **26** - CoNS, **27** - MRSA, MRCoNS,
Interior handle of rear door	**16**, 24, **26**	**16** - MRSA, **26** - CoNS
Ceiling rail	**16**, 19, 20, **21**[b]	**16** - MRSA, **21**
Work area adjacent to patient	**16**, 20	**16** - MRSA
Wall	**15**, 17, **21**[b], 23, **27**	**15** - CoNS, **21**, **27** - MRSA, MRCoNS
Door grip	22, **27**	**27** - MRSA
Bench surface	24	
Floor	**7**, **15**, 17, 24	**7** - MRSA, **15** - CoNS, Pseudomonas spp.
Compartment switches	**6**[c], **16**, 19, 24	**6**, **16** - MRSA
Door handles	17	
Patient's side door handle	**6**[c], 18	6
Air conditioner	18, 22	
Left rear bench seat	19	
Left walk space	19	
Rear seats	**15**	**15** - CoNS, *Acinetobacter* spp.
Workspace deck	**19**, 28	**19** - MRSA
Inside door	**7**	**7** - MRSA
Compartments	28	
Ceiling flap	28	
Rear door	22	
Side door	22	

Table II (*continued*)

Surface sampled	References[a]	References — HAI organism(s) detected
Pulse oximeter	**15**, **16**, 18, **19**, 20, 22, 24	**15** - CoNS, **16** - MRSA, **19** - MRSA
Detector of pulse oximetry	18	
Portable pulse oximeter outer case	19	
Portable pulse oximeter finger sensor	**15**, **19**	**15** - CoNS, **19** - MRSA
Automatic pulse oximeter finger sensor	**19**	**19** - MRSA
Stethoscope	**15**, **16**, 18, 19, 20, 22, 24, **27**	**15** - CoNS, **16** - MRSA, **27** - MRSA, MRCoNS
Stethoscope bell	24	
Stethoscope surface	18	
Stethoscope diaphragm	20	
Stretcher	**6**[c], **7**, **15**, 16, 17, **18**, **19**, 20, **21**[b], 22, **23**, 24, **26**, **27**, **28**	**6**, **7** - MRSA, **15** - CoNS, **18** - MRCoNS, **19** - MRSA, **21**, **23** - MRSA, **26** - CoNS, **27** - MRSA, MRCoNS, *Klebsiella* ESBL, *E. coli* ESBL, **28** - MRSA
Stretcher handle	**7**, 18, 22, **23**, **26**, **27**, **28**	**7** - MRSA, **23** - MRSA, **26** - CoNS, **27** - MRSA, MRCoNS, **28** - MRSA
Stretcher rail/sidebar	**6**[c], **16**, **18**, **19**, 22, 24, **27**	**6**, **16** - MRSA, **18** - MRCoNS, **19** - MRSA, **27** - MRSA, MRCoNS
Stretcher straps	**7**, **16**, 20	**7** - MRSA, **16** - MRSA
Stretcher headrest	**23**, **27**, **28**	**23** - MRSA, **27** - MRSA, MRCoNS, **28** - MRSA
Bed	**15**, **27**	**15** - CoNS, **27** - MRSA, MRCoNS, *Klebsiella* ESBL, *E. coli* ESBL
Stretcher buckle	20, 24	
Stretcher upper and lower seat belt	19	
Stretcher—patient harness	**21**[a]	**21**
Electronic and miscellaneous equipment	**7**, **15**, 16, 17, 18, 19, **27**, **28**	**7** - MRSA, **15** - CoNS, *Acinetobacter* spp., **27** - MRSA, MRCoNS, **28** - MRSA
Clipboard	**16**	**16** - MRSA
Computer	**16**	**16** - MRSA
Microphone	**15**, **16**, 19	**15** - CoNS, *Acinetobacter* spp., **16** - MRSA
Carrying chair	19, **27**, **28**	**27** - MRSA, MRCoNS, **28** - MRSA
Extractor fan	18, 22	
Laptop keypad	19	
Other medical devices	**15**, **16**, **18**, 19, 21, **22**, 24, **27**, **28**	**15** - CoNS, *Klebsiella* spp., *Acinetobacter* spp., **16** - MRSA, **18** - *K. pneumoniae*, **22** - *S. aureus*, *S. marcescens*, *P. aeruginosa*, CoNS, *Legionella*, **27** - MRSA, MRCoNS, *Klebsiella* ESBL, *E. coli* ESBL, **28** - MRSA
IV tray	**16**	**16** - MRSA
Cervical collar	18, 22, **27**	**27** - MRSA, MRCoNS
Direct-current (DC) Cardioversion-defibrillation	**21**[b], **27**	**21**, **27** - *S. aureus*
Cardiac monitor	**16**, **27**, 24	**16** - MRSA, **27** - MRSA, MRCoNS
Portable ventilator	**27**	**27** - MRSA, MRCoNS, *Klebsiella* ESBL, *E. coli* ESBL
Suction devices	**15**, 18, **22**, **27**	**15** - *Klebsiella* spp., **22** - *S. aureus*, *P. aeruginosa*, **27** - MRSA, *Klebsiella* ESBL, *E. coli* ESBL
Water in suction device	**18**, **22**	**18** - *K. pneumoniae* ESBL, **22** - *Legionella*
Bag-valve mask bag	**18**, 22	**18** - *K. pneumoniae* ESBL
Bag-valve mask	18, 22	
AED button	18, 22	
AED handle	18, 22	
ECG device	18, 19, 22, **28**	**28** - MRSA
Facial mask	18, **22**	**22** - *S. aureus, Serratia marcescens*
Sphygmomanometer handle	18	
Intubation tube	18, **22**	**22** - *S. aureus, Serratia marcescens*
Laryngeal mask airway	18, 22	

(*continued on next page*)

Table II (*continued*)

Surface sampled	References[a]	References – HAI organism(s) detected
Laryngoscope	**15**	**15** - CoNS, *Acinetobacter* spp.
Laryngoscope blade	18, 22	
Laryngoscope handle	18, 22	
Nasal prong	18, **22**	**22** - *S. aureus*, CoNS
Oropharyngeal airway	18, **22**	**22** - *P. aeruginosa*
Spine board	18, 22	
Splint	18, 22	
Glucometer	19	
IV box	19	
Tourniquet	28	
Glove packaging	18	

AED, automated external defibrillator; ECG, electrocardiogram; ESBL, extended-spectrum β-lactamase; HAI, healthcare-associated infection; IV, intravenous; MRCoNS, meticillin-resistant coagulase negative *S. aureus*; MRSA, meticillin-resistant *Staphylococcus aureus*.

[a] Bold text refers to studies positive for HAI-causing bacteria.

[b] HAI organism detected, but not specified. HAI organisms detected: MRSA, *S. aureus*, VRE, Enterococcus, Enterobacterales.

[c] HAI organism detected, but not specified. HAI organisms detected: CoNS, meticillin-sensitive *Staphylococcus aureus*, Enterobacterales, non-fermenting Gram-negative bacilli.

demonstrated by increased incidences of carbapenem resistance worldwide [30].

Laboratory methods

Culture analysis was used in all 16 studies. Commonly used media included different iterations of blood agar (e.g., 5% sheep's blood), MacConkey agar, and mannitol salt agar (Table III). Other media were also used in conjunction with those listed above (Table III). Bacto m® *Staphylococcus* broth was used in two studies [7,20], both of which used Polymyxin B and 0.001% tellurite for enrichment. Brain-heart infusion broth was used as an enrichment medium for four of the studies [6,22,23,27], and serum broth was used for media enrichment in one study [21]. In summary, seven studies used enrichment as part of their methods. Each study followed the manufacturer's recommendation for incubation. However, the incubation period for one study was not reported [28] due to the publication being a short report, and the temperature of incubation of another study [22] was not reported. Three studies [17,18,26] did not report specific laboratory analysis methods because sample analysis was outsourced to another laboratory (Table III).

The methods for bacterial identification and susceptibility testing varied throughout the studies. Bacterial identification methods included: selective media, such as HardyCHROM™ MRSA agar and mannitol salt agar with 4 μg/mL oxacillin [15,16,24], Analytical Profile Index (API) [6,27], matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) [21,25], pulsed-field gel electrophoresis (PFGE) [17,28], VITEK® II [22,23] and latex agglutination [7,19,20]. Antimicrobial susceptibility testing (AST) methods included: disc diffusion [6,19,21,27], PCR [7,19,20,25], automated AST with the VITEK® II [22,23] and PFGE [7,17,28]. Sample identification and antibiotic resistance testing methods showed overlap (i.e. the same method used to identify bacterial isolates was sometimes also used to test for antimicrobial susceptibility). Overlap in methods of identification and AST occurred in five studies [17,22–24,28] with VITEK® II [22,23], PFGE [17,28] and HardyCHROM™ MRSA agar [24].

Pathogenic bacteria prevalence

Remarkably, one study found 100% contamination of both ambulances and targeted surfaces [27]. Two other studies also showed 100% ambulance contamination; however, the total number of surfaces that were contaminated was relatively low [22,24]. All ambulances and surfaces in the study with 100% frequency of contamination showed organisms commonly associated with HAIs and other various bacterial species. The pathogenic bacteria detected in this study were: MRSA, MRCoNS, *Klebsiella pneumoniae*, *Klebsiella* ESBL, *E. coli*, and *E. coli* ESBL [27]. From 184 *Staphylococcus* spp. isolates identified, the frequency of MRSA and MRCoNS were 35/184 isolates (19%) and 22/184 isolates (12%), respectively. A total of 49 *K. pneumonia* isolates were detected, of which, 18/49 isolates (37%) were ESBL producing. Similarly, 40 isolates of *E. coli* were detected with 11/40 isolates (3%) classified as ESBL producing. The surfaces that were positive for these bacteria were the blood pressure cuff, oxygen humidifier glass, ambulance wall, door grip, bed, carrying chair, cervical collar, direct-current (DC) cardioversion-defibrillation, portable ventilator, and suction devices (Table II).

In southern Maine, Brown *et al.* [16] sampled 51 ambulances from various sectors (e.g., fire department, private non-profit, hospital-based, and third-service municipal services) to determine whether MRSA could be recovered from ambulances in a primarily rural state. From across the sectors, 25 (49%) ambulances were positive for MRSA. No statistical significance of contamination between the various sectors and annual run volume were found. However, a statistically higher incidence of MRSA from ambulances that used volunteer, pay per-call or part-time coverage compared to 24-h paid coverage was found [16].

In 2012, the prevalence of *S. aureus* isolates in a pre-hospital setting was examined from advanced life support ambulances used by various fire departments in the Chicago metropolitan area [19]. From 34 different Chicago-area municipalities, 26 surfaces were sampled in 71 different ambulances. At minimum, one isolate of *S. aureus* was discovered in 49 of 71 (69%) ambulances. *S. aureus* made up 100 of

Table III
Summary of laboratory methods

Target organism	Sampling methods	Culture medium	Enrichment medium	Identification/AST	Country	Year	Reference
Staphylococcus aureus, pathogenic bacterial contamination	Moistened sterile cotton swabs	Mannitol Salt agar	—	Selective media, Gram staining, catalase and coagulase test, deoxyribonuclease test	Iran	2020	[15]
MRSA	Sterile cotton-tipped applicators saturated in 0.9% NaCl solution	CHROM agar MRSA	—	Colorimetric (selective agar)	USA	2019	[24]
General bacterial contamination	Cotton wool swabs moistened with sterile 0.9% NaCl solution	Blood agar, Mannitol Salt agar, MacConkey agar, Eosin Methylene Blue agar	Brain heart infusion broth	API/disc diffusion	Egypt	2018	[27]
MRSA, VRE, Enterobacterales ESBL	Dip slides/Liquid Amies moistened flocked swab	Blood agar (Columbia agar 5% sheep blood)	Serum broth	MALDI-TOF MS disc diffusion	Denmark	2018	[21]
General microbial contamination	Sterile swabs moistened with Whatman neutralizing solution	Blood agar	Brain heart infusion broth	API/disc diffusion	Spain	2017	[6]
S. aureus, MRSA, *Enterococcus*, VRE	Contact agar plates	Rabbit Plasma Fibrinogen agar, Slanetz agar	—	MALDI-TOF MS MRSA SelectTM, PCR	Denmark	2016	[25]
HAI causing bacteria	RODAC plates	—	—	PFGE	Germany	2015	[28] [a]
Pathogenic bacterial contamination	Soft rayon sterile swabs with thioglycolate fluid	Blood agar, MacConkey agar	—	—	Saudi Arabia	2014	[26] [b]
MRSA, MSSA	Sanicult transport swabs with 1 ml of neutralizing buffer	Bacto m *Staphylococcus* broth	Polymyxin B and 0.001% potassium tellurite	Latex agglutination test/ PCR	USA	2014	[20]
Gram-negative MDRO	Swab transport systems containing liquid Amies medium without charcoal	—	—	PFGE	USA	2013	[17] [b]
S. aureus, MRSA	Pre-moistened cotton swabs	Mannitol salt agar	—	Latex agglutination specific for S. aureus/disc diffusion, PCR	USA	2012	[19]
Pathogenic bacterial contamination	Soft rayon swabs	Blood agar, MacConkey agar	Brain heart infusion broth	VITEK II	South Korea	2012	[22]

MRSA	Cotton wool swabs moistened with 0.9% NaCl solution	Columbia Blood agar, Baird Parker agar	Brain heart infusion broth	VITEK II	Germany	2011	[23]
General bacterial contamination	Soft rayon swabs	Blood agar, MacConkey agar	—	—	South Korea	2011	[18] [b]
MRSA	Sanicult swabs and RODAC plates	Bacto m *Staphylococcus* broth	Polymyxin B and 0.001% potassium tellurite	Rapid latex test/ PCR, PFGE	USA	2011	[7]
MRSA	Dacron swab moistened with phosphate-buffered saline	Mannitol salt agar with 4 μg/mL oxacillin, blood agar (5% sheep blood)	—	Selective agar, catalase and coagulase testing	USA	2010	[16]

API, analytical profile index; AST, antimicrobial susceptibility testing; ESBL, extended-spectrum β-lactamase; HAI, healthcare-associated infection; MALDI-TOF MS, matrix-assisted laser desorption/ionization-time of flight mass spectrometry; MDRO, multi-drug-resistant organism; MRSA, meticillin-resistant *Staphylococcus aureus*; MSSA, meticillin-sensitive *Staphylococcus aureus*; PCR, polymerase chain reaction; PFGE, pulsed-field gel electrophoresis; RODAC, Replicate Organism Detection and Counting; RPF, rabbit plasma fibrinogen; VRE, vancomycin-resistant Enterococci; —, not reported.

[a] Short Report, detailed methods not reported.

[b] Sample analysis outsourced to another laboratory; detailed methods not reported.

the 1125 isolates found in the study. Overall, 77% of the *S. aureus* isolates showed resistance to at least one antibiotic, and 34% showed resistance to two or more. Twelve per cent of *S. aureus isolates* were determined to be MRSA, and the remaining 88% were MSSA. Of the 26 surfaces sampled, nine were found to contain MRSA at least once, with three of the surfaces having more than one MRSA-positive occurrence [19].

Roberts and colleagues [7] suggest that transmission of MRSA between pre-hospital personnel and environmental surfaces is possible. Swab sampling and RODAC plates were used to sample MRSA from fire stations in two northwest fire districts in Seattle, Washington. Various surface areas of the station were sampled, including an ambulance, fire truck/engine, fire protection clothing, living quarters and nasal cavities of healthy personnel. MRSA was detected in 44 of 1064 (4.1%) collected samples. Samples were collected at two different points in time. During the first set of collected samples ($N = 600$), 26 surfaces were positive for MRSA. The ambulance was the highest contributor of MRSA-positive samples (13/26; 50%). The second set of samples collected ($N = 464$) yielded 18 positive results for MRSA with three positive results from the ambulance. MRSA-positive samples were identified primarily using swab sampling (36/44; 75%). Community-acquired and hospital-acquired strains of MRSA were isolated from environmental surfaces and were genetically similar to the *S. aureus* isolates and nasal MRSA isolates collected from EMS providers, suggesting possible transmission between personnel and environmental surfaces [7].

Of the surfaces sampled, the stretcher and its components were the most frequently contaminated surface with organisms capable of causing HAIs, consisting of MRSA, MRCoNS, CoNS, ESBL-producing *Klebsiella* spp. and ESBL-producing *E. coli*. These pathogens were found on the stretcher in at least eight different studies, with one study showing remarkable contamination by detecting MRSA, MRCoNS, ESBL-producing *Klebsiella* and ESBL-producing *E. coli* all on the stretcher [27].

Discussion

After applying the inclusion and exclusion criteria, 16 studies worldwide were determined to have researched the detection of various micro-organisms in the EMS environment. The number of studies per country was as follows: Egypt (one), Iran (one), Saudi Arabia (one), Spain (one), Denmark (two), Germany (two), South Korea (two), and USA (six). One major finding of this review is that studies varied in their scope, geographical location, frequency, and location of collected samples, types of vehicles sampled, and study duration. Microbial assessment also occurred in additional EMS environments outside the ambulance. The locations and total number of samples collected varied to match their respective scope, and as a result, the duration of these studies differed, ranging from a single point in time, to one or more weeks, several months, or up to one year. Another major finding was that collection and analysis methods were consistent among the studies. Culture analysis was used in all the studies, and most researchers used either blood agar, to help organisms grow only when specific nutrients were included, or mannitol salt agar, for the selection of Gram-positive bacteria. The swab method was primarily used for sample collection. The disc diffusion method was used most frequently for antimicrobial susceptibility testing.

Notably, legionella was detected in one study [22]. *Legionella* spp. are Gram-negative bacilli that reside in water and cause infections in humans through inhaled aerosols [31]. They are responsible for respiratory illnesses, such as Legionnaires' disease and Pontiac fever. Legionella bacteria were detected in the water of a suction device. Though inhaled aerosols are the typical route of transmission for infection, infection can also occur through aspiration of contaminated water [32].

Both infected patients and ambulance personnel are potential sources of surface contamination in ambulances, which may result in exposure of patients and ambulance personnel to pathogens during subsequent transportation. Adherence to cleaning protocols and using aseptic techniques can reduce the risk of exposure. However, routine cleaning and aseptic techniques are not always observed in the ambulance setting. The fast-paced environment, quick turnover of emergency calls, and compliance with cleaning protocols pose a challenge in maintaining non-contaminated conditions for EMS providers and patients.

Protocols were established in 2016 under new Danish guidelines for ambulance cleaning. Vikke *et al.* [21] observed that although no statistical significance was found (P=0.068) the data suggest that EMS cleaning protocols were able to be maintained even with the volume of patients throughout the day, and surfaces in the ambulances were able to remain relatively clean. These protocols include thorough cleaning (tidying up the patient area, overall surface wiping and sweeping/washing the floor once per day) and moderate cleaning (wiping high-touch surfaces with detergent or bleach after every patient).

Compliance with cleaning protocols is imperative in reducing the incidence of cross- contamination. Brown *et al.* [16] suggest that cleaning practices among public ambulances used on a 24-h basis were more effective at reducing the incidence of MRSA compared with ambulances that operate on a pay per-call or part-time coverage. In our review, five studies [6,7,15,16,18] revealed contamination with organisms commonly associated with HAIs (MRSA, CoNS and Enterobacterales) in both the patient-care compartment and the driver's cabin. Detecting these bacteria in the driver's cabin suggests cross-contamination from the patient-care compartment, possibly related to poor hand hygiene practices or failure to adhere to standard aseptic technique (e.g., removing gloves after handling a patient). MDROs and pathogenic bacteria found in the ambulance environment are troubling. Cross-contamination can be mitigated by aseptic technique (i.e. changing gloves between patients, cleaning, and disinfection), cleaning and disinfecting the driver's cabin area, and not bringing patient compartment area equipment to the front driver's cabin (e.g., phones, laptops, stethoscopes, etc.). Additionally, laundering EMT clothing frequently would help mitigate cross-contamination.

It is not uncommon for patients receiving emergency care to be in a critical, life-threatening condition. Utilizing ambulance services often coincides with treating vulnerable populations of the young, elderly, immunocompromised or severely injured. An increased risk of harm exists with colonization or infection with MDROs and other pathogenic organisms present in the ambulance. It is evident that with the prevalence of organisms commonly associated with HAIs so frequently present in the patient-care compartment, standard protocols related to cleaning compliance were not regularly met, or that there could be a different source of contamination (e.g., EMS clothing).

The microbiological methods used in the studies reviewed, from sample collection to bacterial identification, appear to be sufficient in recovering micro-organisms in the EMS environment. Although some laboratory methods found in this review were more labour intensive (e.g., API), as new laboratory technologies continue to mature, it is conceivable that sample collection and laboratory processes will be better streamlined with increased efficiency, sensitivity and specificity.

This review identified a high prevalence of organisms commonly associated with HAIs. Therefore, future studies should focus on surfaces most contaminated, expand beyond surface sampling (e.g., air and water) of EMS environments, assess the effectiveness of existing decontamination methods via pre- and post-decontamination studies, evaluate newer methods for decontamination, and provide ongoing education in cleaning compliance among EMS facilities. In addition, assessing the risk of bacterial transmission to patients and EMS personnel will require tracking of individual patients, collection of samples from the ambulance surface(s) and the patient, and conducting bacterial strain typing.

This systematic review had limitations, including a small sample size ($N = 16$ eligible articles). However, we targeted an important question, and believe that we found sufficient evidence to meet our objective and justify our conclusions. Only articles published in English were considered for this review; therefore, relevant studies that were published in other languages may have been missed. When summarizing the methods and findings of all the studies, it was challenging to identify a common thread of methodology or make a direct comparison of studies due to the numerous variances and the style of data reporting. Additionally, some authors did not report detailed methodology in their studies, which resulted in incomplete data to review. In spite of these limitations, the 16 studies captured for this systematic review highlight a need for future research on organisms commonly associated with HAIs in ambulance environments.

Currently, there are no systems in place to evaluate whether a patient who has developed an HAI while in the hospital was exposed during the hospital stay, or in the pre-hospital (e.g., ambulance transport) setting. Therefore, it is recommended that designated subject matter experts in infection prevention are incorporated as liaisons in the pre-hospital setting, acting as a link between the pre-hospital and hospital environments. Similar to the established protocols and processes in the hospital setting, strict uniform protocols for cleaning efficiency should also be implemented in the pre-hospital setting. Education and training for EMS personnel on standard operating procedures for cleaning and disinfection is recommended to optimize consistent and correct methods of cleaning particularly for high-touch surfaces. In order to make technical recommendations involving surface disinfection methods/frequency, use of ultraviolet light, or air scrubbing, it would be necessary to evaluate and compare methods to determine if they are more effective than current practices. Additional research of the EMS environment is also recommended.

In conclusion, organisms most commonly associated with HAIs were detected in the patient-care compartment of

ambulances worldwide and across a variety of different surfaces. MDROs found in the ambulance environment are concerning because of the difficulty in treating the associated infections. Therefore, transport vehicles could potentially serve as a source of transmission for HAIs to patients and EMS personnel. The results of this study suggest that future work is warranted regarding assessing the effectiveness of cleaning protocols and/or the factors that affect decontamination in the ambulance. These investigations could provide scientific data to potentially prevent the transmission of organisms commonly associated with HAIs to transported patients and EMS personnel.

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Appendix A. Supplementary data

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