ORIGINAL ARTICLE

Prevalence of methicillin-resistant Staphylococcus aureus contamination in ambulances in Jeddah, Saudi Arabia

Fadi Jandali Qara^{1*} , Shadi A. Zakai² , Anas F. Hamam³

ABSTRACT

Background: In recent years, multiple reports of an increase in the emergence of community-acquired methicillin-resistant *Staphylococcus aureus* MRSA (CA-MRSA) have arisen. A potential infection risk may be present if a patient were to be transported by an emergency medical services (EMS) unit that previously transported a patient harboring CA-MRSA. We, therefore, sought to investigate whether there is a certain prevalence of CA-MRSA contamination among ambulances operating in the city of Jeddah, Saudi Arabia.

Methods: This was an observational, cross-sectional survey study involving ambulances (types I to IV) in service in Jeddah from September to November of 2018. Five areas were chosen to be swabbed in each ambulance enrolled in our study.

Results: A total of 425 samples were collected from 85 ambulances operating in three different health care sectors. Our results showed the overall contamination to be 338 samples (79.5%), with the highest contamination rate found on the stretcher grips and the blood pressure cuff sites (both n = 70 samples; 16.47%). Overall, only three samples yielded *S. aureus* bacteria, with none being MRSA.

Conclusion: Even though we have an overall bacterial contamination rate of 79.5% in the areas surveyed on EMS ambulances, it is not clear that this contamination has a pathological potential to cause disease. The failure to isolate a single MRSA sample from the 425 taken suggests there is no MRSA problem.

Keywords: Ambulance, methicillin-resistant Staphylococcus aureus, MRSA, emergency medical services.

Introduction

Microbiological contamination refers to the non-intended or accidental introduction of infectious material, such as bacteria, yeast, mould, fungi, virus, prions, protozoa, or their toxins and by-products [1,2]. As a major human pathogen, Staphylococcus aureus has already been associated with a variety of clinical infections. Furthermore, a genomic mutation in S. aureus led to resistance to methicillin and other antibiotics. Infection by methicillin-resistant S. aureus (MRSA) takes place commonly in the skin and subcutaneous tissues, causing necrotizing fasciitis, cellulitis, and diabetic foot ulcers. MRSA has been also associated with osteomyelitis, septic arthritis, community-acquired necrotizing pneumonia, and hospital-acquired ventilator-associated pneumonia. The infection by MRSA can cause death when the bacterial infection enters the bloodstream and/or causes bacterial endocarditis, particularly among hospitalized patients with unfavorable responses to vancomycin treatment [3].

Resistant staphylococcal strains were first reported in hospitalized patients in large acute care hospitals and fall under the umbrella of hospital-acquired MRSA (HA-MRSA). Additionally, community-acquired MRSA strains have emerged with distinct characteristics from those of HA-MRSA in terms of clinical features, antibiotic susceptibility, and molecular biology [4,5]. Approximately 1% to 2% of individuals can carry MRSA in their noses and skins without experiencing apparent clinical symptoms. Indeed, the epidemiological patterns of MRSA show a notable variation globally as well as within specified regions or countries. For instance, the estimated number of invasive MRSA cases in the United

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Received: 12 February 2020 | Accepted: 27 April 2020



States (US) recently decreased by 31% during the period between 2005 and 2011, and this reduction was more marked in hospitalized patients as compared with among healthy individuals residing in the community [6]. In Europe, the prevalence of MRSA was reported as 40% in the United Kingdom, 41% in Ireland, 44% in Greece, and 45% in Italy, respectively, indicating a north to south gradient existed across the continent [7]. In Saudi Arabia, the MRSA prevalence was 2.3% to 47.2% in the Eastern province [8], 43% to 61.7% in the Assir region [9,10], and 38.8% to 53.1% in Makkah [11,12]. As a part of a recent systematic review and meta-analysis, Yousef et al. [13] revealed that the lowest prevalence rate was observed in Dahran and the highest rates were noted in Riyadh (5.97% and 94.59%, respectively).

The knowledge about the epidemiological aspects of MRSA could be further improved by expanding insight about the potential objects that could be contaminated with virulent strains. Actually, MRSA has already been isolated in prior research from a number of medical devices, including thermometer probes, stethoscopes, bed rails, pulse oximeters, and pagers [14]. In addition, ambulances represent a crucial element that facilitates the journey of patients (and, thus, any diseases they may be carrying) from their community to health care facilities and, hence, the risk of contamination during or at the end of the trip may be increased. Furthermore, several factors can increase the risk of the compromise of paramedical staff and patients, such as acquiring infections from undiagnosed patients, airspace limitations, high patient turnover, and the limited time available for adequate cleaning and disinfection of the ambulance before returning to duty.

However, despite the above, no data is currently available about the role of ambulances as reservoirs for MRSA in Saudi Arabia. Given that approximately 93% of MRSA isolates in Saudi Arabia are caused by a single clone [15], it is plausible that several environmental and host-related factors play major roles in the prevalence of this strain. For the purpose of the research, the continuous surveillance of MRSA and other infections in the community resembles a starting point in prevention and protection efforts aimed against nosocomial infections.

In this context, we sought to investigate the prevalence of MRSA in a representative sample of the ambulance fleet in Jeddah, Saudi Arabia to further reveal the risk factors for MRSA infection and inform future preventive plans accordingly.

Methods

A cross-sectional study was conducted involving ambulances from across both government and private settings in Jeddah, Saudi Arabia during the period between September 2018 and December 2018. Ethical approvals were obtained from the Unit of Biomedical Ethics at King Abdulaziz University, Ministry of Health, Saudi Red Crescent Authority (SRCA) and all tertiary private hospitals in the city.

The surveyed communities ranged from rural areas to crowded regions with populations of more than 10,000 people. All I–IV type ambulances operating in Jeddah, with the following structures or characteristics were included: a rear ambulance door in the back cabin, stretcher grip, blood pressure cuff, on/off button for the blood pressure measurement system, and an oximeter device. Ambulance vehicles other than I–IV types, those missing any of the above five areas, those under maintenance, and those from services that refused to participate were excluded from this study.

Sampling sites were swabbed using swabs moistened with sterile normal saline (0.9% NaCl). Blinding for the swab sites, and the healthcare sector operating the ambulance, was done at the level of the Microbiology team, the data processor, the statistician, and the manuscript writer. All swabs were taken and transported by a single collector. More specifically, five selected sites were swabbed as follows: (1) the inner side of the grip of the right rear door in the back cabin, swabbed in a vertical position with rolling and up/down movements; (2) the inner side of the bottom stretcher grip that raises up the stretcher, swabbed in a vertical position with rolling and right-to-left movements: (3) the inner side of the blood pressure cuff, swabbed in a horizontal position all over the surface area that touches the patient arm with rolling and zigzag-like movements; (4) the on/off button of the blood pressure cuff, swabbed in a horizontal position with rolling and circular movements all over the button; and (5) the two inner sides of the oximeter device, swabbed in a horizontal position all over the inner surface with rolling and forward/backward movements (Figure 1). These sites were selected based on literature and after consensus between the researchers.

All samples were transported to the Microbiology Laboratory Department, at King Abdulaziz University Hospital, Jeddah, to be processed within a maximum 2

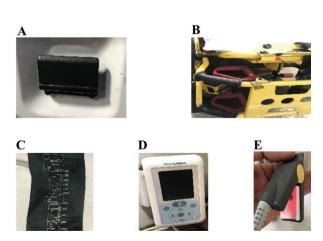


Figure 1. The sampling sites selected for swabbing within the ambulance. (A) The grip of the right rear door in the back cabin, (B) the bottom stretcher grip, (C) the inner side of the blood pressure cuff, (D) the on/off button of the blood pressure cuff, and (E) the inner sides of the oximeter.

hours after collection. The obtained samples were streaked onto plates containing mannitol salt agar, which has been shown to be highly sensitive and selective for MRSA [16] due to the fermentation of mannitol by *S. aureus*. The samples were subsequently incubated aerobically for 96 hours at 37°C. Single circular yellow colonies were then subcultured into oxacillin-supplemented blood agar and incubated at 37°C for 24 hours. *Staphylococcus aureus* was identified by Gram stain, catalase test, and slide coagulase test from single colonies grown on the blood agar. The presence of MRSA was then evaluated by oxacillin supplementation along with incubation for 24 hours. The persistence of the colonial growth indicated an MRSA-positive isolate.

Statistical analysis was performed using the Statistical Package for the Social Sciences version 21.0 for Windows software program (IBM Corp., Armonk, NY). Categorical variables are presented as frequencies and percentages. A chi-squared test was used to investigate statistical differences in MRSA frequency according to the type of the managing hospital—categorized as either managed by the government, the SRCA, or privately—and sampling sites within a given vehicle. A *p*-value of less than 0.05 was deemed to be statistically significant.

Results

A total of 135 ambulances were initially selected to be tested for MRSA contamination. Eighty-five (63.0%) vehicles met the inclusion criteria and were subsequently investigated further. Of the 85 included ambulances, 39 (45.9%) were managed by the government, 32 (37.5%) were managed by the SRCA, and 14 (16.5%) were managed by private hospitals. The total number of collected swabs was 425, with 338 (79.5%) of them vielding bacterial growths. Nevertheless, no MRSA was isolated from any of these 338 samples. The highest contamination rate, regardless of the swab site, was observed in ambulances managed by the SRCA (95%), followed by those managed by government (80%), while the ambulances of private hospitals presented the lowest contamination rate (42.9%), with the difference ultimately being statistically significant (p < 0.001) (Table 1).

The analysis of microbial growth by hospital type as stratified by swab site showed highest contamination rates (90.6%–100.0% depending on swab site) were present in SRCA-operated ambulances, which was higher than rates observed in either government operated (71.8%–84.6%) or private hospital operated (35.7%–42.9%) ambulances (Table 2 and Figure 2).

However, no statistically significant difference was found among the various contamination rates of different swab sites in government (p = 0.455), SRCA (p = 0.315), or private (p = 0.965) ambulances (Table 3).

Discussion

Improving the knowledge about the microbial status of ambulances represents a first key step in better controlling or blocking the infection transmission process between the community and health care settings. The primary objective of this study was to investigate the hypothesis that ambulances represent an important reservoir of MRSA and/or other microorganisms and to assess whether the bacterial load in government ambulances differ from those in ambulances in the private or SRCA sectors by obtaining microbial swabs from different components of a representative sample of vehicles. Ultimately, we determined that the presently implemented infection control measures are appropriate given the observation of a zero frequency of MRSA isolates among all collected samples. However, other bacterial growths were detected in 79.5% of the samples and their frequencies were significantly higher in the ambulances of government and SRCA hospitals than private institutions.

Our finding of an absence of MRSA contamination was in agreement with the outcomes of another recent Saudi study conducted in Riyadh that investigated 10 ambulances operated by the SRCA [17]. Following on the review of three areas with possible high microbial loads, including the interior handle of the rear door, oxygen flow meter knob, and the stretcher handle, the authors found no growth of MRSA although other skin flora and environmental microorganisms were successfully grown

. Growth of MRSA and other microorganisms by hospital type and ambuid	iunce site.

Parameter	Category	MRSA growth			Growth of other microorganisms		
		Freq.	%	p-value	Freq.	%	<i>p</i> -value
Hospital type	Government	0	0.0	-	156	80.0	< 0.001*
	Private	0	0.0		30	42.9	
	SRCA	0	0.0		152	95.0	
Swab site*	1	0	0.0	-	67	78.8	0.663
	2	0	0.0		70	82.4	
	3	0	0.0		70	82.4	
	4	0	0.0		68	80.0	
	5	0	0.0		63	74.1	

^{*}Swab sites: 1, inner side of the grip of the right rear; 2, inner side of the bottom stretcher grip; 3, inner side of the blood pressure cuff; 4, on/off button of the blood pressure cuff; 5, two inner sides of the oximeter device.

Court aits	Hospital	.,	Contamination rate			
Swab site		N	Freq.	%	<i>p</i> -value	
Inner side of the grip of the right rear	Government	39	30	76.9		
	Private	14	6	42.9	<0.001*	
	SRCA	32	31	96.9		
Inner side of the bottom stretcher grip	Government	39	33	84.6		
	Private	14	6	42.9	<0.001*	
	SRCA	32	31	96.9		
Inner side of the blood pressure cuff	Government	39	31	79.5	<0.001*	
	Private	14	7	50.0		
	SRCA	32	32	100.0		
	Government	39	34	87.2	<0.001*	
On/off button of the blood pressure cuff	Private	14	5	35.7		
	SRCA	32	29	90.6		
	Government	39	28	71.8		
Two inner sides of the oximeter device	Private	14	6	42.9	0.003*	
	SRCA	32	29	90.6		
	Government	39	156	80.0	< 0.001*	
Total	Private	14	30	42.9		
	SRCA	32	152	95.0		

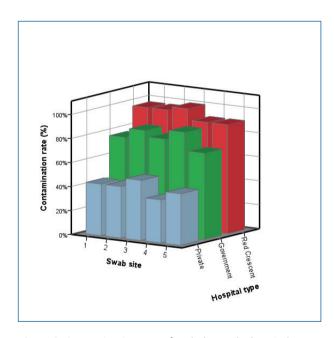


Figure 2. Contamination rate of ambulances by hospital type as stratified by swab site. Bars represent the percentage of swabs with positive microbial growth according to site and type of hospital. Swab sites: 1, inner side of the grip of the right rear; 2, inner side of the bottom stretcher grip; 3, inner side of the blood pressure cuff; 4, on/off button of the blood pressure cuff; 5, two inner sides of the oximeter device.

and appeared susceptible to the fumigation procedures. In the present study, we supported the validity of such findings by investigating additional likely (i.e., due to heavy use) sites of potential microbial contamination and

a greater number of ambulances in the Western region of the Kingdom. In other reports in the literature, MRSA and other *Staphylococcus* strains have been isolated from the stretcher mattress [18–20], stretcher handrail [18,19,21], door handle [22,23], preparation area [18,19,21], steering wheel [24,25], and oxygen flow apparatus [19,21,22]. Other evidence indicated that the frequently touched areas on medical devices, such as intravenous equipment [21,24], cardiac monitors [19,21], and blood pressure cuffs could act as reservoirs for MRSA.

Interestingly, some studies from other Asian and European countries have reported no MRSA isolates were located in ambulances following investigation, including in 117 vehicles in Korea [23], 30 vehicles in Thailand[20], and 50 samples in Denmark [26] although other microbial contaminants such as *Enterococcus* and methicillin-susceptible *S. aureus* were observed. Notably, the bacterial contamination was higher after the ambulance was in service for certain period of time as compared with at the start of service, and this was especially true regarding the contamination of medical instrument surfaces.

Nonetheless, in the literature, MRSA contamination has been consistently reported in ambulance fleets in several countries. For example, 10 of 21 ambulances in the emergency medical services fleet in Washington DC, US showed significant rates of MRSA contamination although these results should be interpreted with caution due to limitations in the microbiological methodologies used [18]. Specifically, the authors suggested MRSA as a finding if the cultures showed positive colonial growth on oxacillin agar with 6.5% NaCl, positive mannose

Table 3. Other microorganism growth by ambulance site as stratified by hospital type.

Hospital	Swab site*	N	Contamination rate			
			Freq.	%	p-value	
Government	1	39	30	76.9		
	2	39	33	84.6		
	3	39	31	79.5	0.455	
	4	39	34	87.2		
	5	39	28	71.8		
	1	14	6	42.9		
	2	14	6	42.9		
Private	3	14	7	50.0	0.965	
	4	14	5	35.7		
	5	14	6	42.9		
	1	32	31	96.9	0.315	
	2	32	31	96.9		
SRCA	3	32	32	100.0		
	4	32	29	90.6		
	5	32	29	90.6		
	1	85	67	78.8	0.663	
Total	2	85	70	82.4		
	3	85	70	82.4		
	4	85	68	80.0		
	5	85	63	74.1		

^{*}Swab sites: 1, inner side of the grip of the right rear; 2, inner side of the bottom stretcher grip; 3, inner side of the blood pressure cuff; 4, on/off button of the blood pressure cuff; 5, two inner sides of the oximeter device.

fermentation, and positive catalase testing. Therefore, it is not surprising that MRSA might be overestimated in such a study. In southern Maine, US, approximately 50% of ambulances showed at least one area with MRSA strains, and the frequency of positive isolates presented no significant difference between fire-based and non-fire-based ambulances and was not dependent upon the annual call volume [21]. Also in the US, Rago et al. [24] found that only 12% of total isolates were MRSA.

Given the lower prevalence of MRSA in some European countries like Germany [27] when compared with the US, it is plausible that ambulance contamination rates would be altered accordingly. Eight of 89 German ambulance car cabins (9%) were positive for MRSA based on samples swabbed from the headrests and the handles of the stretcher, indicating the necessity of implementing infection control techniques specifically in areas in the vicinity of patients [28]. A relatively similar percentage (7%) was reported in another German cross-sectional study employing a sample of 150 "ready-for-service" ambulances [19].

As such, the US appears to have a greater burden of MRSA-contaminated ambulances when compared with Asian and northern European countries. Still, the fact that microbial contamination is restricted to the patient-centered equipment indicates an effective use of protective equipment by the ambulance personnel, the continuous monitoring of MRSA-infected patients, and

the implementation of specialized cleaning of ambulance vehicles following the transportation of patients in countries with a low burden [28]. For example, Alrazeeni et al. [17] revealed that nine out of 10 ambulances studied yielded no microbial growth after fumigation with 6% hydrogen peroxide at the site of the oxygen knob and the stretcher, while six ambulances presented without contamination at the interior handle of the rear. On the other hand, 6 to 10 ambulances were contaminated with a mixed growth of bacteria in these three locations before fumigation. Similarly, laundering was associated with a significant reduction of vegetative pathogenic bacterial contamination on the uniforms of ambulance staff, yet the degree of *Clostridium difficile* contamination was not remarkably reduced [29].

The results presented in this study should be carefully considered while implementing infection control measures in pre-hospital settings. Ambulances represent an accelerating setting subjected to heavy use throughout the day and night, with rapid patient turnover rates. Ambulance personnel are typically thrust into a variety of conditions and environments, including homes, nursing homes, highways, and agricultural environments that makes maintaining pathogen-free vehicles a real challenge. Nonetheless, it seems that the regular cleaning of the equipment and fumigation of the ambulance air are effective means for reducing the potential of bacterial contamination and colonization at least at the national level.

On the other hand, other bacterial growths were detected in significantly higher rates in the ambulances of SRCA and governmental hospitals than private institutions. These differences may be related to higher usage frequency in SRCA and governmental ambulances, compared with private hospitals' ambulances, or to differences in users' sociodemographic factors. However, being out of the scope of this study, such factors were not explored and may be subject to another study to determine the factors and the health implications of such contaminations.

Therefore, standard procedures for the cleaning, decontamination, and disinfection of ambulances and their equipment should be continued not only to hamper the chances of MRSA transmission but also to reduce the overall load of other types of bacteria. Specified time-tabled cleaning schedules are ideal to be followed by the relevant infection control members alongside the appropriate use of chemical disinfectants. In addition, patients' relatives should be asked to avoid nonessential contact inside the ambulances to decrease airborne and shed bacterial concentrations [30,31]. Finally, adherence to infection control guidelines should be emphasized in government settings to resolve the variable patterns of bacterial contamination between the government and private sectors.

The present study is not without limitations. The results were limited to a distinct geographical region and, hence, the outcomes cannot be further generalized to other areas in Saudi Arabia or to other areas of the world. More comprehensive studies that involve multiple regions could better reveal a map of MRSA contamination according to a specific geographical distribution. The cross-sectional design may also have had an impact on our ability to identify possible risk factors of increased colonization of other bacteria and the discrepancy in their distribution between the private and government vehicles. Furthermore, we did not examine the used interventional cleaning procedures or the levels of crew adherence to disinfection protocols to offer suitable recommendations to other sectors accordingly. Finally, conducting prospective studies concerned with bacterial contamination in the emergency medical service might reveal a time-dependent existence or seasonal variation in MRSA prevalence.

Conclusion

In consideration of the deficient level of international evidence regarding pre-hospital hygiene and the levels of bacterial contamination and colonization, we pursued the identification of contamination levels of ambulance vehicles with MRSA in a cross-sectional study in Jeddah, Saudi Arabia. The results revealed the use of efficient infection control programs as indicated by a lack of MRSA growth in all isolates. However, the finding of other types of bacterial contamination underlines the importance of maintaining adherence to the specified cleaning guidelines, particularly in ambulances managed

by the Saudi government and the SRCA. Conducting more comprehensive studies that encompass multiple Saudi regions, investigating the potential leading risk factors of increased contamination, and evaluating the used sanitation procedures are warranted.

Acknowledgment

The authors would like to express gratitude to Mr. Hany Y. M. Abdullah for his important role in the success of this research

List of Abbreviations

CA-MRSA Community acquired-MRSA
EMS Emergency medical services
HA-MRSA Hospital acquired-MRSA
MRSA Methicillin-resistant *S. Aureus*SRCA Saudi Red Crescent Authority

Conflict of interest

The authors declared that they have no conflict of interest.

Funding

None.

Consent for publication

Not applicable.

Ethical approval

Ethics approval was sought from the three concerned authorities:

Ethical approval was granted by the Unit of Biomedical Ethics and Research Committee of King Abdulaziz University, Jeddah, Saudi Arabia. (Reference No: 588-19).

Ethical approval was also granted by the Unit of Research and Studies Administration of Directorate of Health Affairs, Ministry of Health, Jeddah, Saudi Arabia. (Study No: 00943) (Reference No: A00609)

Ethical approval was also granted by Director of the Department ambulance of Saudi Red Crescent Authority, Jeddah, Saudi Arabia (Reference No: 17/D).

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