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ORIGINAL RESEARCH

Ambulance vehicles as a source of multidrug-resistant infections: a multicenter study in Assiut City, Egypt

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Background: Ambulances may represent a potential source of infection to patients, patients' relatives, and paramedical staffs. In this study, we analyzed the extent of bacterial contamination in ambulance vehicles and measured the degree of antimicrobial resistance among isolated pathogens.

Materials and methods: Twenty-five vehicles were included and 16 sampling points were swabbed in each vehicle. Then the swabs were immediately transferred to the laboratory to identify bacterial contaminants utilizing standard microbiological procedures and API® systems. Antibiotic susceptibility testing and screening for methicillin-resistant staphylococci and extended spectrum β-lactamases (ESBLs)-producing Gram-negative rods were carried out.

Results: A total of 400 samples were collected, 589 bacteria were isolated and 286 (48.6%) of the isolates were potentially pathogenic. The highest contamination rate with pathogenic bacteria was detected in suction devices (75.8%) and stethoscopes (67.7%). Staphylococci were the most frequently detected microorganisms (n=184) followed by *Klebsiella* spp. (49), *Escherichia coli* (40), *Citrobacter* spp. (7), and *Proteus* spp. (6). Staphylococci were mostly sensitive to vancomycin, whereas Gram-negative bacteria were sensitive to imipenem. Overall, 46.1% of *Staphylococcus aureus* were methicillin resistant, whereas 20.4% of the coagulase-negative staphylococci were methicillin resistant. Moreover, 36.7% of *Klebsiella* spp. and 27.5% of *E. coli* were ESBL producers.

Conclusion: Our study provides evidence that ambulances represent a source of prehospital multidrug-resistant infections.

Keywords: ambulance, contamination, resistance, methicillin-resistant *Staphylococcus aureus*, extended spectrum β-lactamases

Introduction

Ambulance vehicles are an integral part of emergency medical services. Emergency ambulances aim to help critically ill patients and to prevent the development of life-threatening complications in seriously injured cases.[1] Ambulances can respond to thousands of cases per year and after each mission, each ambulance vehicle must be cleaned and decontaminated to be ready for use for the next mission. Effective cleaning protocols for this emergency medical environment are essential in order to avoid the presence of pathogenic microorganisms that represent a potential risk of infection for patients or even the accompanying emergency medical professionals. Although different infection control procedures are employed, ambulances remain a potential source of infection to patients, relatives of the patients, and paramedical staff during transport to a health care facility, or during an interfacility transfer.[1,2] Recent research has demonstrated that ambulance

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surfaces that are directly surrounding patients are frequently contaminated with microorganisms.[1] Moreover, Eibicht and Vogel reported that ambulance cars were contaminated with methicillin-resistant *Staphylococcus aureus* (MRSA) after even a short transport of MRSA-colonized patients.[3] Multidrug-resistant bacteria as MRSA are common pathogens isolated from hospitalized patients as well as outpatient settings. Nowadays, the spread of some multiresistant clones in the health care settings and the emergence of community-acquired MRSA are causes of major concern for public health specialists and clinicians.[4,5]

Since patients transported on rescue vehicles are generally critically ill, great care should be taken to avoid the presence of contaminating bacteria that are resistant to antibiotics and therefore difficult to treat such as MRSA or the extended spectrum β-lactamase (ESBL)-producing Gram-negative rods. Of great concern is the fact that previous studies have reported that MRSA colonization was detected among emergency services personnel.[6]

Microorganisms were detected not only in the interior of ambulances but also on the emergency services equipment. Some reports showed that medical devices such as stethoscopes showed a high rate of MRSA contamination.[7] Other studies by Brown et al[8] and Roline et al[9] showed that around half of samples from emergency ambulances tested positive for MRSA contamination. Therefore, microbiological evaluation of ambulance vehicles is an essential infection control step that must be considered in order to develop valuable risk reduction interventions.

To date, there has been no data about contamination of ambulance vehicles in Egypt. Therefore, the goal of this observational, cross-sectional study was to determine the extent of bacterial contamination in different ambulance vehicles in Assiut city, Egypt and to describe the degree of antimicrobial resistance for the isolated pathogens.

Materials and methods

In our study, we aimed to determine the extent of bacterial contamination in different ambulance vehicles in Assiut city and Assiut University hospitals in Egypt and to describe the degree of antimicrobial resistance for the isolated pathogens. This cross-sectional study was carried out in the period from January to June 2016. The investigations were carried out with the full cooperation of the Ministry of Health and University hospital officers.

Sampling process

A total of 25 rescue stations throughout Assiut city were included in the study. Unannounced visits were performed. The ambulance crews had no advance knowledge about the visits. Sixteen sampling points were selected based on their well-known high frequency of contact by emergency personnel and patients. These areas were as follows: carrying handle of cart; sidebar of cart; DC shock apparatus; stethoscope; cervical collar; inside wall of the vehicle; emergency personnel seats; portable ventilator; blood pressure cuff; monitor; door grip; oxygen humidifier glass; suction device; headboard of patient stretcher; and steering wheel.

Ambulance car surfaces were swabbed with cotton wool swabs moistened with sterile 0.9% NaCl solution that has been shown to be superior in recovering bacteria from ambulance surfaces to sponges and dry cotton wool swabs.[3] During the sampling process, swabs were passed over the entire sampling point and rotated to collect as much material as possible. Swabs were inserted into sterile tubes containing normal saline to avoid desiccation during transport.

Microbiological analysis and identification of contaminating microorganisms

Upon arrival at the microbiology laboratory, swabs were immediately transferred into brain heart infusion broth (Becton Dickinson GmbH, Heidelberg, Germany) and incubated for 20–24 h at 37°C (5% CO_2). Thereafter, the enriched cultures were streaked onto microbial culture plates of blood agar, mannitol salt agar, MacConkey's agar, and eosin methylene blue agar to be identified utilizing standard microbiological procedures. All positive cultures were stained by Gram stain for preliminary identification of Gram-positive and Gram-negative microorganisms. Catalase and coagulase production were used to classify Gram-positive cocci. Staphylococci were identified by standard protocols including the colony morphology, Gram staining, catalase test, and tube coagulase test.[10] The API® system (API® 20E and API® 20NE, bioMérieux, Marcy l'Etoile, France) were used to further identify Gram-negative organisms.

After isolation of the contaminating bacteria, antibiotic sensitivity for 15 antibiotics (ampicillin, amoxicillin/clavulanic acid, ceftriaxone, cefotaxime, ceftazidime, cefpodoxime, imipenem, amikacin, gentamicin, tetracycline, ciprofloxacin, norfloxacin, chloramphenicol, trimethoprim/sulfamethoxazole, and vancomycin) was tested by the disc diffusion method on Thermo Scientific™ Oxoid™ Mueller-Hinton agar (Thermo Fisher Scientific, Waltham, MA, USA) using the modified Kirby–Bauer disk-susceptibility method.[11] Only Staphylococci were tested for vancomycin sensitivity. Inhibition zone diameters were measured and results

were interpreted according to the susceptibility breakpoints recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines.[12]

In addition, standard microbiological techniques were employed to rapidly screen for methicillin resistance in staphylococci and ESBL-producing Gram-negative rods. These organisms were selected because these pathogens have well-known robust antibiotic resistance profiles and they can cause serious life-threatening nosocomial infections.

Screening and confirmatory tests for ESBL production in Gram-negative rods

Testing for ESBL production is considered for all Gram-negative rods according to the recommendations of the CLSI guidelines.[12] Initially, isolates were screened for ESBL production by detecting microorganisms that exhibited reduced susceptibility to one or more of cefpodoxoime, ceftazidime, ceftriaxone, and cefotaxime. These bacteria were considered as potential producers of ESBL. Confirmation of ESBL production was performed phenotypically by the combined disc synergy testing between ceftazidime versus ceftazidime-clavulanate and cefotaxime versus cefotaxime-clavulanate where a ≥5 mm increase in the zone diameter for the antimicrobial agent tested in combination with β-lactamase inhibitor versus its zone when tested alone indicates ESBL production. In addition, minimal inhibitory concentration (MIC) values for ceftazidime and cefotaxime in the presence of clavulanic acid are reduced by ≥3 two-fold dilutions as tested by the Etest® (bioMérieux, Marcy l'Etoile, France).[13]

Screening for methicillin resistance among staphylococcal isolates

For the detection of methicillin resistance among staphylococci, suspected colonies on mannitol salt agar were subcultured on oxacillin resistance screening agar base (Oxoid™ Thermo Fisher Scientific, Waltham, MA, USA) that contains 6 µg/mL oxacillin.[14,15] For confirmation of methicillin resistance, MRSA-screen latex agglutination test was carried out. This test was used for the detection of penicillin-binding protein 2a (PBP2a), and based on agglutination of latex particles sensitized with monocloclonal antibodies against PBP2a in accordance with the manufacturer's protocol (Denka Seiken Co, Niigata, Japan).[16] In addition, MIC values for cefoxitine were tested using the commercially available cefoxitine Etest. MIC values >4 µg/mL were considered positive for *mecA*-mediated resistance.[12]

Results

Analysis of microbial samples

In our study, a total number of 25 ambulance cars were screened for bacterial contamination without the crews' advance knowledge. Samples were taken from 16 sites within each vehicle. In total, 400 samples were collected and examined. Remarkably, all sites within ambulance vehicles showed bacterial growth. A total of 589 bacteria were isolated. About half of the isolates 286 (48.6%) were potentially pathogenic organisms as shown in Table 1. When comparing different areas within each vehicle, it was clear that suction devices (75.8%) and stethoscopes (67.7%) showed the highest pathogenic ratios, whereas the lowest contamination rates with pathogenic bacteria were detected in steering wheels and DC shock apparatus.

Isolated microorganisms according to site of collection

With respect to the isolated bacteria, Staphylococci (184) were the most frequently detected microorganisms from the collection sites followed by *Klebsiella pneumoniae* (49) and *Escherichia coli* (40). Only a few sites were contaminated with *Citrobacter* spp. (7) and *Proteus* spp. (6). In addition, all specimens showed contamination with non pathogenic contaminants such as *Bacillus* spp., *Corynebacterium* spp., and *Micrococcus* spp. Of great concern is the fact that the headboard of the patient stretcher showed the highest yield of staphylococcal contamination (20). On the other hand, most of the *K. pneumoniae* and *E. coli* isolates were detected in suction devices (14) and beds (11), respectively (Table 2). Of the 184 isolated staphylococci, 76 (41.3%) were identified as *S. aureus* and 108 (58.7%) were coagulase-negative staphylococci (CNS).

Rate of microbial resistance

Antibiotic sensitivities of isolated organisms showed that the isolated staphylococci were mostly sensitive to vancomycin (100%), *Klebsiella* spp. and *E. coli* were sensitive to imipenem (95.9% and 92.5%, respectively); however, *Proteus* spp. and *Citrobacter* spp. were the least resistant organisms as all strains were sensitive to the third-generation cephalosporins, cefotaxime, ceftriaxone, ceftazidime, and cefpodoxime in addition to imipenem and amikacin antibiotics (Table 3).

Further testing of staphylococci for methicillin resistance showed that about half of *S. aureus* isolates (35/76, 46.1%) were MRSA, whereas 22/108 (20.4%) of the CNS were methicillin-resistant coagulase-negative staphylococci

Table 1 Bacterial contamination of different sites in ambulance vehicles

Site	Potential pathogenic bacteria n (%)	Non pathogenic bacteria n (%)	Total number of isolated bacteria per site
Carrying handle of cart	21 (63.6)	12 (36.4)	33
Sidebar of cart	14 (48.3)	15 (51.7)	29
Bed	32 (54.2)	27 (45.8)	59
DC shock apparatus	2 (8.3)	22 (91.7)	24
Stethoscope	21 (67.7)	10 (32.3)	31
Cervical collar	22 (59.5)	15 (40.5)	37
Inside wall of the vehicle	14 (43.8)	18 (56.3)	32
Chair	17 (42.5)	23 (57.5)	40
Portable ventilator	29 (53.7)	25 (46.3)	54
Blood pressure cuff	8 (24.2)	25 (75.8)	33
Monitor	13 (34.2)	25 (65.8)	38
Door grip	15 (40.5)	22 (59.5)	37
Oxygen humidifier glass	27 (61.4)	17 (38.6)	44
Suction device	25 (75.8)	8 (24.2)	33
Headboard of patient stretcher	25 (62.5)	15 (37.5)	40
Steering wheel	1 (4.0)	24 (96.0)	25
Sum	286 (48.6)	303 (51.4)	589

Table 2 Frequency of different isolated microorganisms in each site of the vehicles

Site	Potentially pathogenic bacteria					Non pathogenic bacteria		
	Staphylococcus spp.	*Klebsiella pneumoniae*	*Escherichia coli*	*Proteus* spp.	*Citrobacter* spp.	*Corynebacterium* spp.	*Bacillus* spp.	*Micrococcus* spp.
Carrying handle of cart	14	2	5	0	0	5	4	3
Sidebar of cart	14	0	0	0	0	0	15	0
Bed	12	7	11	2	0	2	25	0
DC shock apparatus	2	0	0	0	0	7	9	6
Stethoscope	18	0	0	0	3	0	10	0
Cervical collar	17	0	3	0	2	3	8	4
Inside wall of the vehicle	10	0	2	2	0	0	13	5
Chair	14	1	0	0	2	0	15	8
Portable ventilator	9	9	9	2	0	0	25	0
Blood pressure cuff	8	0	0	0	0	12	13	0
Monitor	13	0	0	0	0	12	13	0
Door grip	15	0	0	0	0	0	17	5
Oxygen humidifier glass	12	11	4	0	0	5	12	0
Suction device	5	14	6	0	0	0	8	0
Headboard of patient stretcher	20	5	0	0	0	5	10	0
Steering wheel	1	0	0	0	0	0	24	0
Sum	184/286 (64.3%)	49/286 (17.1%)	40/286 (13.9%)	6/286 (2.1%)	7/286 (2.5%)	51/303 (16.8%)	221/303 (73%)	31/303 (10.2%)

(MRCNS). All sites were contaminated with either MRSA or MRCNS except for the DC shock apparatus and steering wheels.

This high level of methicillin resistance among staphylococci diverted our attention to further test the isolated Gram-negative rods for production of the ESBL enzyme. A total of 18/49 (36.7%) of *Klebsiella* spp. and 11/40 (27.5%) of *E. coli* were ESBL producers. However, no ESBL-producing organisms were detected among *Proteus* spp. and *Citrobacter* spp.

When comparing the frequency of MRSA, MRCNS, and ESBLs among the different sites of vehicles, there were no pronounced trends in their distribution (Figure 1). However when taken together, portable ventilators and beds had higher counts, whereas DC shock apparatuses and steering wheels had neither of them.

Table 3 Antibiogram for the microorganisms isolated from different sites in ambulances

Antibiotic		*Staphylococcus* spp.		*Klebsiella pneumoniae*		*Escherichia coli*		*Proteus* spp.		*Citrobacter* spp.	
		n=184	%	n=49	%	n=40	%	n=6	%	n=7	%
Ampicillin	s	40	21.7	4	8.2	5	12.5	2	33.3	3	42.9
	r	144	78.3	45	91.8	35	87.5	4	66.7	4	57.1
Amoxicillin/clavulanic acid	s	66	35.9	7	14.3	7	17.5	4	66.7	5	71.4
	r	118	64.1	42	85.7	33	82.5	2	33.3	2	28.6
Ceftriaxone	s	155	84.2	25	51.0	19	47.5	6	100	7	100
	r	29	15.8	24	49.0	21	52.5	0	0.0	0	0.0
cefotaxime	s	160	87.0	23	46.9	25	62.5	6	100	7	100
	r	79	42.9	26	53.1	15	37.5	0	0.0	0	0.0
Ceftazidime	s	113	61.4	23	46.9	23	57.5	6	100	7	100
	r	71	38.6	26	53.1	17	42.5	0	0.0	0	0.0
Cefpodoxime	s	99	53.8	18	36.7	23	57.5	6	100	7	100
	r	85	46.2	31	63.3	17	42.5	0	0.0	0	0.0
Imipenem	s	150	81.5	47	95.9	37	92.5	6	100	7	100
	r	34	18.5	2	4.1	3	7.5	0	0.0	0	0.0
Amikacin	s	110	59.8	33	67.3	35	87.5	6	100	7	100
	r	74	40.2	16	32.7	5	12.5	0	0.0	0	0.0
Gentamicin	s	100	54.3	20	40.8	13	32.5	5	83.3	3	42.9
	r	84	45.7	29	59.2	27	67.5	1	16.7	4	57.1
Tetracycline	s	40	21.7	5	10.2	4	10.0	1	16.7	1	14.3
	r	144	78.3	44	89.8	36	90.0	5	83.3	6	85.7
Ciprofloxacin	s	50	27.2	30	61.2	10	25.0	4	66.7	5	71.4
	r	134	72.8	19	38.8	30	75.0	2	33.3	2	28.6
Norfloxacin	s	55	29.9	33	67.3	15	37.5	5	83.3	4	57.1
	r	129	70.1	16	32.7	25	62.5	1	16.7	3	42.9
Chloramphenicol	s	14	7.6	5	10.2	4	10.0	5	83.3	5	71.4
	r	170	92.4	44	89.8	36	90.0	1	16.7	2	28.6
Trimethoprim/sulfamethoxazole	s	23	12.5	9	18.4	6	15.0	4	66.7	6	85.7
	r	161	87.5	40	81.6	34	85.0	2	33.3	1	14.3
Vancomycin	s	184	100	na	na	na	na	na	na	na	na
	r	0	0.0	na	na	na	na	na	na	na	na

Abbreviations: s, sensitive; r, resistant; na, not applicable.

Discussion

This study is the first work to describe the level of bacterial contamination in ambulance vehicles in Assiut city in Egypt. There seems to be a failure in effective cleaning and disinfection of the vehicles, demonstrating a possible lack of consistency in frequency and/or method of cleaning.

Bacterial contamination of inanimate surfaces and equipment in the patient zone and health care area is a major health problem worldwide as it may play a role in cross-transmission of pathogens that may lead to infection or colonization. This colonization may generate a reservoir of potential multidrug-resistant pathogens.[2] Biofilm formation is one of the principle factors that enable bacteria to survive on inanimate surfaces and equipment. Bacterial biofilm may reduce the efficacy of terminal cleaning procedures as it enables bacteria to resist disinfectants and survive in the environment for a long time.[17]

A total of 25 ambulance vehicles were examined, 400 swabs were cultured, and 286 potential pathogenic organisms were isolated. Sites where the highest frequency of pathogenic bacteria were detected included beds, portable ventilators, oxygen humidifier glass, suction devices, headboards of patient stretchers, cervical collars, stethoscopes, and the carrying handles of carts. The presence of pathogenic organisms in the equipment and sites was expected because they are usually touched with the hands and come frequently in contact with patients or their body secretions such as pus, blood, and sweat. The relatively lower frequency of pathogenic bacteria detected on DC shock apparatus and steering wheels is probably due to the infrequent use of them compared with other equipment and the fact that steering wheels does not come in direct contact with patients. Consistent with our results, the SEKURE study that analyzed the

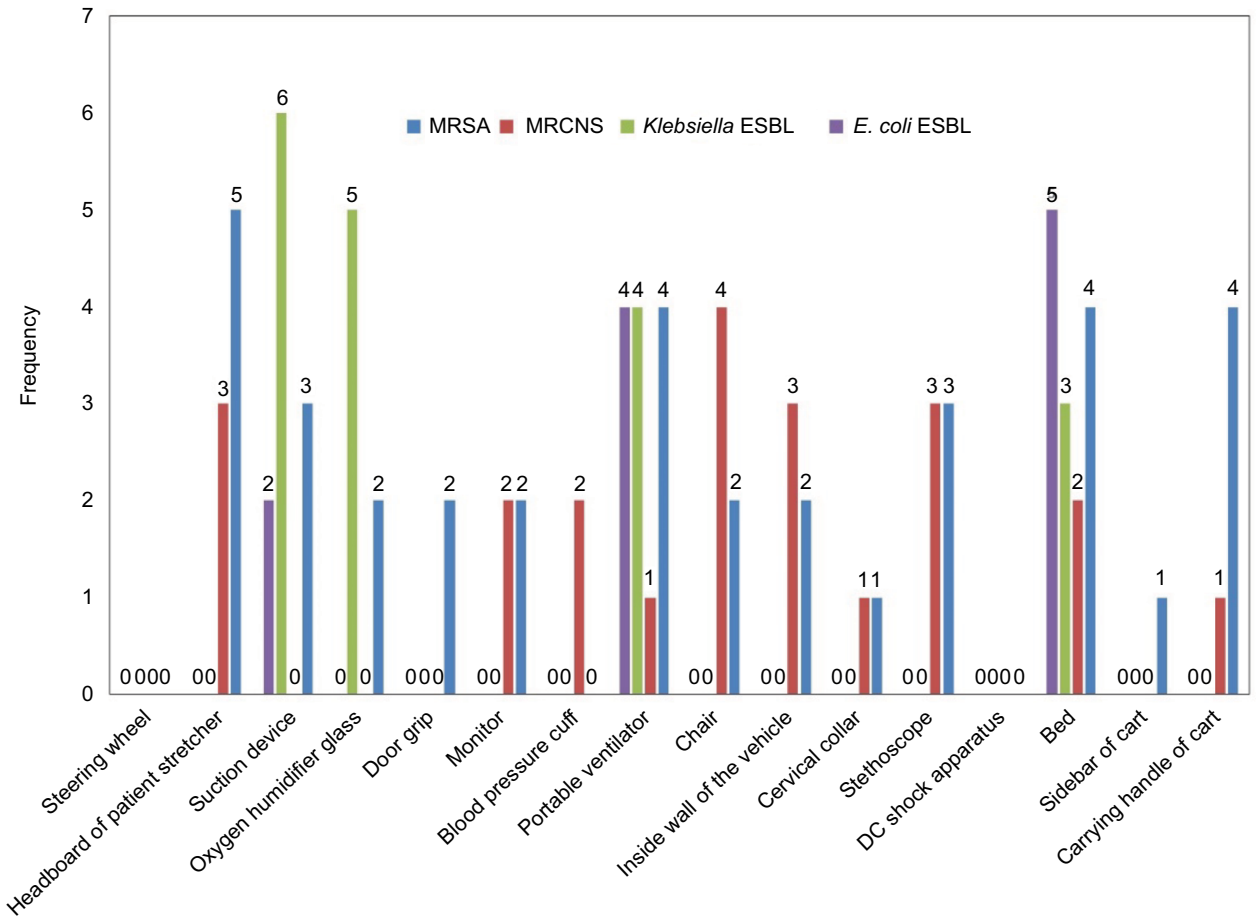

Figure 1 Frequency of methicillin-resistant staphylococci and ESBL-producing *Klebsiella* spp. and *Escherichia coli* in each site of ambulance vehicles.
Note: When comparing the frequency of MRSA, MRCNS, and ESBLs among the different sites of vehicles, there were no pronounced trends in their distribution. However when taken together, portable ventilators and beds had higher counts, whereas DC shock apparatus and steering wheels had neither of them.
Abbreviations: MRSA, methicillin-resistant *Staphylococcus aureus*; MRCNS, methicillin-resistant coagulase-negative staphylococci; ESBL, extended spectrum β-lactamase.

bacterial contamination of German ambulances has reported that contact surfaces directly surrounding patients or staff were most frequently contaminated with microorganisms.[1]

In total, 184 isolates of staphylococci, 49 of *K. pneumoniae*, 40 *E. coli*, 6 *Proteus* spp., and 7 *Citrobacter* spp. were detected. From this abundant distribution of staphylococci and coliforms, it may be inferred that there was no time-tabled organized cleaning schedules. Furthermore, such a high level of pathogenic microorganisms is worrying since these organisms have been implicated in different serious community and nosocomial infections.[18–21] Similar to our findings, other studies have pointed out the widespread microbial populations in ambulances and their role as a potential source of prehospital acquired infections.[1,8,22–26]

The presence of pathogenic organisms in rescue vehicles is also very dangerous, especially when transporting immunocompromised and severely ill patients who are more liable to infections. Moreover, such a high frequency of microorganisms is a potential risk of transmission not only to patients but also to emergency personnel. In addition, patients transported by ambulance may act as a vector and disseminate those disease-causing microorganisms to hospital environments and therefore contribute to the development of health care associated infections.

In the present study, antibiogram for the isolated environmental pathogens was determined. Alarmingly, isolated staphylococci showed high levels of resistance to different antibiotics, such as chloramphenicol (92.4%), trimethoprim/sulfamethoxazole (87.5%), ampicillin (78.3%), and tetracycline (78.3%), while no resistance to vancomycin was observed. A similar high level of resistance was also observed among *Klebsiella* spp. and *E. coli* but not among *Proteus* spp. and *Citrobacter* spp. About 90% of *Klebsiella* spp. and *E. coli* were resistant to ampicillin, tetracycline, and chloramphenicol, and about half of them were resistant to the third-generation cephalosporins while *Proteus* spp. and *Citrobacter* spp. showed only remarkable resistance to ampicillin antibiotic (66.7% and 47.1%, respectively) probably

due to the limited frequency of *Proteus* spp. and *Citrobacter* spp. detected. Detection of such high counts of multidrug-resistant staphylococci, *Klebsiella* spp., and *E. coli* may be explained by the absence of infection control programs that monitor the hygienic measures in our ambulances leading to the consequent existence and spread of these organisms. If these organisms initiate an infection, only limited therapeutic choices will be available.

Since 64.3% of the isolates were identified as *Staphylococcus* spp., the potential for the existence of methicillin resistance was high. Further testing of these colonies detected methicillin resistance not only among *S. aureus* but also among *S. epidermidis* strains. About half of the *S. aureus* were MRSA and 20% of *S. epidermidis* were MRCNS. In addition, our study was the first study that described the prevalence of ESBL among environmental samples from emergency ambulances. ESBL-producing *K. pneumoniae* and *E. coli* were isolated from suction devices and portable ventilators. These multidrug-resistant bacteria may contribute to life-threatening respiratory tract infections if these types of equipment are reused for critically ill patients without being disinfected. No ESBL bacteria were detected among *Proteus* spp. and *Citrobacter* spp., most likely due to the limited number of isolates.

Both methicillin-resistant staphylococci and ESBL colonies were recovered from different sites within the ambulances, with the exception of DC shock apparatus and steering wheels that contained methicillin-sensitive *S. epidermidis* isolates that were probably skin contaminants. Our results showed no relationship between sites inside ambulances and methicillin resistance or ESBLs distribution indicating a possible cross-contamination within the vehicle.

Detection of MRSA, MRCNS, and ESBL raises concerns since these pathogens may cause severe life-threatening complications. It was shown that MRSA was detected in ambulance vehicles immediately after transport of MRSA-colonized or -infected patients.[3] Other studies showed that ambulances have a significant degree of MRSA contamination and may therefore represent an important factor for transmission of serious infections to patients.[9,27] Generally, ESBL and MRSA testing in the ambulance environment is useful for infection control purposes. Documenting these potential hazards may promote the development of best practices to reduce risks of transmission to transported patients.

Interestingly, studies have shown that a high density of bacterial contamination was not only detected in ground emergency ambulances[1,8,9,28–30] but also detected in Emergency Service Helicopters.[31,32]

Based on our observations, it can be concluded that the level of microbial contamination in ambulances within Assiut city in Egypt is not acceptable. It is highly recommended that standard operating procedures of cleaning, decontamination, and disinfection of emergency vehicles and their equipment be implemented. This includes decreasing the contamination rate to the environment by colonized patients and contaminated health care workers by employing regular time-tabled cleaning schedules that are carried out by specialized infection control members, the use of appropriate disinfectants, appropriate cleaning of medical equipment in vehicles, management of water quality introduced into vehicles, avoiding non essential contact of patients' relatives in the ambulance environment to limit concentrations of shed and airborne bacteria.[33,34]

Additional studies are also planned to evaluate and validate the disinfection protocols. Further research should be carried out to analyze the effect of seasonal climate changes on the prevalence of pathogens in ambulances. The reason for that is the well-known effect of temperature and environmental conditions on microbial growth. Our planned future studies will highlight the difference in microbial populations in the summer versus the winter.

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Disclosure

The authors report no conflicts of interest in this work.

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