**ABSTRACT**

The objectives of this study were to evaluate infection control and the incidence of bacterial pathogens in Emergency Medical Service (EMS) ambulances in Riyadh, Saudi Arabia. The effectiveness of fumigation techniques used for these ambulances to minimize the spread of infection to transported patients and pre-hospital care providers was also assessed.

Methods: Based on previous literature review indicating a higher propensity of microbial load, 3 areas within the ambulance, such as, stretcher handle, oxygen flow meter knob, and interior handle of the rear door were selected for specimen collection. Swab samples were collected both in the day and night shift, after the intended disinfection and cleaning (before and after fumigation). Micro-organisms were identified using standard procedures. This phase-I study was conducted at the Emergency Medical Services Department, Prince Sultan Bin Abdulaziz College of Emergency Medical Services, Al Malaz, King Saud University, Riyadh, Saudi Arabia between October and November 2013, wherein a total of 10 ambulances from the Saudi Red Crescent Authority in Riyadh were selected for inclusion in the study.

Results: The specimens from all 10 ambulances showed similar results. In post disinfection and before fumigation, swab samples showed positive cultures that grew moderate to large quantities of environmental and skin flora. However, almost all organisms were susceptible to the fumigation technique.

Conclusion: This study confirms the importance of evaluating the frequency and efficiency of various fumigation techniques as an ambulance is a potential reservoir for microbial transmission to patients and staff.

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Health care associated infections are acquired in a health care setting, and despite the presence of many disinfection methods, microbial contamination remains a significant health concern throughout the world. Ambulances can conceivably be a potential source of different pathogenic microbes by virtue of their role in transporting patients from a scene to a healthcare facility, or during an inter-facility transfer. This creates a scenario wherein not only the patients but also the paramedical staff and relatives of the patients may be exposed to various pathogens, some of which may cause infections and diseases. Although some universal precautions and disposable equipment reduces risk to patients and providers, the ambulance remains vulnerable to bacterial contamination from blood, secretions, and other potential infectious material. Therefore, many different infection control procedures are being employed to prevent ambulances from potentially transmitting, either to medical personnel or patients, or relatives of patients. Studies indicated that infections acquired from ambulances cause a significant financial burden, which is most likely due to the presence of contaminated devices within the ambulances. Infections could thus arise from the home, ambulances, or hospitals. Although many hospital-based infection control programs are being used at present, ambulance disinfection has not been widely stressed upon as an important part of public health administration. The Ambulance Service (Emergency Medical Service [EMS]) in Saudi Arabia is managed by the Saudi Red Crescent Society Authority (SRCSA). By 2009, there were approximately 1,300 ambulances in the SRCSA, and 447 EMS centers run by 5,507 staff in the country. Research conducted regarding pre-hospital infection should not only include assessments of prevalence of microbes from various locations in an ambulance, but also the effect of sterilization, or disinfection techniques in causing a decrease, or removal of various pathogens. In this scenario, it is important to have an evidence-based and cost-effective approach. Such a holistic approach will enable the best control of probable nosocomial infections that may arise from pre-hospital infection due to exposure in ambulances. A regional study examined the levels of bacterial contamination in Welsh ambulances over a 12-month period on a monthly schedule. The results showed a variety of microbes were present in the samples before cleaning of emergency vehicles - most important though is the observation of fresh contamination in ambulances of previously uncontaminated zones in the vehicle due to cleaning methods. Unacceptable levels of microbes have been found re-emphasizing the need for more stringent infection control programs. The current study is based on a hypothesis that the EMS ambulances of the Kingdom of Saudi Arabia (KSA) can carry pathogenic bacteria hazardous both to the paramedical personnel, as well as, patients that are transported within these ambulances to the hospitals. The relation between disinfection and cleaning procedures along with the use, and effect of fumigation of ambulances in order to minimize further spread of infections to patients and paramedical personnel needs to be closely investigated. Therefore, the purpose of the current study is to understand, identify pathogens, and recommend appropriate solutions for a pathogen-free environment in the pre-hospital setting in KSA.

Methods. This was a surveillance-based prospective experimental study conducted in an EMS setting. The current study (Phase 1) is part of a project, which comprised 3 phases in total (1 - identification of microorganisms and efficacy of fumigation technique in ambulances; 2 - identification of effective disinfectant techniques in pre-hospital setting; and 3 - identification of any incidents of nosocomial infections transported through ambulances). In Phase 1, conducted between October to November 2013, 10 busy ambulances operating both in day and night shifts with SRCA in Riyadh region were included.

Selection of sampling sites. A total of 3 areas within the ambulances were selected for specimen collection, that is; stretcher handle, oxygen flowmeter knob, and door handle of the ambulance (Figure 1). These locations were selected based on confirmed previous literature review and experience for their increased propensity to microbial contamination. A sample of an ambulance operating under the SRCA, and model fumigating equipment currently used is presented in Figure 2. The ambulances were swabbed both in the day and night upon readiness to return to service, after all the intended cleaning and decontamination with multi-organism surface disinfectant spray (AZO, Synergy Health plc, Chorley, UK) had been completed.

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Ambulances regularly used these disinfectants for cleaning, however, in case of death or known infectious patient transportation, fumigation is employed. Samples were obtained from the same ambulance, from the same site using the same technique by the same individual before, and after fumigation. This study was blinded and therefore, the Emergency Medical Technicians and the ambulance cleaners were unaware of the study, or the sampling times, and sampling sites. Two microbiology lab technicians were incharge of collecting the samples at the same time (for example, at 6 AM, and 6 PM). The specimens were maintained at an ambient environmental temperature compatible with the interior patient compartment of the ambulance. All collected samples were transported appropriately and cultured for identification on the same day of collection. All samples were coded with a unique identification number to avoid any study bias, and all experiments were conducted by a qualified microbiologist. All samples were computed, and all results were documented before analyses. Factors that may have affected the study results were also taken into account (Table 1). In addition, the effectiveness of the adopted fumigation or disinfection techniques within these ambulances was also explored.

**Data collection and processing.** Sampling was carried out by surface swabbing using soft rayon sterile swabs with thioglycolate fluid (COPAN Italia S.P.A., Brescia, Italy). The samples were cultured using a standard culturing medium (nutrient agar, blood agar plates, and MacConkey agar plates in an Heraeus incubator [Thermo Scientific, Asheville, USA] at 37°C) as per the standard protocols, and then screening and confirmatory testing was performed for identification of specific pathogens.11 We also studied the rate of growth, whether it is minimal or positive. Samples were collected after the disinfection of ambulances (that is, before fumigation), and after fumigation with 6% hydrogen peroxide ($H_2O_2$) for up to 2 hours. All tests were performed by Al Borg Laboratories, Riyadh, KSA.

**Results. Demographics.** A total of 10 busy ambulances operating both in the day and night shift were included in this preliminary study. All ambulances had similar configuration as per the recommendations of Saudi EMS.

**Figure 1** - Sampling sites within the ambulance: A) oxygen knob; B) door handle; and C) stretcher handle.

**Figure 2** - A typical Emergency Medical Service (EMS) ambulance along with fumigation equipment: A) typical ambulance; B) disinfectant spray; and C) humidifier.
Evaluation of ambulance fumigation ... Alrazeeni & Al Sufi

Isolated micro-organisms according to site of collection. Micro-organisms isolated from all the 3 sites of all the ambulances were included in this study. The most common organisms isolated include Bacillus species (sps), coagulase negative Staphylococci, and Enteric bacteria (Tables 2-4). Both Gram negative non-lactose fermenting bacteria and Gram positive bacteria were isolated.

Microbial prevalence before fumigation. A total of 9 ambulances were contaminated before fumigation, but after general cleaning from samples obtained from the oxygen knob (Table 2). No microbial growth from swabs collected from the oxygen knob was found only in one ambulance. At least 8 ambulances were contaminated before fumigation at the stretcher handle. More varieties of organisms were isolated from handles of ambulances (Table 3). A total of 6 ambulances were contaminated with Bacillus sps from the door specimen, with each 3 ambulances being contaminated with coagulase negative Staphylococci, and mixed growth. All ambulances were found to be contaminated at the interior handle of the rear door (Table 4).

Microbial prevalence after fumigation. The fumigation technique used was successful in reducing most of the bacterial contamination. A total of 9 ambulances showed no growth of micro-organisms at the site of the oxygen knob. A similar number of ambulances showed no growth from the specimen collected from the stretcher. A total of 6 ambulances were found to be not contaminated at the interior handle of the rear door of the ambulance. Growth was heavy or moderate in all cultures before fumigation with less growth after fumigation.

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### Table 1 - Areas of sample collection and other factors included in a study in Saudi Arabia.

| Variables                        | Considerations                                      |
|----------------------------------|-----------------------------------------------------|
| Area of obtained sample for culture | Stretcher handle                                   |
| Ambulance                        | Extent and effectiveness of fumigation procedures   |
| Patients                         | Immune function and type of illness, frequency of patient transfer |
| Personnel                        | Health status and procedure compliance             |
| Condition of scene               | Sanitary conditions at the area                     |

### Table 2 - Microbial contamination of the oxygen knob of the ambulance before and after fumigation.

| Ambulance | Before fumigation | After fumigation |
|-----------|-------------------|------------------|
| Day       |                   |                  |
| A         | Bacillus species  | No growth        |
| B         | No growth         | No growth        |
| C         | Bacillus species  | No growth        |
| D         | Bacillus species  | No growth        |
| E         | Mixed growth      | No growth        |
| Night     |                   |                  |
| F         | Bacillus species  | No growth        |
| G         | Bacillus species  | No growth        |
| H         | Bacillus species, coagulase negative staphylococci | Bacillus species |
| I         | Bacillus species  | No growth        |
| J         | Coagulase negative staphylococci | No growth |

Mixed growth - growth of enteric bacteria along with Gram positive bacteria. Growth was heavy or moderate in all cultures before fumigation with less growth after fumigation.

### Table 3 - Microbial contamination on the stretcher handle before and after fumigation.

| Ambulance | Before fumigation | After fumigation |
|-----------|-------------------|------------------|
| Day       |                   |                  |
| A         | Mixed growth      | No growth        |
| B         | Bacillus species  | Bacillus species |
| C         | No growth         | No growth        |
| D         | Gram positive bacteria | No growth |
| E         | Mixed growth      | No growth        |
| Night     |                   |                  |
| F         | Coagulase negative staphylococci | No growth |
| G         | No growth         | No growth        |
| H         | Bacillus species, coagulase negative staphylococci | No growth |
| I         | Gram negative non-lactose fermenting | No growth |
| J         | Bacillus species  | No growth        |

Mixed growth - growth of enteric bacteria along with Gram positive bacteria. Growth was heavy or moderate in all cultures before fumigation and very less growth was observed after fumigation.

### Table 4 - Microbial contamination on the interior handle of the rear door of the ambulance before and after fumigation.

| Ambulance | Before fumigation | After fumigation |
|-----------|-------------------|------------------|
| Day       |                   |                  |
| A         | Bacillus species  | No growth        |
| B         | Mixed growth      | Bacillus species |
| C         | Bacillus species  | No growth        |
| D         | Mixed growth      | No growth        |
| E         | Mixed growth      | Bacillus species |
| Night     |                   |                  |
| F         | Bacillus species  | No growth        |
| G         | Bacillus species, coagulase negative staphylococci | No growth |
| H         | Bacillus species  | Bacillus species |
| I         | Coagulase negative staphylococci | No growth |
| J         | Coagulase negative staphylococci, Bacillus species | Bacillus species |

Mixed growth - growth of enteric bacteria along with Gram positive bacteria. Growth was heavy or moderate in all cultures before fumigation and very less growth was observed after fumigation.
handle of the rear door post-fumigation. A similar density of growth and types of microbes was found in samples collected both in the day and night.

Rate of microbial prevalence according to the site of collection. Approximately 90% (oxygen knob), 80% (stretcher handle), and 100% of the ambulances were contaminated at the oxygen knob, handle, and interior handle of the rear door with predominantly Bacillus sps before fumigation. The absence of growth was observed in <25% of the ambulances before fumigation (Figure 3). Fumigation was found to be effective in decreasing microbial contamination with approximately 90% ambulances being disinfected at the oxygen knob and handle, and 60% ambulances disinfected at the door (Figure 4). Post-fumigation, a drastic increase in clean and disinfected ambulances was obtained with only 10% of ambulances being infected at the oxygen knob and stretcher, and only 40% ambulances being infected near the interior handle of the rear door (Figure 5).

**Discussion.** This study was planned to understand, identify pathogens, and recommend appropriate solutions for a pathogen-free environment in 10 ambulances in KSA. The pathogens isolated, such as Bacillus, Staphylococci, and Enterococci can pose substantial risk for nosocomial infections. There was a decrease in the growth of these microbes following fumigation with 6% H₂O₂. This is a well-known disinfectant for its broad anti-microbial properties, and is recommended by the Food and Drug Authority to be used as a liquid chemical sterilant. It has been shown that concentrations of 3%, 10%, and 15% H₂O₂ are very efficacious against a broad spectrum of pathogens. A concentration of 6% H₂O₂ for 2 hours used in this study was standardized by our SRCA ambulances, and was found to show promising levels of chemical sterilization.

The incidence of microbes in the included EMS ambulances was as high as 100%, of which some isolated organisms belonged to potential pathogenic strains while others were normal flora. The prevalence rates of growth observed from the 3 different sampling sites were in the range of 80-100% before fumigation. In post-fumigation, there was approximately a 60-90% decrease in the incidence of microbes. This indicates the significance of disinfection and sterilization techniques in prevention of disease transmission. These organisms could be potential nosocomial pathogens not only for patients who have weak immune systems, but also for personnel. The highest percentage of contamination was as expected near the door, where 90% of microbial growth was found.

Environmental flora, such as Pseudomonas could not be isolated in this study. Temperature is thought to
play an important role in influencing microbial growth and contamination. However, there was little difference in the density of microbial populations, or types of pathogens isolated from swab samples in the day versus the night. Although it can be assumed that the extreme heat common in KSA climate could form a protective mechanism against potential pathogens, this cannot be conclusively established in this study, and our planned future studies may highlight this perspective.

Identification of some Enterococci and Staphylococci raises special concerns since ambulance crew and personnel may be infected during the transfer rides from the sampling sites. In addition, these pathogens can also be transmitted to new patients, or relatives of subjects who may travel along with the patient to the hospital, or the respective destinations. In this first phase of study, we have collected samples from 3 sites per ambulance, totalling 30 sites before fumigation, and 30 sites after fumigation from 10 ambulances with a total of 60 samples. We plan to extend this study to a larger number of samples and additional sites for assessing the overall efficacy of the fumigation technique using other chemicals for ambulances. It is important to understand that ambulances can be a potential source of contamination, and therefore, more intense infection control mechanisms and disinfection practices need to be employed.

This study is important because although studies have assessed sparse infection control within hospitals, literature exists regarding the probability of transmission of infection within the EMS and ambulances. A surveillance study identified Enterococci, Bacilli, and dermal flora, and a corresponding reduction in microbial counts after cleaning. Similar results were observed in this study. A few studies showed approximately 50% of ambulances to be contaminated by methicillin resistant Staphylococci. However, methicillin resistant Staphylococci were not identified in this study.

It has been widely understood that ambulances could be a potential source of disease transmission to patients and staff. We recommend additional studies, be planned in the next 2 phases of this project with more ambulances to further understand the nature of contamination, and the efficacy of fumigation techniques. We support the recommendations that comprehensive education infection control programs that help in understanding disease transmission and etiology of infections is important for paramedics. This will help in decreasing the transmission of infection due to better infection control processes by all the staff and cleaning personnel. There is a definite need for stricter implementation of ambulance disinfection program with more frequency. Many occupationally acquired infections can be limited by proper awareness program, training initiatives, and stringent guidelines for ambulances.

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References

1. Galtelli M, Deschamp C, Rogers J. An assessment of the prevalence of pathogenic microorganisms in the rotor wing air ambulance: one program’s findings. Am J Infect Control 2006; 25: 81-84.
2. Pearse J. Infection control manual. A practical guide for the prevention and control of infection in the health care setting. Houghton (South Africa): Jacana Education; 1997.
3. Stone PW, Braccia D, Larson E. Systematic review of economic analyses of health care-associated infections. Am J Infect Control 2005; 33: 501-509.
4. Pirtet D. Infection control and quality health care in the new millennium. Am J Infect Control 2005; 33: 258-267.
5. Greenwood D, Slack RB, Peutherer JF. Hospital infection. In: Greenwood D, editor. Medical microbiology: a guide to microbial infections: pathogenesis, immunity, laboratory diagnosis, and control. 16th ed. Edinburgh (UK): Churchill Livingstone; 2002. p. 662-669.
6. World Health Organization. Department of Communicable Disease, Surveillance and Response. [accessed 2010 Jun 1]. Prevention of hospital-acquired infections. A practice guide. 2nd ed. Geneva (CH): WHO; 2002. Available from: http://www.who.int/csr/resources/publications/whocdscsreph200212.pdf
7. Pirtet D, Tarara D, Wenzel RP. Nosocomial bloodstream infection in critically ill patients. Excess length of stay, extra costs, and attributable mortality. JAMA 1994; 271: 1598-1601.
8. Nigam Y, Cutter J. A preliminary investigation into bacterial contamination of Welsh emergency ambulances. Emerg Med J 2003; 20: 479-485.
9. Roline CE, Crumpecker C, Dunn TM. Can methicillin-resistant Staphylococcus aureus be found in an ambulance fleet? Prehosp Emerg Care 2007; 11: 241-244.
10. Alves DW, Bissell RA. Bacterial pathogens in ambulances: results of unannounced sample collection. Prehosp Emerg Care 2008; 12: 218-224.
11. Madigan M, Martinko J, editors. Brock Biology of Microorganisms. 13th ed. New Jersey (NJ): Pearson Education; 2011. p. 1096.
12. Rutala AW, Weber DJ, the Healthcare Infection Control Practices Advisory Committee (HICPAC), CDC. Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008. Available from: http://www.cdc.gov/hicpac/pdf/guidelines/disinfection_nov_2008.pdf
13. Jarvis WR. The epidemiology of colonization. *Infect Control Hosp Epidemiol* 1996; 17: 47-52.

14. National Nosocomial Infections Surveillance System. National Nosocomial Infections Surveillance (NNIS) System Report, Data summary from January 1992 to June 2002, issued August 2002. *Am J Infect Control* 2002; 30: 458-475.

15. Roline CE, Crumpecker C, Dunn TM. Can methicillin-resistant *Staphylococcus aureus* be found in an ambulance fleet? *Prehosp Emerg Care* 2007; 11: 241-244.

16. Herold BC, Immergluck LC, Maranan MC, Lauderdale DS, Gaskin RE, Boyle-Vavra S, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. *JAMA* 1998; 279: 593-598.

17. Kowalski TJ, Berbari EF, Osmon DR. Epidemiology, treatment, and prevention of community-acquired methicillin-resistant *Staphylococcus aureus* infections. *Mayo Clin Proc* 2005; 80: 1201-1207.

18. Shaban R, Creedy D, Clark M. Paramedic knowledge of infectious disease aetiology and transmission in an Australian Emergency Medical System. *Australasian Journal of Paramedicine* 2003; 1: 10. Available from: [http://ro.ecu.edu.au/jephc/vol1/iss3/10](http://ro.ecu.edu.au/jephc/vol1/iss3/10)

19. Pearson L. Infection Control: Science, Management and Practice. In: McCulloch J, editor. London (UK): Whurr Publishers; 2000.

20. McDonell A. Issues of infection control in prehospital settings. *Journal of Emergency Primary Health Care* 2008; 6: 1.

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