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Microbial Air Quality and Bacterial Surface Contamination in Ambulances During Patient Services

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ABSTRACT

Objectives: We sought to assess microbial air quality and bacterial surface contamination on medical instruments and the surrounding areas among 30 ambulance runs during service. ***Methods*:** We performed a cross-sectional study of 106 air samples collected from 30 ambulances before patient services and 212 air samples collected during patient services to assess the bacterial and fungal counts at the two time points. Additionally, 226 surface swab samples were collected from medical instrument surfaces and the surrounding areas before and after ambulance runs. Groups or genus of isolated bacteria and fungi were preliminarily identified by Gram's stain and lactophenol cotton blue. Data were analyzed using descriptive statistics, *t*-test, and Pearson's correlation coefficient with a *p*-value of less than 0.050 considered significant. ***Results*:** The mean and standard deviation of bacterial and fungal counts at the start of ambulance runs were 318±485cfu/m^3 and 522±581cfu/m^3, respectively. Bacterial counts during patient services were 468±607cfu/m^3 and fungal counts were 656±612cfu/m^3. Mean bacterial and fungal counts during patient services were significantly higher than those at the start of ambulance runs, p=0.005 and p=0.030, respectively. For surface contamination, the overall bacterial counts before and after patient services were 0.8±0.7cfu/cm^2 and 1.3±1.1cfu/cm^2, respectively (p<0.001). The predominant isolated bacteria and fungi were *Staphylococcus* spp. and *Aspergillus* spp., respectively. Additionally, there was a significantly positive correlation between bacterial (r=0.3, p<0.010) and fungal counts (r=0.2, p=0.020) in air samples and bacterial counts on medical instruments and allocated areas. ***Conclusions*:** This study revealed high microbial contamination (bacterial and fungal) in ambulance air during services and higher bacterial contamination on medical instrument surfaces and allocated areas after ambulance services compared to the start of ambulance runs. Additionally, bacterial and fungal counts in ambulance air showed a significantly positive correlation with the bacterial surface contamination on medical instruments and allocated areas. Further studies should be conducted to determine the optimal intervention to reduce microbial contamination in the ambulance environment.

In health care facilities, including ambulances, there are several sources of infectious agents including patients, staff, and the environment itself (including the air and on devices/instruments used) which can become contaminated.[1-4] Healthcare personnel working in the ambulances may be at risk to blood-borne, airborne, droplet, and direct contact infections due to medical and health care practices administered to the patient (sometimes over a long period of time) in a closed air ventilation system and limited air space.[5,6] These conditions may increase the risk of airborne and droplet infections, such as influenza, avian flu, severe acute respiratory syndrome (SARS), chickenpox, pulmonary tuberculosis, meningococcal meningitis, and Ebola.[7-10] Personnel may be exposed to unrecognized or undiagnosed patients with these infections. A previous study showed that bus drivers in air conditioned buses were at risk to airborne and droplet infections due to the unhygienic condition of the air and poor ventilation.[11,12] Patients with coughing, sneezing, or any other medical procedures that expel oral fluids into the air can generate aerosols in the ambulance air.[13-15] Millions of tiny droplets of water and mucus are expelled at approximately 100 meters per second, larger droplets are deposited on surface environments, and smaller droplets dry rapidly to droplet nuclei of less than 5μm and

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contain virus particles or bacteria that can survive in the air and could be inhaled into the respiratory tract causing respiratory infections.[15,16] Moreover, non-pathogenic microorganisms, especially airborne bacteria and fungi, may cause and trigger asthma or allergies among susceptible individuals and infections in persons with weak physical and immune conditions.[17,18]

In ambulance services, inadequate cleaning with disinfectants of medical instruments and the internal surfaces may increase the risk to healthcare personnel working in ambulances. Our study sought to assess the microbial air quality and levels of bacterial surface contamination on medical instruments and in the surrounding areas in selected ambulances during patient services of a provincial hospital network in north-eastern Thailand.

METHODS

A cross-sectional study of 30 ambulance runs was conducted to assess microbial air quality (total bacterial and fungal counts) and bacterial surface contamination on medical instruments and surrounding areas during patient services of a provincial hospital network in north-eastern Thailand. The study was performed over a two-month period. Ethical approval was given by the ethical committee of Mahidol University, Faculty of Public Health (Reference No. MUPH 2009-184).

Air samples in the studied ambulances were collected for four minutes using the BioStage Impactor QuickTake 30 sample pump (SKC Inc, USA) with a fixed air flow rate of 28.3L/min. The air collection technique followed the active air sampling method.[19] Duplicate air samples were collected upon starting ambulance runs (1–2 air collection points), and during patient services in ambulances (2–4 air collection points). Approximately, 30–40 minutes were taken in patient service in each ambulance run, and about eight minutes were taken for each point of air sample collection. Outdoor air samples used for comparison were collected before and after ambulance services. Total bacterial count was obtained in plate count agar (PCA), and total fungal count was cultivated in 4% sabouraud dextrose agar (SDA). The equipment and devices for air sample collection were non-critical.

A total of 106 air samples (53 for bacterial counts and 53 for fungal counts) were collected at the start of ambulance runs and 212 air samples (106 for bacterial counts and 106 for fungal counts) collected during patient services in ambulances and were analyzed in this study. For outdoor air, 120 air samples (60 for bacterial counts and 60 for fungal counts) were included. Air samples for bacteria were incubated at 37°C for 48 hours and those for fungi were incubated at room temperature for five days with daily observation. After incubation, the bacterial and fungal colonies were counted and calculated to express as a colony forming unit/m^3 (cfu/m^3) using the following formula:

$$\text{Microbial count (cfu/m}^3\text{)} = \frac{\text{Total colonies} \times 10^3}{\text{Air flow rate} \times \text{collection time (minutes)}}$$

Therefore, in our study:

$$\text{Microbial count (cfu/m}^3\text{)} = \frac{\text{Total colonies} \times 10^3}{28.3 \times 4}$$

After counting the bacteria and fungi, the identification of colonies was performed according to their colony appearance, Gram's stain, and microscopic morphology following Larone's guide.[20] Additionally, 226 surface swab samples were collected from medical instruments and allocated areas in studied ambulances before and after patient services to culture total bacteria using a sterile wet swab. Direct plating of surface swabs were cultivated using PCA and incubated at 37°C for 48 hours before counting.

The American Conference of Governmental Industrial Hygienist (ACGIH) committee suggested that the presence of bacterial or fungal counts exceeding 500cfu/m^3 in an office workplace was an indication of poor ventilation or an unsanitary condition and needed remedial action.[21] Our study followed the ACGIH recommended guideline for air quality interpretation. For medical instrument surfaces and allocated area surface swabs, a total bacterial count ≥5cfu/cm^2 indicated high bacterial surface contamination.[22,23]

Data from the microbial air quality assessment and surface swab cultures were analyzed by descriptive statistics, including percentages, means, and standard deviations (SD). The comparison between microbial counts in air samples collected at starting ambulance runs and those collected during patient services was analyzed using *t*-test and proportional *Z* test. For

bacterial surface contamination, the comparison between mean surface swab counts of before and after patient services was analyzed using paired *t*-test or Wilcoxon test. The correlation between bacterial and fungal counts in air samples and the bacterial surface contamination on medical instruments and allocated areas in studied ambulances was analyzed using Pearson's correlation coefficient. The significance level was set at $p<0.050$.

RESULTS

All study ambulances had a driver's compartment and an aligned window with at least a 150 square inch opening for checking and communications. They had emergency medical sets, such as a cardiopulmonary resuscitation set, and various medical equipments and medication. Basic and advanced supplies, such as vascular access, ringer's lactate or normal saline solution, antiseptic solution, intravenous catheters, needles and syringes, airway and ventilation equipment (e.g. laryngoscope handle, laryngoscope blades, endotracheal tube) were provided in all studied ambulances. Additionally, equipment for cardiac care (a portable, battery-operated monitor/defibrillator) and other advance equipment, such as a nubulizer, glucometer or blood glucose measuring device, pulse oximetry with pediatric and adult probes were available.

A total of 106 indoor air samples were collected at the start of ambulance runs, and 212 indoor air samples collected during patient services from the 30 ambulance runs enrolled in the study to investigate bacterial counts and fungal counts. It was found that the mean±SD of bacterial counts at starting ambulance runs was 318±485cfu/m^3, and that of fungal counts was 522±581cfu/m^3. During patient services the bacterial and fungal counts were 468±607cfu/m^3 and 656±612cfu/m^3, respectively. The mean bacterial and fungal counts during patient services in the studied ambulance runs were significantly higher than those of bacterial and fungal counts at the start of the runs, $p=0.005$ and $p=0.030$, respectively. The data are shown in Table 1.

When the bacterial and fungal counts were described in detail and compared with the recommended ACGIH guidelines level, it was found that 17.0% of air samples collected at the start of ambulance runs (9/53 samples) and 26.4% of samples collected during patient services (28/106 samples) had bacterial counts more than the ACGIH recommended level (>500cfu/m^3). This was also the case for fungal counts, with 37.7% of samples (20/53) collected at start of runs and 50.9% of samples (54/106) collected during services with fungal counts more than the recommended level [Table 2]. Both bacterial and fungal counts in air samples collected during patient services showed significantly higher percentages of high levels than those of air samples at the start of ambulance runs, $p<0.010$ [Table 2].

The bacterial and fungal colonies isolated from air samples in studied ambulances were preliminary identified. It was found that most isolated bacterial colonies (47.8–51.6%) were *Staphylococcus* spp., 35.2–37.8% were gram negative bacilli, and 13.2–14.4% were gram positive bacilli. The majority of isolated fungal colonies (55.6–58.7%) were

Table 1: Distribution of microbial counts (cfu/m^3) of air samples collected from 30 studied ambulance runs (measurements were taken at the start of the run and during patient services).

Parameters	Start of run Number (%) of microbial counts (cfu/m^3) in each run n=53			During patient services Number (%) of microbial counts (cfu/m^3) in each run n=106			Number (%) of microbial counts (cfu/m^3) in outdoor air n=60	
	≤300	301-500	>500	≤300	301-500	>500	Before run >1000	After run >1000
Bacterial counts Mean±SD	23(76.6)	2(6.7) 318±485*	5(16.7)	19(63.3)	4(13.4) 468±607*	7(23.3)	4(13.3) 537±577	4(13.3) 518±589
Fungal counts Mean+SD	16(53.3)	4(13.4) 522±581**	10(33.3)	11(36.6)	5(16.7) 656±612**	14(46.7)	10(33.3) 1191±908	12(40.0) 1221±997

** Statistically significant difference by t-test, p=0.005*
*** Statistically significant difference by t-test, p=0.030*
Remarks: One to two air samples per ambulance run were collected at the start of runs, and three to four were collected during patient services. Two air samples per ambulance run were collected from outdoor air (one before the ambulance run and one after).

Table 2: Number and percentage of air samples of two levels of bacterial and fungal counts (≤500 and >500 cfu/m^3) collected from the ambulances.

Ambulance runs	Number (%) of air samples with bacterial counts (cfu/m^3)		Number (%) of air samples with fungal counts (cfu/m^3)	
	≤500	>500	≤500	>500
At start*n=53	44(83.4)	9(17.0)	33(62.3)	20(37.7)
During patient services n=106	78(73.6)	28(26.4)	52(49.1)	54(50.9)
p-value**	<0.010		<0.010	

One to two air samples per ambulance run were collected at the start of runs and three to four air samples were collected during patient services

*** By proportional Z test*

Aspergillus spp., and 36.3–37.2% were *Penicillium spp.* The distribution of genus or groups of isolated bacteria and fungi from in-ambulance air was similar proportion with that from outdoor air [Table 3].

A total of 452 surface swab samples before and after ambulance services (226 each) were collected from medical instrument surfaces and allocated areas in the studied ambulances. These included 44 samples each from stethoscopes and the adjustment knob of oxygen flows, 18 samples from long spinal boards, 60 samples each from stretcher mattresses, and air-flow adjustment fins. It was found that the mean±SD of overall bacterial surface counts before and after patient services were 0.8±0.7cfu/cm^2 and 1.3±1.1cfu/cm^2, respectively, p<0.001. However, no surface swab samples had bacterial counts more than the recommended count (≥5cfu/cm^2) [Table 4].

The correlation between bacterial and fungal counts in air samples and the bacterial surface contamination on medical instruments and allocated areas in the ambulances was analyzed [Table 5]. It was found that bacterial and fungal counts in ambulance air revealed a significantly positive correlation with the bacterial surface contamination on medical instruments and allocated areas in ambulances (r=0.3, p<0.010 and r=0.2, p=0.020, respectively).

Table 3: Number and percentage of bacterial and fungal groups isolated from studied air samples in the ambulance and outdoor air samples.

Microbial identification	Number (%) of isolated colonies in ambulance air	Number (%) of isolated colonies in outdoor air
Before ambulance runs		
Bacteria	n=91	n=84
Staphylococcus spp.	47(51.6)*	40(47.6)*
Gram negative bacilli	32(35.2)	29(34.5)
Gram positive bacilli	12(13.2)	15 (17.9)
Fungi	n=124	n=98
Aspergillus spp.	69(55.6)	52(53.1)
Penicillium spp.	46(37.2)	26(26.5)
Fusarium spp.	4(3.2)	5(5.1)
Septate hypha fungi	2(1.6)	13(13.3)
Rizopus and Mucor	3(2.4)	2(2.0)
During ambulance runs		
Bacteria	n=90	n=84
Staphylococcus spp.	43(47.8)*	38(46.4)*
Gram negative bacilli	34(37.8)	28(32.1)
Gram positive bacilli	13(14.4)	18(19.7)
Fungi	n=121	n=98
Aspergillus spp.	71(58.7)	54(55.1)
Penicillium spp.	44(36.3)	25(25.5)
Fusarium spp.	2(1.7)	6(6.1)
Septate hypha fungi	1(0.8)	10(10.2)
Rizopus and Mucor	3(2.5)	3(3.1)

The majority (81–86%) were Staphylococcus aureus

Table 4: Mean and standard deviations (SD) of surface swab counts in studied ambulance before and after patient services.

Surface swabs collected from	Before patient services		After patient services		*p*-value
	Number of samples	Mean±SD cfu/cm^2	Number of samples	Mean±SD cfu/cm^2	
Stethoscope	44	0.8±0.9	44	1.7±1.7	0.002*
Oxygen flow knob	44	0.3±0.5	44	1.6±1.9	0.003*
Long spinal boards	18	0.3±0.4	18	1.0±1.4	0.063^W
Stretchers	60	0.2±0.3	60	0.7±1.2	0.010*
Air-flow fins	60	0.6±0.7	60	1.6±1.6	<0.001*
Total	226	0.8±0.7	226	1.3±1.1	<0.001*

Statistical significance by paired t-test at p=0.050

^{W}No statistical significance by Wilcoxon at p=0.050

Remark: No surface swab samples had bacterial counts more than the recommended count (≤5cfu/cm^2).

Table 5: The correlation between bacterial counts or fungal counts in air samples and the bacterial surface contamination on medical instruments and surrounding areas among studied ambulances.

Microbial counts in air samples	Surface swab counts on medical instruments and surrounding areas
Bacterial count	r = 0.3, p <0.010*
Fungal count	r = 0.2, p =0.020*

**statistically significant correlation at p=0.050*

DISCUSSION

An ambulance is a self-propelled vehicle specifically designed to transport critically sick or injured people to a medical facility. Ambulance personnel frequently have to take rapid action and provide medical care under life-or-death circumstances, and may be exposed to communicable diseases in the course of their duties.[2,5] The risk towards these infections or diseases depends on a source of infectious agents, a susceptible host with a portal of entry receptive to the agent, and a mode of transmission.[6,9] Contaminated environments, especially air and surface areas may be major sources of infectious agents.[5,6,9]

This short-term study of microbial counts in indoor air samples collected from 30 ambulance runs found that between 17.0% to 50.9% of air samples had a high level bacterial or fungal count indicating poor ventilation or unsanitary conditions.[21] These high microbial counts in air samples occurred in some ambulance runs, which had more patient care activities. A previous study in dental clinics revealed that higher microbial counts were found during activities of works and after work.[14] For surface swab samples, results revealed that bacterial counts on medical instruments and allocated areas in studied ambulances after patient services were significantly higher than those before patient services (p<0.050), with the exception of the long spinal board (p>0.050). A preliminary report demonstrated that several species of nosocomial pathogens were isolated in ambulances and formidable antibiotic resistance patterns.[24] In addition, a study in German ambulances found methicillin resistant *Staphylococcus aureus* (MRSA) contamination in 18 sampling surfaces of 11 ambulances out of a total 150 studied ambulances.[25]

The United States Environmental Protection Agency (EPA) registered disinfectants or detergents/disinfectants that best met the overall needs of the healthcare facility for routine cleaning and disinfection should be selected. Routine surface cleaning is recommended to control the spread of pathogens in hospital environments, including ambulances. This was supported by Andersen et al,[26] who found that moist and wet mopping is the most effective cleaning method to reducing bacteria on the floor. However, a preliminary investigation in Welsh emergency ambulances found that most sites within ambulances were contaminated with several species of bacteria before cleaning, and even after cleaning many sites were still contaminated.[27]

Although ambulance personnel regularly wore surgical masks to protect themselves from droplet infections, the evidence from a previous study in healthcare workers in a hospital who regularly wore surgical masks while taking care of patients showed high accumulation of bacteria and fungi on the inside and outside areas of the used surgical masks suggesting that they were unsuitable for preventing the infections.[11,28] Additionally, several factors influenced the microbial load in indoor air, such as the number of patients, the air conditioning systems, and the ventilation. Some physical factors including heat, temperature, and humidity were also reported.[12,29,30] Both personnel and patients are a potential source of microorganisms as they shed the microorganisms from the skin and the respiratory tract.[17,31] Temperatures below 16°C and above 25°C caused a reduction in the concentration of airborne fungi. Furthermore, maintaining the relative humidity between 30–60% would help control mold and dust mites.[29,32,33] Generally, most airborne bacteria and fungi do not affect healthy humans, but may affect human health including allergies and non-specific symptoms in susceptible persons, such as young children, the elderly, and immune-compromised individuals.[17,18,34,35]

Due to the high levels of microbial counts found in some ambulance runs, the air ventilation in ambulances should be improved. Previous studies demonstrated that the air-conditioned buses with open exhaust ventilation fans had significantly lower bacterial and fungal counts than those without open exhaust ventilation fans,[12] and that the total number of microorganisms would be reduced after starting the air conditioning system for a few minutes.[30] Additionally, an evaluation of ambulance decontamination using gaseous chlorine dioxide

(ClO_2) demonstrated that ClO_2 gas could reduce a variety of bacteria in ambulances at least six-log.[36]

Most of isolated bacterial colonies from air samples (49.7%) were *Staphyloccoccus* spp., and most of the majority (81.6%) were *Staphylococcus aureus*. The most isolated fungal colonies (57.1%) were *Aspergillus* spp., and *Penicillium* spp. However, this bacterial air quality assessment and identification did not cover anaerobic and higher bacteria. According to European Commission Classification, most isolates from this study were considered potential candidates for causing sick building syndromes and associated with clinical manifestations of allergy, rhinitis, asthma, and conjunctivitis.[32] A survey of airborne fungi in buildings and outdoor environments in the US (2002) found that *Aspergillus* spp. was the most common fungi,[37] which was the same in our study. Moreover, our study found that bacterial and fungal counts in ambulance air had a significantly positive correlation with the bacterial surface contamination on medical instruments and allocated areas in ambulances. This evidence corresponded with the study of Nzeako et al,[38] which demonstrated the correlation between sedimentation plate count and surface swab of fungi in hospital wards.[38] In recent years, several studies on the application of ATP-activity for monitoring microbial loads in environmental samples (including surface and device swabs, aqueous humidifier samples, and ready-to-eat foods) were evaluated and was reported as a useful tool as it gave a good correlation with the standard methods.[39-41]

The intervention program for reducing the microbial concentration in the air and on the surface areas in ambulances should emphasize the need for a ventilation improvement strategy, and improvement in the cleaning program of the ambulance environment, including medical instruments, seats, the floor, and air conditioning units. Additionally, the use of standard precautions, including hand hygiene and effective personal protective equipment (PPE) will prevent cross infection and save ambulance personnel's lives.

CONCLUSION

This study revealed high microbial contamination (bacterial and fungal counts) in ambulance air during ambulance services and higher bacterial contamination on medical instrument surfaces and allocated areas after ambulance services compared to the start of ambulance runs. The predominant isolated bacteria and fungi in air samples were *Staphylococcus* spp. and *Aspergillus* spp., respectively. Additionally, bacterial and fungal counts in ambulance air showed a significantly positive correlation with the bacterial surface contamination on medical instruments and various areas tested in the studied ambulances. Future studies should be conducted to determine the optimal intervention to reduce the microbial contamination in ambulance air and surfaces.

Disclosure

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