Risk Stratification-based Surveillance of Bacterial Contamination in Metropolitan Ambulances

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Received: 19 June 2010
Accepted: 26 October 2010

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Key Words: Bacterial Infections; Contamination; Ambulances

INTRODUCTION

Ambulances can possibly be a source for various pathogens to be transmitted because they transport many patients with various diseases or infections. To prevent the ambulance from being a source for transmission of infection to patients or ambulance crews, strict infection control protocols should be implemented and monitored. Ambulance services are increasingly being recognized around the world as being an important part of public health systems. However, although hospital-based infection control programs are being currently emphasized (1-3), prehospital infection control has not been recognized as an essential part of public health. Existing research related to prehospital infection have usually been regarding the prevalence of pathogens in samples from the surface of ambulances or devices, contamination rates of specific pathogens, and the possibility of sterilization for the cultured microorganisms (4-6). To prevent the ambulance from being a source of contamination, we should have an evidence-based and cost-effective infection control protocol for ambulances and its equipment. Medical devices and material are usually classified into three categories (critical, semi-critical, and non-critical) according to the likelihood of being contaminated (7). For example, devices like the blade of laryngoscope, being directly in contact with the airway mucous membrane of patients, are considered as a critical device. Because ambulance devices and materials are too many and very various, this schematic approach according to this risk stratification-based surveillance (RSS) for contamination will be very helpful for the implementation and quality assurance of infection control.

Few studies on the prevalence of microorganism according to RSS (Critical, Semi-critical, Non-critical) have been done. This RSS-based prevalence will help ambulance authorities make a cost-effective infection control guideline and monitoring system.

This study aimed to know the prevalence of microorganism contaminated in ambulance devices and material according to RSS.

MATERIALS AND METHODS

Study design and setting
This study was a surveillance and descriptive study. This study was done in a metropolitan emergency medical service (EMS),
which is a single tiered, fire-based, basic life support (BLS) EMS. The metropolitan city has about 10 million population, 250,000 annual ambulance transports in 2008, and 117 ambulances for prehospital transport.

The metropolitan ambulance authority (city fire department) follows the national standard operating procedures (SOP) for infection control which was first made by the national headquarters of the fire department in 2005. The national SOP included the goal of infection control, role of EMS authority, infection control committee and education program, environmental control of the ambulance station, personal protective equipment, field precautions and post-return precautions. This was revised to be stricter in January 2008. According to these SOP, ambulance crews should wash the decontaminated surface of ambulances using an appropriate cleaner, sterilize the devices, and change the material if disposable.

**Selection of sampling sites**

We used a convenience sampling method. Sampling time also decided with a convenience method. Among 117 ambulances, 13 ambulances (11.3%) were selected. For each ambulance, the same thirty three sampling sites were decided (total 429 sites), according to risk stratification. Each sampling was also categorized according to type of device: airway devices, breathing devices, circulation devices, other devices, and ambulance apparatus. Driver site was used as a control (Table 1). Sampling was done with blinding to ambulance crews in April, 2009.

**Data collection and processing**

Sampling was done by surface swabbing using soft rayon swabs (COPAN Italia S.p.A., Brescia, Italy). The samples were put on the blood agar plate and MacConkey agar plate and then screening was done for Methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin-resistant *Enterococcus* (VRE) contamination. Fluid samples were put into an aseptic container and transported to the microbiology laboratory center of the study institution for cultivation. Fluid from the oxygen filter tank or suction bottle was filtered using an analytical test filter funnel with 0.2 µm size and samples from surface of the filter were swabbed and tested for *Legionella* antigen. After one night, the fluids were also put on blood agar and MacConkey agar plates and then screening was done for MRSA and VRE contamination. Interpretation was done by the certified board of the division of microbiology (laboratory medicine) as a routine clinical interpretation.

**Outcome measures and primary data analysis**

We investigated the positive rate for whole bacteria including

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**Table 1. Sampling sites according to risk stratification for contamination**

| Devices          | Critical                   | Semi-critical                     | Non-critical                     |
|------------------|----------------------------|----------------------------------|----------------------------------|
| Airway devices   | LMA cuff                   | Laryngoscope blade               | Laryngoscope handle             |
|                  | Intubation tube            | Suction tip                      |                                  |
|                  |                            | Water in suction bottle          |                                  |
|                  |                            | Oropharyngeal airway             |                                  |
| Breathing devices|                           | Nasal prong                      | ECG line                         |
|                  |                            | Facial mask                      | Button of AED                    |
|                  |                            | BVM mask                         | Handle of AED                    |
|                  |                            | BVM bag                          | Handle of sphygmomanometer      |
|                  |                            | Oxygen gate connector            | Detector of pulse oxymetry       |
|                  |                            | Oxygen generator                 |                                  |
|                  |                            | Water in oxygen generator        |                                  |
|                  |                            | Bottom of oxygen generator-inside|                                  |
| Circulation devices|                         |                                  |                                  |
|                  | ECG line                   |                                  |                                  |
|                  | Handle of AED              |                                  |                                  |
|                  | Handle of sphygmomanometer|                                  |                                  |
| Other devices    | Splint for upper extremity | Splint for lower extremity       |                                  |
|                  | Cervical collar            | Spine board                      |                                  |
|                  | Spine board                | Patient’s side door handle-1     |                                  |
|                  |                             | Patient’s side door handle-2     |                                  |
|                  |                             | Stretcher car handle             |                                  |
|                  |                             | Stretcher car side bar           |                                  |
|                  |                             | Surface of stethoscope           |                                  |
| Ambulance apparatus|                       | Extractor fan                    |                                  |
|                  |                            | Air conditioner                  |                                  |
| Control          | Steering wheel             | Steering wheel                   |                                  |
|                  | Driver’s side door handle  |                                  |                                  |

LMA, laryngeal mask airway; BVM, bag valve mask; ECG, electrocardiography; AED, automatic external defibrillator.
MRSA and VRE for the 429 samples according to risk stratification groups (Critical, Semi-critical, and Non-critical). We calculated the positive culture rate and its 95% confidence intervals (95% CIs) for descriptive analysis.

Ethics statement
This study was exempted for review by the institutional board review of the Seoul National University Hospital because this study did not enroll human subjects or animals.

RESULTS

Demographics of participating ambulance
All ambulances participating in this study had very similar configuration to type II of Federal Specification for Ambulances KKA-1822 of the USA (8) and were made in Korea. Demographics on patient transport of participating ambulances of 2008 were described in Table 2. Daily average transport volume per an ambulance was 6.6 per a day (range 4.8-8.4). Male was 42.3% (range 34.0%-46.7%) and mean age was 50.8 ± 21.4. Proportion of respiratory symptom or fever was 6.6% and 1.7%, respectively.

Prevalence rate of microorganisms according to sampling site
The total positive culture rate among 429 samples was 214 (49.9%, 95% CI; 45.1%-54.7%), which was the highest for circulation devices (69.2%, 95% CI; 56.6%-80.1%) and the lowest in ambulance apparatus (19.2%, 95% CI; 6.6%-39.4%) (Table 3). Critical, semi-critical, and non-critical devices showed 15.4% (95% CI, 4.4%-34.9%), 41.0 (95% CI, 33.2%-49.2%), and 59.1% (95% CI, 44.0%-57.9%), respectively. Pathogens were found on the following four sites; 1) Extended spectrum beta lactamase (ESBL) positive- *Klebsiella pneumoniae* in the water of suction bottles (airway devices), 2) ESBL positive- *K. pneumoniae* in the Bag-Valve Mask (BVM) bag (breathing devices). 3) Methicillin resistant coagu-

Table 2. Demographic findings of transported patients by ambulance in 2008

| Ambulance | Call volume, total | Average, daily | Male | Age (yr) (mean ± SD) | Symptom (%) | Pain, NOS | Fever | Others |
|-----------|-------------------|----------------|------|---------------------|-------------|-----------|-------|--------|
|           | No.               | No.            | No.  | %                   | Respiratory | Cardiovacular | Neurologic | Gastrointestinal | Pain, NOS | Fever | Others |
| Total (n = 117) | 250,596           | 5.9            | 109,446 | 43.7 | 49.7 ± 21.8 | 6.4          | 4.2       | 24.0   | 18.6   | 46.3  | 2.1 | 18.0 |
| Target ambulance (n = 13) | 31,382            | 6.6            | 13,281 | 42.3 | 50.8 ± 21.4 | 6.6          | 4.4       | 22.7   | 16.8   | 47.3  | 1.7 | 17.5 |
| A         | 3,072             | 8.4            | 1,044  | 34.0 | 49.1 ± 19.1 | 7.0          | 4.4       | 24.8   | 20.0   | 46.9  | 1.6 | 16.3 |
| B         | 2,113             | 5.8            | 866    | 41.0 | 51.0 ± 21.1 | 4.9          | 3.8       | 20.6   | 16.6   | 59.2  | 0.9 | 13.6 |
| C         | 2,087             | 5.7            | 865    | 41.4 | 48.3 ± 20.8 | 7.0          | 4.8       | 22.5   | 17.3   | 58.7  | 1.7 | 4.3  |
| D         | 1,750             | 4.8            | 689    | 39.4 | 46.0 ± 20.3 | 4.5          | 4.9       | 27.1   | 19.9   | 50.4  | 1.0 | 22.4 |
| E         | 2,227             | 6.1            | 1,036  | 46.5 | 49.9 ± 22.3 | 6.1          | 5.1       | 20.7   | 16.0   | 38.7  | 3.9 | 16.5 |
| F         | 2,648             | 7.3            | 1,211  | 45.7 | 51.6 ± 21.3 | 8.0          | 5.1       | 27.6   | 20.2   | 41.4  | 1.1 | 21.2 |
| G         | 2,036             | 5.6            | 785    | 38.6 | 51.2 ± 20.8 | 5.5          | 3.5       | 19.9   | 11.6   | 58.4  | 1.0 | 9.2  |
| H         | 2,603             | 7.1            | 1,042  | 40.0 | 53.2 ± 19.3 | 5.8          | 3.3       | 19.4   | 13.7   | 38.8  | 1.0 | 26.7 |
| I         | 2,661             | 7.3            | 1,242  | 46.7 | 52.1 ± 23.1 | 4.9          | 5.3       | 21.9   | 14.8   | 40.7  | 2.2 | 19.9 |
| J         | 2,815             | 7.7            | 1,310  | 46.5 | 53.4 ± 23.0 | 8.5          | 4.2       | 25.8   | 16.7   | 45.7  | 1.8 | 13.7 |
| K         | 2,905             | 8.0            | 1,311  | 45.1 | 51.8 ± 22.7 | 7.2          | 4.4       | 21.4   | 18.1   | 45.6  | 1.9 | 15.8 |
| L         | 2,339             | 6.4            | 1,007  | 43.1 | 52.0 ± 21.3 | 8.1          | 4.2       | 21.0   | 16.9   | 51.7  | 1.5 | 12.1 |
| M         | 2,126             | 5.8            | 873    | 41.1 | 49.0 ± 20.9 | 6.5          | 3.9       | 22.0   | 15.8   | 46.9  | 2.9 | 14.4 |

NOS, not otherwise specified.
Cultured microorganism and features

From 429 sampling sites, 624 sample cultures were investigated. Positive rate for any microorganism was 63.5% (396/624). Of these, four pathogens were identified. The others were environmental or normal flora, which are all susceptible to antibiotics (Table 4).

When we described the positive rate according to type of devices and participating ambulance, positive rate was 69.2% (9/13 ambulances) for airway devices which are a kind of critical devices, 92.3% (12/13 ambulances) for breathing devices which are a kind of semi-critical devices (Table 5).

**DISCUSSION**

The prevalence rate for microorganisms in a metropolitan ambulance surveillance was 49%, of which a few were pathogenic and most environmental or normal flora. This prevalence rate is not likely to be important, unless in critical or semi-critical devices. Medical devices in ambulance are classified into critical, semi-critical, and non-critical. Critical devices like intubation equipment, which should be sterilized until use, showed a 15.4% positive rate. 45.2% of semi-critical devices sampled were also positive. This finding is a surrogate marker for poor infection control for ambulance equipment (1, 2). Critical and semi-critical devices should be sterilized to clear up all microorganisms. A disposable device will be an alternative option for this goal (5). Non-critical devices include any external monitor apparatus for ECG, defibrillator, and so on. These devices are not important even though there are any microorganisms contaminated. Risk stratification-based surveillance (RSS) will guide us to make a feasible approach for maintenance of disinfection and quality.

Ambulance apparatus or driver’s sides are classified into non-critical devices, generally not needing any sterilizing. For example, ambulance driver’s sides showed very high contamination rate (73.1%). These findings are not serious.

Environmental microorganisms also will be problematic for immune compromised patients (9). However, environmental flora like *Acinetobacter* or *Pseudomonas*, which usually grow in soil or water, were found in this study. Those flora mean that minimum cleaning and washing for the ambulance was insufficient. This finding suggests disinfection for ambulances was poor.

Four pathologic microorganisms were indentified (0.9%). MRSA was from the driver’s site and MRCoNS was from the stretcher bar. These pathogens should not be present in ambulances and devices. Ambulance crews as well as drivers take part in transfer of patients, which can deliver pathogen to new patients from these side devices. MRSA has been known to be a common pathogen in hospital-based surveys, particularly in intensive care units (10). In recent reports, nosocomial MRSA infections are spreading to community, which are very different

### Table 4. Identification of microorganism: environmental and normal flora

| Classification* | Name of bacteria | Count, No. | Subtotal, No. (%) |
|-----------------|-----------------|------------|-------------------|
| Total           |                 | 423        | 423 (100.0)       |
| GNR-F           | Enterobacter cloacae | 1          | 16 (3.8)          |
|                 | Klebsiella pneumoniae | 2          |                   |
|                 | Lecleria adcarboxyiata | 1          |                   |
|                 | Pantoea agglomerans | 5          |                   |
|                 | Pantoea species    | 5          |                   |
|                 | Serratia marcescens | 1          |                   |
|                 | unidentified Gram (+) bacilli, fermentor | 1          |                   |
| GNR-NF          | Acinetobacter baumannii | 3          | 64 (15.1)         |
|                 | Acinetobacter haemolyticus | 1          |                   |
|                 | Acinetobacter radiodurans | 5          |                   |
|                 | Acinetobacter species | 2          |                   |
|                 | Chryseomonas luteola | 8          |                   |
|                 | Deflta acidovorans | 6          |                   |
|                 | Pseudomonus aeruginosa | 1          |                   |
|                 | Pseudomonus oxihabitans | 15         |                   |
|                 | Pseudomonus species | 5          |                   |
|                 | Pseudomonus stutzeri | 5          |                   |
|                 | Sphingomonas paucimobilis | 3          |                   |
|                 | Stenotrophomonas maltophilia | 3      |                   |
|                 | unidentified Gram (+) bacilli, non-fermentor | 7          |                   |
| GPC-E           | Enterococcus casseliflavus | 1          | 5 (1.2)          |
|                 | Enterococcus faecalis | 4          |                   |
| GPC-M           | Kocuria varians | 1          | 54 (12.8)         |
|                 | Kytococcus species | 1          |                   |
|                 | Micrococcus species | 52         |                   |
| GPC-S           | Coagulase negative Staphylococcus | 38       | 46 (10.9) |
|                 | Staphylococcus aureus | 4          |                   |
|                 | Staphylococcus hominis | 2          |                   |
|                 | Staphylococcus simulans | 1          |                   |
|                 | Staphylococcus warneri | 1          |                   |
| GPR-B           | Aneurinbacillus species | 2          | 141 (33.3)       |
|                 | Bacillus cereus | 2          |                   |
|                 | Bacillus circularis | 2          |                   |
|                 | Bacillus firmus | 3          |                   |
|                 | Bacillus lenta | 5          |                   |
|                 | Bacillus species | 118         |                   |
|                 | Brevibacillus species | 5          |                   |
|                 | Geobacillus species | 2          |                   |
|                 | unidentified Gram (+) bacilli | 1          |                   |
|                 | unidentified Gram (+) branched bacilli | 1          |                   |
| GPR-C           | Brevibacterium species | 10         | 58 (13.7)       |
|                 | Cellulomonas species | 12         |                   |
|                 | Corynebacterium species | 30         |                   |
|                 | Corynebacterium ulcerans | 1          |                   |
|                 | Rhodococcus species | 5          |                   |
| GPR-L           | Lactobacillus species | 4          | 4 (0.9)        |
|                 | Legionella antigen | 13         | 13 (3.1)        |
| Mold            | Mold | 18          | 22 (5.2)        |
| Yeast           | Candida species | 3          |                   |

*Microorganisms were classified as follows. GNR-F, Gram-negative rods-fermentor; GNR-NF, Gram-negative rods-nonfermentor; GPC-E, Gram positive coccus-enterococcus; GPC-M, Gram-positive cocci-micrococcus; GPC-S, Gram-positive coccus-staphylococcus; GPR-B, Gram-positive rods-bacillus; GPR-C, Gram-positive rods-coveryebacterium; GPR-L, Gram-positive rods-Lactobacillus; L-ag, Legionella antigen.
from hospital-acquired MRSA in terms of molecular analysis or clinical risk factors and features (11, 12). In this study, only one sample was MRSA positive, which was not identified on the basis of molecular biologic analysis.

The positive rate for MRSA in an ambulance sample conducted in the USA was 12.4%, which was very high compared to that of our study. However, our study was conducted using samples of devices and ambulance apparatus, not from the human body including hands, which are very relevant for MRSA infection. For future studies, to investigate the positive rate of MRSA, we should test samples from hands of ambulances crews.

Another pathologic microorganism was K. pneumoniae, which was extended spectrum beta lactamase (ESBL) positive. These bacteria were cultured from water in the suction bottle and surface of a bag-valve-mask bag, which means these can cause sepsis in patients directly. K. pneumoniae also may cause septicemia and septic shock in the immune compromised host.

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This study has limitations. The number of selected ambulances was 13 (11.1%), which was conveniently sampled. Therefore, study results could be biased from selection method. In particular four ambulances were washed using alcohol and tap water before sampling, which could have affected the results as rou-

**Table 5. Prevalence of microorganism culture by ambulance, equipment and device**

| Equipment and devices | Sample No. | Positive sample No. | Positive ambulance % | A | B | C | D | E | F | G | H | I | J | K | L |
|-----------------------|------------|---------------------|----------------------|---|---|---|---|---|---|---|---|---|---|---|---|
| Airway devices        |            |                     |                      |   |   |   |   |   |   |   |   |   |   |   |   |
| Laryngoscope blade    | 105        | 37                  | 35.2                 | 9 | 69.2 | + | + | + | + | + | + | + | + | + |
| Laryngoscope handle   | 15         | 7                   | 46.7                 | 5 | 38.5 | + | + | + | + | + | + | + | + | + |
| LMA cuff              | 15         | 5                   | 33.3                 | 3 | 23.1 | + | + | + | + | + | + | + | + | + |
| Intubation tube       | 15         | 4                   | 26.7                 | 2 | 15.4 | + | + | + | + | + | + | + | + | + |
| Suction tip           | 14         | 4                   | 28.6                 | 3 | 23.1 | + | + | + | + | + | + | + | + | + |
| Water in suction bottle | 18     | 9                   | 50.0                 | 4 | 30.8 | + | + | + | + | + | + | + | + | + |
| Oropharyngeal airway  | 13         | 4                   | 30.8                 | 4 | 30.8 | + | + | + | + | + | + | + | + | + |
| Breathing devices     |            |                     |                      |   |   |   |   |   |   |   |   |   |   |   |   |
| Nasal prong           | 17         | 8                   | 47.1                 | 4 | 30.8 | + | + | + | + | + | + | + | + | + |
| Face mask             | 14         | 5                   | 35.7                 | 4 | 30.8 | + | + | + | + | + | + | + | + | + |
| BVM mask              | 17         | 10                  | 58.8                 | 6 | 46.2 | + | + | + | + | + | + | + | + | + |
| BVM bag               | 17         | 13                  | 76.5                 | 9 | 69.2 | + | + | + | + | + | + | + | + | + |
| Oxygen gate connector | 15         | 4                   | 26.7                 | 2 | 15.4 | + | + | + | + | + | + | + | + | + |
| Oxygen generator       | 15         | 6                   | 40.0                 | 4 | 30.8 | + | + | + | + | + | + | + | + | + |
| Water in oxygen generator | 28     | 14                  | 50.0                 | 12 | 92.3 | + | + | + | + | + | + | + | + | + |
| Bottom of oxygen generator inside | 17 | 11 | 64.7 | 7 | 53.8 | + | + | + | + | + | + | + | + | + |
| Other devices         |            |                     |                      |   |   |   |   |   |   |   |   |   |   |   |   |
| Circulation devices   | 104        | 84                  | 80.8                 | 12 | 92.3 | + | + | + | + | + | + | + | + | + |
| ECG Line              | 17         | 12                  | 70.6                 | 8 | 61.5 | + | + | + | + | + | + | + | + | + |
| Handle of AED         | 20         | 15                  | 75.0                 | 8 | 61.5 | + | + | + | + | + | + | + | + | + |
| Button of AED         | 23         | 21                  | 91.3                 | 11 | 84.6 | + | + | + | + | + | + | + | + | + |
| Handle of sphygmomanometer | 26     | 21                  | 80.8                 | 8 | 61.5 | + | + | + | + | + | + | + | + | + |
| Detector of pulse oxymeter | 18 | 15 | 83.3 | 10 | 76.9 | + | + | + | + | + | + | + | + | + |
| Surface of stethoscope | 14         | 5                   | 35.7                 | 4 | 30.8 | + | + | + | + | + | + | + | + | + |
| Ambulance apparatus   |            |                     |                      |   |   |   |   |   |   |   |   |   |   |   |   |
| Extractor fan         | 13         | 4                   | 30.8                 | 4 | 30.8 | + | + | + | + | + | + | + | + | + |
| Air conditioner       | 14         | 2                   | 14.3                 | 1 | 7.7 | + | + | + | + | + | + | + | + | + |
| Steering wheel        | 20         | 16                  | 80.0                 | 9 | 69.2 | + | + | + | + | + | + | + | + | + |
| Driver’s side handle  | 21         | 18                  | 85.7                 | 10 | 76.9 | + | + | + | + | + | + | + | + | + |

LMA, laryngeal mask airway; BVM, bag valve mask; ECG, electrocardiography; AED, automatic external defibrillator.
tine practice. However, the positive rate of normal flora was similar in medical devices between pre-washed ambulances and non-washed ambulances because the most of medical devices were not washed or sterilized.

Different EMS systems and infection control guidelines may be there, which also make us this result to be generalized to external world. This study was not related with infection rate or contamination rate for patients transported by these ambulances. Therefore these results are not related with clinical outcomes.

The risk stratification-based surveillance for contamination in metropolitan ambulances showed very high prevalence of environmental and normal flora infection in critical and semi-critical devices. And a few pathogens were also found. All kinds of pathogens are important to infection control for non-critical devices in ambulance as well as semi-critical, or critical. For critical devises, all normal flora are serious in terms of contaminated devices and should be targeted for being sterilized.

To prevent the ambulance from being a source of contamination, more strict infection control and monitoring protocol should be implemented.

ACKNOWLEDGMENTS

All authors had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. We would like to thank and acknowledge the contributions of Emergency Medical Technicians (Doo Ho Kim and Yeon Jeong Cho) of Seoul National University Hospital on sampling and the Division of Rescue and Emergency Medical Service, National Emergency Management Agency for cooperation.

This study was financially supported by the office of Mrs. Ae Joo Lee, a member of National Assembly. All authors (Noh H, Shin SD, Kim NJ, Oh HS, Joo SJ, Kim JJ, Ro YS, and Ong ME) are not related with any other conflicts of interest in this study.

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Risk Stratification-based Surveillance of Bacterial Contamination in Metropolitan Ambulances

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This study was performed to know the risk-stratification-based prevalence of bacterial contamination of Seoul Metropolitan City-Fire department’s ambulance vehicle surfaces, equipment, and materials. Total 13 out of 117 ambulances (11.1%) were sampled and 33 sites per each ambulance were sampled and specific identification was performed. We categorized sampling sites into risk stratification-based groups (Critical, Semi-critical, and Non-critical equipment) related to the likelihood of direct contact with patients’ mucosa. The prevalence (positive/number of sample) of bacterial contamination in critical, semi-critical airway, semi-critical breathing apparatus group was as high as 15.4% (4/26), 30.7% (16/52), and 46.2% (48/104), respectively. Despite current formal guidelines, critical and semi-critical equipments were contaminated with pathogens and normal flora. This study suggests the need for strict infection control and prevention for ambulance services.