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RESEARCH ARTICLE

Effectiveness of Fumigation Techniques Against Microbial Spectrum in Ambulances

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ABSTRACT

This study presents a microbial infection risk in the pre-hospital environment especially in an ambulance. Ambulances are at a severe risk for nosocomial infection. The objective behind this study was to estimate the contaminants i.e. microbiological spectrum in ambulance to analyse the effectiveness of disinfection in controlling these contamination. The most common organisms isolated from the ambulances were coagulase positive *Staphylococcusaureus*, *Pseudomonas aeruginosa*, *Bacillus species*, *Escherichia coli*, *Listeria species*, *Enterobacter aerogens*, *Salmonellaspecies*, *Klebsiella* and coagulase negative *Staphylococci*. The samples were collected from 10 different ambulances which were active in service, before and after disinfection. The samples were maintained in ambient temperature using thermo-electrical cooler and were transported to the laboratory. Through this project, we recommend the application of disinfection techniques to reduce the infection in the ambulances.

Keywords- Fumigation, Disinfection, Microbiological Analysis, Nosocomial infection

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INTRODUCTION

An environment associated with health care can harbor a wide spectrum of infectious microbes. The pre-hospital environment especially the ambulance is at a severe risk for spread of nosocomial infections. Ambulances can conceivably be a potential source of different pathogenic microbes by virtue of their role in transporting patients to a health- care facility, or during an inter-facility transfer. This creates a scenario wherein not only the patients but also the para-medical staff and relatives of the patients may be exposed to various pathogens, which may lead to infections and diseases. Some disposable equipment and universal precautions reduces the risk to patients, but the ambulance still remains vulnerable to contamination from secretions, blood and other infectious material (Daifallah, 2014). Disinfection of an ambulance is a necessity in order to avoid the spread of nosocomial infection. In some areas and sectors of India it has been observed that the disinfection practice is not being practiced properly; hence this study has been undertaken to estimate the Microbiological spectrum in ambulance and the effectiveness of fumigation in controlling these contamination (Galtelli and Rogers, 2006 and Stone et al., 2005)
A regional study (Nigam and Cutter, 2003) examined the levels of bacterial contamination in ambulances over a 3-month period on a monthly schedule. The results showed presence of different microbes in the collected samples before cleaning the emergency vehicles. Through this study fresh contamination was observed in ambulances of previously uncontaminated zones due to the cleaning methods. Unacceptable levels of microbes have been found hence there is a need for more precise and stringent infection control program.

This project is based on a hypothesis that the ambulances can carry pathogenic bacteria which are hazardous to the personnel (paramedical staff and the patients); being transported to the hospitals. The relation between disinfection, cleaning procedures and the effect of fumigation of ambulances in order to minimize spread of infections to the personnel travelling in the ambulance needs to be closely investigated. Hence, this study has been undertaken to estimate the microbiological spectrum and the effectiveness of fumigation in controlling these contamination in ambulance.
In this study a total of ten ambulances which were active in service were selected. A total of 4 areas within the ambulance (such as stretcher handle, oxygen flow-meter knob, Body surface of the ambulance and the door handle) were included for sample collection. The samples were collected before and after the fumigation process.

The fumigation process was same for all the ambulances (i.e. the use of potassium permanganate and formaldehyde in ratio of 2:5)

The samples were maintained at ambient temperature by the help of thermo-electric cooler and transported to the laboratory.

The collected samples were inoculated in different culture medium for the isolation of microbes and the positive cultures were tested further to confirm the bacterial and fungal isolates. The most common organisms isolated were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus species*, *Salmonella species*, *Escherichia coli*, *Aspergillus species*, *Candida albicans* and some Enteric bacteria.

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After fumigation, no growth in the microbial isolates was observed. Through this study, we recommend the use of fumigation technique to reduce the infection in the ambulances.

EXPERIMENTAL METHODS

Collection of sample's

The samples for analyses were collected from four different places such as stretcher handle, oxygen flow meter knob, door handle and body surface of each ambulance. The samples were collected using swab technique. Post swab sample collection and air monitoring was performed in each of the ambulance with the help of Anderson Air Sampler.

The study comprises of two segments. In the first segment, the samples were collected before fumigation (at the evening after completion of work). The second set of samples was collected from the ambulance after being fumigated with potassium permanganate and formaldehyde in the ratio of 2:5 for 6 hours.

In total 8 swab samples and 20 plates were collected from each ambulance (in both first and second segment). The samples were maintained in ambient temperature using a thermo-electrical cooler and were transported to laboratory for further analysis. All the samples were coded with a unique identification number to avoid any study bias.

Analysis of the Collected Samples

All the swab samples collected before and after fumigation were cultured in a nutrient rich media. The samples were then isolated on Nutrient Agar, MacConkey Agar, Baired Parker Agar, Eosin Methylene Blue Agar, PALCOM Agar, Plate Count Agar, Blood Agar, Potato Dextrose Agar, Sabouraud Dextrose Agar, Violet Red Bile Glucose Agar, Violet Red Bile Lactose Agar and Xylose Lysine Tergitol-4 Agar. These isolated samples along with the plates from air monitoring were incubated at the 37 ^{o}C for 24-48 hours (for bacteria) and 25 ^{o}C for 5 days (for fungi.) The colonies obtained were further screened and confirmation test were performed to identify the microorganism.

RESULTS

Results of Swab analysis

Microorganisms isolated from all the 4 sites of all the ambulances are included in this study. The most common organisms isolated include *Bacillus species, Staphylococcus aureus, Escherichia coli, Listeria species, Salmonella species, Aspergillus niger* and *Candida albicans.* Both Gram negative non-lactose fermenting bacteria and Gram positive bacteria were isolated.

To analyze the efficacy of fumigation procedure on the growth of contaminants, total bacterial count in samples from all ambulances were considered.

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Table-1: The detailed description regarding the Total Bacterial Count and the different microbes isolated from Site 01- Door Handle is illustrated.

Ambulance	Site 01- Door Handle			
	Total Bacterial Count		Microbes Isolated	
	BF	AF	BF	AF
A	17000	NG	*Bacillusspecies, Escherichia coli, Staphylococcus Species, Salmonellaspecies, Candida species, Mucorspecies*	NG
B	46000	NG	*Bacillusspecies, Listeriaspecies, Staphylococcus species, Klebsiella species, Aspergillus species*	NG
C	38000	NG	*Bacillusspecies, Escherichia coli, Staphylococcus species Candida species, Klebsiella species,*	NG
D	11000	NG	*Bacillusspecies, Pseudomonas species, Staphylococcus species, Candida albicans, Enterococcus faecalis, Enterococcus aerogens*	NG
E	73000	NG	*Escherichia coli, Enterococcus aerogens, Staphylococcus aureus, Salmonella species, Listeria species, Pseudomonas species*	NG
F	28000	NG	*Bacillusspecies, Pseudomonas aeruginosa, Staphylococcus aureus, Listeria species, Salmonella species*	NG
G	37000	NG	*Staphylococcus aureus Escherichia coli, Salmonellaspecies, Candida species, Listeria species.*	NG
H	52000	NG	*Bacillusspecies, Staphylococcusaureus, Salmonellaspecies, Candida species, Listeria species, Pseudomonas species*	NG
I	9600	NG	*Staphylococcus aureus, Enterococcus aerogens, Pseudomonas species, Mucor species*	NG
J	98000	NG	*Staphylococcus aureus, Listeria species, Escherichia coli, Salmonellaspecies, Candida species, Listeria species, Klebsiella species*	NG

Table-2: The detailed description regarding the Total Bacterial Count and the different microbes isolated from Site 02- Stretcher Handle is illustrated.

Ambulance	Site 02- Stretcher Handle			
	Total Bacterial Count		Microbes Isolated	
	BF	AF	BF	AF
A	7600	NG	*Escherichia coli, Staphylococcus aureus, Candidaspecies, Salmonellaspecies.*	NG
B	6600	NG	*Bacillusspecies, Candidaspecies, Escherichia coli.*	NG
C	9900	NG	*Staphylococcusaureus, Escherichiacoli, Candidaspecies*	NG
D	8600	NG	*Pseudomonasaeruginosa, Staphylococcusaureus, Listeria species*	NG
E	7500	NG	*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*	NG
F	9600	NG	*Staphylococcus a*ureus, *Escherichia coli,*	NG

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			Salmonella species, Enterococcus faecalis	
G	8700	NG	*Staphylococcus aureus, Escherichia coli,Listeriaspecies*	NG
H	5100	NG	*Bacillusspecies, Staphylococcus aureus, Escherichia coli*	NG
I	1100	NG	*Staphylococcus aureus, Listeria species, Escherichia coli*	NG
J	6500	NG	*Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus species*	NG

Table-3: The detailed description regarding the Total Bacterial Count and the different microbes isolated from Site 03- Oxygen Flow Meter Knob is illustrated.

Ambulance	**Site 03-Oxygen Flow Meter Knob**			
	Total Bacterial Count		**Microbes Isolated**	
	BF	**AF**	**BF**	**AF**
A	1600	NG	*Staphylococcus a*ureus, *SalmonellaspeciesBacillusspecies, Salmonellaspecies,*	NG
B	1550	NG	*Staphylococcus aureus, Listeriaspecies*	NG
C	1400	NG	*Escherichia coli, Bacillusspecies*	NG
D	1300	NG	*Pseudomonas aeruginosa, Bacillusspecies,*	NG
E	1800	NG	*Escherichia coli, Bacillusspecies*	NG
F	2100	NG	*Staphylococcus aureus, Bacillus species*	NG
G	1900	NG	*Staphylococcus aureus, Bacillus species*	NG
H	900	NG	*Staphylococcus aureus, Bacillus species*	NG
I	250	NG	*Staphylococcus aureus, Bacillus species*	NG
J	1000	NG	*Staphylococcus aureus, Bacillus species*	NG

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Table-4: The detailed description regarding the Total Bacterial Count and the different microbes isolated from Site 04- Body Surface of the Ambulance is illustrated

Ambulance	Site 04- Body Surface of the Ambulance			
	Total Bacterial Count		Microbes Isolated	
	BF	AF	BF	AF
A	860000	NG	*Staphylococcus aureus, Bacillus species, Aspergillus species, Escherichia coli, Salmonellaspecies, Candida species, Mucor species*	NG
B	950000	NG	*Staphylococcus aureus, Bacillusspecies, Escherichia coli, Listeriaspecies, Staphylococcus species, Klebsiella species, Aspergillus speciesPseudomonas aeruginosa,Mucorspecies,*	NG
C	290000	NG	*Escherichia coli, Bacillus species, Aspergillus species, salmonella, staphylococcus species, Candida species,*	NG
D	170000	NG	*Pseudomonas aeruginosa,Staphylococcus aureus Bacillus species, Aspergillus species,*	NG
E	880000	NG	*Escherichia coli,Pseudomonas aeruginosa,Staphylococcus aureus, Bacillusspecies, Aspergillus species, Klebsiellaspecies*	NG
F	110000	NG	*Bacillusspecies, Escherichia coli, Listeriaspecies, Staphylococcus species, Klebsiella species, Salmonella species, Enterococcus faecalis,*	NG
G	172000	NG	*Staphylococcus aureus Bacillus species, Aspergillus species,*	NG
H	950000	NG	*Salmonella Bacillus species, Aspergillus species,*	NG
I	15000	NG	*Staphylococcus aureus Bacillus species, Aspergillus species,*	NG
J	368000	NG	*Pseudomonas aeruginosa Bacillus species, Aspergillus species,*	NG

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Result of Air Monitoring

Table-5: The detailed description regarding the Total Bacterial Count and different microbes found in the Air after Air Monitoring in all the Ambulances is illustrated

Ambulance	Air Monitoring Results			
	Total Bacterial Count		Microbes Isolated	
	BF	AF	BF	AF
A	TNTC	NG	*Staphylococcus aureus,Escherichia coli,Bacillusspecies, Candida albicansAspergillusspecies, Bacillusspecies, Staphylococcus Species, Salmonellaspecies, Candida species, Mucorspecies*	NG
B	TNTC	NG	*Staphylococcus aureus, Candida albicans, Pseudomonas aeruginosa, Bacillus species*	NG
C	TNTC	NG	*Escherichia coli, Bacillus species, Candida albicans*	NG
D	TNTC	NG	*Pseudomonas aeruginosa, Candida albicans, Staphylococcus aureus, Bacillus species*	NG
E	TNTC	NG	*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus species, Candida albicans*	NG
F	TNTC	NG	*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus species, Candida albicans*	NG
G	TNTC	NG	*Staphylococcus aureus, Bacillus species, Candida albicans*	NG
H	TNTC	NG	*Salmonella species, Bacillus species, Candida albicans*	NG
I	TNTC	NG	*Staphylococcus aureus, Bacillus species, Candida albicans*	NG
J	TNTC	NG	*Pseudomonas aeruginosa, Bacillus species, Candida albicans*	NG

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CONCLUSION

The concern for the health care environment that may harbor a substantial reservoir of infectious agents has been vigorously examined by many microbiologist and infectious disease experts. This study was accordingly designed to isolate the different microbes which can be found contaminating the ambulances and implement appropriate solutions for procuring a pathogen free area/environment in Ambulances.

We isolated and identified pathogenic microbial bacteria such as; Bacillus, Staphylococci, Escherichia coli, Salmonella and Enterococci which can pose substantial risk for nosocomial infections. A reason to this could be the low knowledge about disinfection and shortfalls in the practices for disinfection and sterilization control procedures by the staff members. From this study it is evident that following fumigation with potassium permanganate and Formaldehyde (in a ratio of 2:5), there was a decrease in the growth of these microbes. 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. To overcome the occurrence of infection due to lack of fumigation procedures, there must be implementation of a stringent institutional policy by microbiologists.

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