ORIGINAL ARTICLE

Microbiological Load of Ethylene Oxide Sterilized Medical Devices and its Elimination by Cobalt 60 Source

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Abstract

Objective: To determine the residing microbial flora of ethylene oxide (EtO) sterilized medical devices and optimization of safe dose of gamma radiation (Cobalt 60 source) for the complete elimination of microbial load.

Study Design: Experimental study.

Place and Duration of Study: Department of Biotechnology, Lahore College for Women University, Lahore, Pakistan from September 2014 to June 2015.

Methodology: Thirty-six samples of EtO sterilized medical devices of same batch of three different companies were collected for this study. Isolation and enumeration of microbes were done by using different selective and differential media. Gram staining and biochemically characterization by API 20 (Bio Merieux, France) kit was done for identification of the microorganisms. The medical devices having high microbial load were sent to Pakistan Radiation Services (PARAS) for gamma irradiations at 3 different selected doses (20 KGy, 25 KGy, and 30 KGy).

Results: Different types of Gram positive bacteria (*Staphylococcus epidermidis, Staphylococcus aureus* and *Bacillus subtilis*) were isolated from the EtO sterilized samples. Gram negative bacteria and fungi were not detected on these medical devices. Gamma irradiations results showed that 30 KGy was optimized dose for complete elimination of microbial flora on endotracheal, Nelaton, and tracheostomy tubes.

Conclusion: Gamma radiations (Co 60 source) effectively decontaminate the microbial flora on the equipment previously sterilized by the ethylene oxide gas; and 30 KGy is the optimized dose for all these medical devices.

Key Words: *Endotracheal tube. Nelaton tube. Tracheostomy tube. Gamma radiation. Pakistan Radiation Services (PARAS). Decontamination.*

INTRODUCTION

The area of medical sterilization has become complex due to increase in the need to protect patients from exposure to infectious microorganisms found on medical devices used during their treatment.[1] The rate of occurrence of surgical site infections among individuals undergoing surgery is 2 - 5% in developed countries whereas much higher rates are reported ranging from 12% to 39% in developing countries.[2]

Endotracheal and Nelaton tubes are special type of catheters. Endotracheal tube is used to provide positive pressure ventilation during anesthesia and is also helpful in protecting the airway of patient from aspiration of gastric contents. On the other hand, Nelaton tube is commonly used for the drainage of urine and gastric juice.

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Tracheostomy tube is particularly used in intensive care units and is considered as semi-critical item. It is used for multiple medical applications and is performed on people who require prolonged mechanical ventilation.[3] These tubes can act as reservoirs for the infectious microorganisms. Hence, proper decontamination of these medical instruments is essential for preventing the patients from secondary infections.

In Pakistan, little work has been reported on estimation of microbial flora on medical devices. However, higher rate of nosocomial infections are reported in tertiary care hospitals.[4] Almost half of the nosocomial infections are related with the use of invasive medical device.[5] There are many methods of sterilizations of medical devices. Most of pharmaceutical and medical devices are sterilized by gamma radiation. Radiation treatment is better than ethylene oxide (EtO) sterilization, as it leaves no toxic residue on product as well as has good penetration power.[6] However, half of disposable healthcare products are sterilized by gamma radiations worldwide.[7]

The aim of this study was to estimate the microbial load on medically important invasive devices and optimizing the safe dose of gamma radiation for complete elimination of all microbial contaminants. This will contribute considerably to the advancement of heath-care standards in Pakistan by providing sterilization facilities to locally produced medical and surgical devices.

METHODOLOGY

The research study was conducted at the Department of Biotechnology, Lahore College for Women University, Lahore, Pakistan, from September 2014 to June 2015. Approval of this study was obtained from the institutional Ethical and Research Board of the University. Samples from 3 pharmaceutical companies selected on the basis of demand in local hospitals of Lahore, Pakistan. A total of 36 EtO sterilized medical devices (endotracheal, Nelaton, and tracheostomy) of same batch of 3 different companies were collected.

Standard plate count (SPC) method was used for isolation and total viable count (CFU/mL) of micro-organism. Each sample was rinsed thoroughly in a 250 mL beaker containing 100 mL of sterile 0.9% saline solution. About 100 µl of this solution was spread on sterilized nutrient agar plates. Plates were aerobically incubated at 37°C for 24 hours. After incubation, total viable count was determined by counting the colonies on Nutrient agar.

Mac-Conkey agar was used for gram negative lactose and non-lactose fermenter bacteria. In addition, fungal contamination was checked on potato dextrose agar. Bacteria were initially identified on the basis of morphology and gram staining, and biochemical characterization was done by API 20 identification system (Bio Merieux, France).

The devices showing high microbial load were sent for irradiation at 20, 25 and 30 KGy. 25 KGy is considered a standard dose for sterilization of medical devices.[8] For gamma radiation, samples were sent to Pakistan Radiation Services (PARAS). After irradiation of samples, colony forming units (CFU)/mL was again determined. On the basis of results, gamma radiations dose was optimized for complete elimination of micro-flora.

All the data obtained was analyzed using SPSS software (version 20). The data in each group was normally distributed and one-way analysis of variance (ANOVA) was used to compare the value of CFU/ml of irradiated and non-irradiated samples along with negative and positive control. The values were expressed as mean ±SD. A p-value of lower than 0.05 is considered as statistically significant.

RESULTS

The average number of colonies on nutrient agar for 3 companies (A, B and C) of endotracheal, Nelaton, and tracheostomy tube were recorded before and after selected doses (20, 25 and 30 KGy) of Cobalt 60 source radiations (Table I). Comparative analysis of micro flora among the devices from each company revealed that microbial load was high in both endotracheal and Nelaton tubes of company A, whereas tracheostomy tubes of company B was the most contaminated one. However, no microbial growth was observed on Mac-

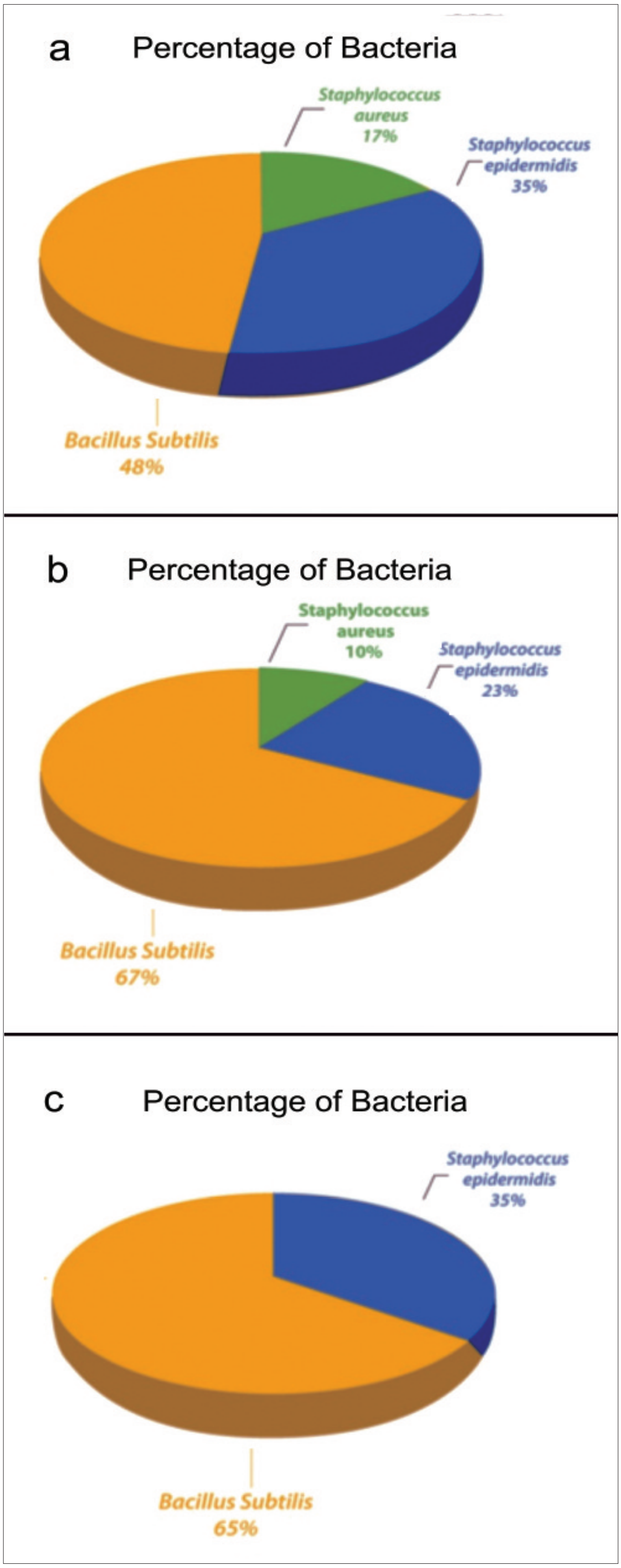

Figure 1: (a) Percentage of three different types of bacteria in samples of company A, B and C of endotracheal tube. **(b)** Percentage of three different types of bacteria in samples of company A, B and C of Nelaton tube. **(c)** Percentage of two different types of bacteria in samples of company A, B and C of tracheostomy tube.

Table I: Bacterial population (CFU/sample) of radiated and non-radiated endotracheal, Nelaton, and tracheostomy tubes.

Medical device	Results		Bacterial population (CFU/sample) mean ±SD		
			Company A	Company B	Company C
Endotracheal tube	Results before radiation		4x10^3 ±0.4161	2.4x10^3 ±0.2575	3x10^3 ±0.468^3
	Results after radiation	20 KGy	4x10^3 ±0.4000	Nill	Nill
		25 KGy	2x10^3 ±0.2828	Nill	Nill
		30 KGy	Nill	Nill	Nill
Nelaton tube	Results before radiation		5.33x103 ±0.6108	3x10^3 ±0.4384	2x10^3 ±0.4195
	Results after radiation	20 KGy	5.11x10^3 ±0.4898	Nill	Nill
		25 KGy	4.23x103 ±0.6324	Nill	Nill
		30 KGy	Nill	Nill	Nill
Tracheostomy tube	Results before radiation	3x10^3 ±0.4384	7x10^3 ±0.6722	2.33x10^3 ±0.3771	
	Results after radiation	20 KGy	Nill	6x10^3 ±0.6324	Nill
		25 KGy	Nill	3x10^3 ±0.4000	Nill
		30 KGy	Nill	Nill	Nill

Results are mentioned as mean ±Standard deviation.

Conkey and potato dextrose agar, indicating the absence of gram negative bacterial and fungal contamination on these medical devices.

Results of this study showed that *Staphylococcus epidermidis, Staphylococcus aureus* and *Bacillus subtilis* were common microbial flora on endotracheal, Nelaton, and tracheostomy tubes and their relative frequencies are mentioned in (Figure 1a, 1b and 1c), respectively. Prevalence of *Bacillus* spp. was found to be high among all microbial flora (Figures 1a, 1b and 1c).

After gamma radiation, it was found that bioburden was high at 20 KGy, whereas subsequent reduction was observed at 25 KGy and complete elimination of the mirobes was achieved at 30 KGy for all selected samples (Table I).

DISCUSSION

Almost half of all nosocomial or healthcare associated infections are related with the use of invasive devices.[5] A number of factors such as endotracheal and tracheostomy intubation, as well as urinary catheterization contributes significantly to the development of nosocomial infections among critically ill patients.[9] Improper sterilization of medical devices is another major factor in elevating the risk of nosocomial infections. Healthcare associated infections (HAIs) are seen worldwide; but in developing countries like Pakistan, rate of nosocomial infections is high.

In view of this situation, one approach is use of gamma radiation as method for effective sterilzation of surgical devices. It is gaining extensive consideration in many parts of the world. A number of developed nations have adopted this method as the ultimate procedure for sterilization of medical devices.[8] Trends are also shifting toward this sterilization technique among developing countries as well. Gamma radiations have high peneration power and leave no toxic residues, unlike in EtO sterilization. EtO is highly toxic, carcinogenic, irritating, water and organic solvent soluble, and explosive. These disadvantages of EtO may limit its usage for sterilization of medical devices, and hence an increase in the shift towards gamma radiation.[10] In Pakistan, considerable work has been reported on the optimization of gamma radiation dose of cotton, bandages, sanitary pads, and needles; but not on the medical devices under study.

In this study, gram positive cocci and rods were commonly observed bacteria whereas no gram negative bacteria and fungal contamination were found. The presence of both gram negative and positive bacteria is reported on medical devices.[11] Gram negative are mostly pathogenic microorganisms which play a major role in nosocomial infections. *Staphylococci (S.)* particularly *Staphylococcus epidermidis* is causative agent of many infections by contaminated temporarily and permanently inserted devices.[11,12] *S. aureus* also contribute in major health problems like sepsis, thrombosis, endocarditis etc. Normally these bacteria are commonly present on skin and mucous membrane. However, they are opportunistic pathogens and may cause serious health issues in patients and may be associated with high morbidity and motility in the healthcare units.[13] On the other side, gram positive rods, i.e. *Bacillus* spp. are also involved in different infections.

These results revealed complete elimination of isolated gram positive *Staphylococci* was achieved at 25 KGy but gram positive bacilli (*Bacillus subtilis*) were able to resist this radiation dose. The ability of *B. subtilis* to produce spores and their thick cell wall enables them to persist in extreme conditions, making it difficult to be eliminated.[14,15] Therefore, radiation dose was raised to 30 KGy at which *B. subtilis* and all other bacteria were effectively eliminated. While recommended dose for medical devices is 25 KGy,[8] it may be high or low due to the nature of the contaminants.

The facility of sterilization can contribute considerably to the advancement of healthcare standards in Pakistan by providing sterilization facilities to locally produced medical and surgical devices. It also reduces cost of care, since secondary infections among patients may complicate their illness and recovery, and it may also result in prolonged hospital stay.[16]

CONCLUSION

On the basis of results, it is suggested that 30 KGy is the optimized dose for complete sterilization of medical devices (endotracheal, Nelaton, and tracheostomy tubes) which may help reduce the equipment-born infections in the healthcare units of Pakistan.

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