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**REDUCING THE RISK OF NOSOCOMIAL ILLNESSES BY TARGETING THE
VIABLE MICROFLORA ON OXYGEN MASK AND NASAL OXYGEN CANNULA
THROUGH COBALT-60 SOURCE**

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ABSTRACT

Medical and surgical devices that are being used in healthcare facilities need to be sterilized properly because number of people die every year and are succumbed to different infections. Therefore, this study focuses on determining the bioburden of nasal oxygen cannula and oxygen mask of three different commonly used companies of Pakistan to optimize gamma radiation dose for complete elimination of microflora present on these medical devices. Samples were checked for microbial load by using basic media (nutrient agar, MacConkey agar, potato dextrose agar). Microflora was identified by biochemical characterization using API for gram positive (bioMerieux). The results showed the presence of *Staphylococcus epidermidis*, *Bacillus subtilis* and *Staphylococcus aureus* species on samples. Samples showing maximum bioburden were sent to PARAS (Pakistan Radiation Services) for irradiation at different selected doses of gamma rays; 15, 20, 25 and 30 kGy to examine the most appropriate dose for the proper sterilization. It was observed that nasal oxygen cannula and oxygen mask were completely sterilized at 25 and 30 kGy respectively. Hospital acquired infections are considered fatal all over the world but

these are given less emphasis in developing countries like Pakistan where there are no strict infection control practices hence this negligence towards sterilization of medical equipments results in high frequency of nosocomial infections. Therefore, this study was designed to alleviate the risk of nosocomial infections and complications of surgery.

Keywords: Bioburden, gamma radiation, oxygen mask, nasal oxygen cannula, immunocompromised

INTRODUCTION

Effective and proper sterilization of medical devices utilized in hospitals is of critical importance for the improvement of health care facilities as it directly correlates with patient safety and disease transmission. Hospital acquired infections have significant ramifications for patient morbidity and mortality. Sterilization of medical equipments is done to prevent transmission of microorganisms and to avoid outbreaks and other infections [1].

Approximately 6% of patients acquire nosocomial infections and incidence rate is increasing day to day. Common nosocomial diseases are associated with urinary tract, respiratory tract, surgical wounds due to use of intravenous cannulas [2]. In low income countries rate of nosocomial infections in newborns is 3-20 times higher than in developed countries [3].

Mostly microorganisms attach to surfaces of indwelling medical devices and form biofilms which usually include gram-positive or gram negative bacteria and yeasts. The

most common gram positive bacteria isolated from medical equipments are *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus viridans*, *Enterococcus faecalis* and gram-negative bacteria *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli* [4]. *Candida* species is most common fungal species that form adhesive layer on surface of medical equipments [5].

Nasal oxygen cannula is a tubing with two prongs that fit inside nose openings and are used in oxygen therapy of patients suffering from respiratory diseases. The surface irregularities of nasal endoscopes and other medical equipments give rise to microbial adherence of pathogens [6]. Oxygen masks are mostly used in intensive care units for treating the patients in critical condition suffering from serious ailments [7].

Gamma radiation sterilization is gaining world wide acceptance because it has been proved to be a useful method for the industrial sterilization of disposable medical

products. Gamma radiation leads to destruction of microorganisms by targeting their DNA, thus disabling them to reproduce and survive. In this process materials to be sterilized are bombarded with high energy gamma rays. The collision reactions result in ionization and formation of free radicals which are responsible for destruction of chemical bonds ultimately breaking down the microbial DNA [8]. Formerly, EtO (ethylene oxide) sterilization was used to sterilize medical products but this gas is toxic and carcinogenic and it can handle low volume of International Atomic Energy Agency recommended that the effective dose of sterilization is 25kGy (2.5Mrads) to attain sterility assurance level of 10^{-6} but this dose varies depending upon bioburden [10].

Hospital acquired infections caused by improperly sterilized medical devices are major cause of morbidity and mortality in Pakistan [11]. This study was carried out to estimate microbial load on surgical devices (nasal oxygen cannula and oxygen mask) for optimization of gamma radiation dose to completely sterilize these medical equipments for safe usage. Complete sterilization of medical devices through gamma rays is of utmost importance as it prevents the growth and spread of diseases. Gamma radiation sterilization is very

products so, later on trends shifted towards use of gamma radiation because of its ability to handle large volumes of packaged products of different densities at different doses. Through gamma radiation sterilization heat sensitive products can be managed [9].

After realizing the importance and beneficial effects of gamma radiation sterilization on medical equipments, PAEC (Pakistan Atomic Energy Commission) established facility PARAS (Pakistan Radiation Services) in 1987 for sterilization of disposable medical equipments.

penetrating and effective and an optimized dose of proper sterilization is investigated in this study which assures the safety of public health.

MATERIALS AND METHODS

Survey and Samples collection

There are many different pharmaceutical companies manufacturing and distributing nasal oxygen cannula and oxygen mask in Pakistan. Nine samples of three companies were selected on the basis of their usage and cost in private and public sector hospitals. All samples were mentioned EtO (ethylene oxide) sterilized.

Isolation and enumeration of microflora

To culture microflora different types of media e.g, nutrient agar, MacConkey agar and potato dextrose agar were used to

determine total bacterial count, total coliform and total fungal count respectively. The experiment was performed in triplicate. Positive and negative controls were also run along with the experiment. Normal saline (0.9%) solution was prepared and passed through the interior cross section or working area of samples. 100µl of passed saline solution was spreaded over the prepared media (nutrient, MacConkey and potato dextrose agar) and incubated for 24-48 hours . After colony count, morphology of colonies was recorded. The resulting colonies were further proceeded for microscopy and biochemical characterization to identify the residing microflora on the samples.

Microscopy

Gram positive and gram negative bacteria were differentiated using gram staining procedure based on their cell-wall structure. The smear of microbial colony to be identified was prepared, dried, heat-fixed and flooded with several reagents like crystal-violet, iodine, ethanol and safranin. After staining, the slide was observed under microscope and results were recorded [12].

Biochemical characterization using API for gram positive (bioMerieux)

API test was performed by preparing a suspension of microbial colony from freshly streaked plates. For the preparation of

bacterial suspension three colonies were picked from freshly streaked agar plates and transferred to 2ml of saline solution. With the help of micropipette, 100µl of suspension was poured into each of well. The strip was incubated at 37°C for 48 hours after which results were observed [13].

Dose optimization of gamma radiation

The effective dose of sterilization for medical device is 25 kGy (2.5 Mrads) to attain sterility assurance level of 10^{-6} but this dose varies depending upon bioburden, resistance of microbial population and material compatibility [10] . Four different radiation doses 15, 20, 25 and 30 kGy were selected to radiate the samples in order to determine which dose was most effective for complete elimination of microbial load. Bioburden of the irradiated samples were determined after radiation. The saline was passed through the internal tubing of the samples. Saline was spreaded over the media followed by incubation at 37°C and results were determined after 24-48 hours. Significant reduction of bioburden was noticed with the increase in radiation dose.

Statistical analysis

All data were analysed using Co-stat software and results are mentioned as \pm standard deviation.

RESULTS & DISCUSSION

Results of the study showed that gram positive bacteria cocci and rods are present on non-irradiated samples and viable count of each sample is mentioned in table 1.

Total viable count after radiation at different selected doses (15, 20, 25, 30 kGy) are described in (Table 2).

Table 1: Average bacterial count of non-irradiated samples on nutrient agar

Sample	Nasal Oxygen Cannula			Oxygen Mask		
Company	A	B	C	A	B	C
CFU/ml	$7 \times 10 \pm 0.344$	$2 \times 10 \pm 0.0942$	$8 \times 10 \pm 0.5827$	$3 \times 10 \pm 0.623$	$4 \times 10 \pm 0.617$	$2 \times 10 \pm 0.374$

Comparison of three different companies shows that maximum microbial count was present on company C and company B of

nasal oxygen cannula and oxygen mask respectively (Fig 1a and Fig 1b).

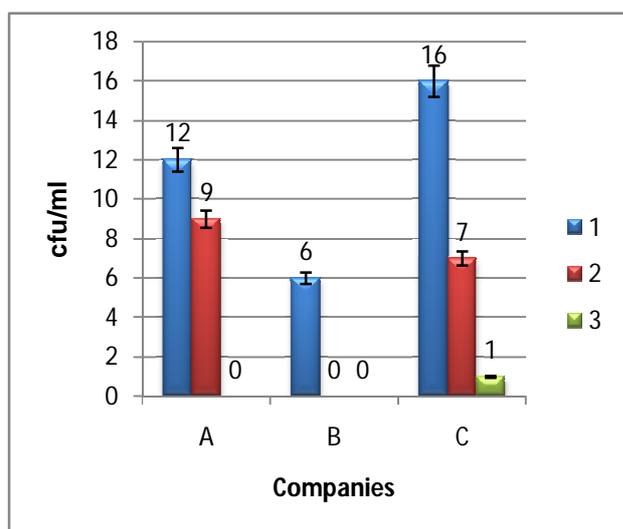


Fig 1a: The complete microbial count over all samples of nasal oxygen cannula belonging to company A, B and C

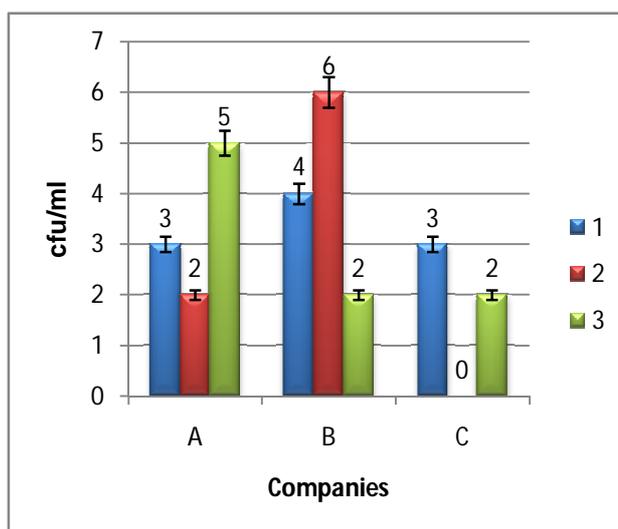


Fig 1b: The complete microbial count over all samples of oxygen mask belonging to company A, B and C

Table 2: Enumeration of bacterial count over irradiated samples

Sample	Doses	CFU/ml
Nasal Oxygen Cannula	15 kGy	$4 \times 10 \pm 0.6324$
	20 kGy	$1 \times 10 \pm 0.2828$
	25 kGy	NIL
Oxygen Mask	20 kGy	$6 \times 10 \pm 0.4$
	25 kGy	$3 \times 10 \pm 0.6324$
	30 kGy	NIL

Results exhibited bioburden decreases with the increase in radiation dose and 25 kGy and 30 kGy are optimized doses for

sterilization of nasal cannula and oxygen mask respectively (Fig 2a and 2b).

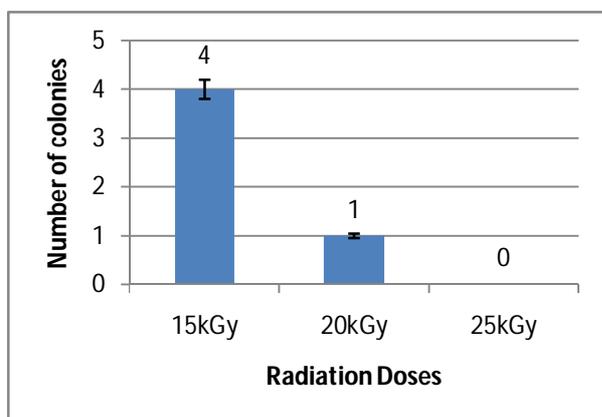


Fig 2a: Comparing bioburden on irradiated samples of nasal oxygen cannula

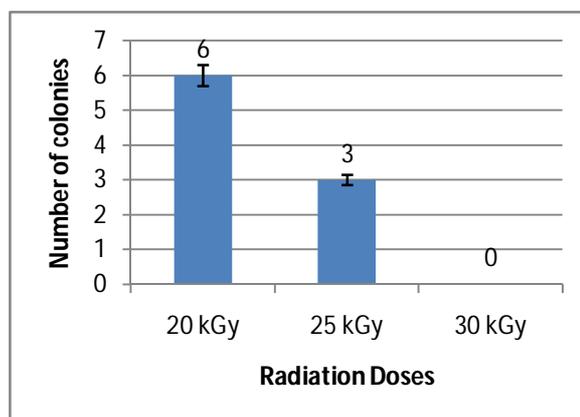


Fig 2b: Comparing bioburden on irradiated samples of oxygen mask.

No microbial growth observed on MacConkey agar and Potato dextrose agar which indicated that gram-negative bacteria and fungi was absent on all samples of nasal oxygen cannulas and oxygen masks.

aureus and *Bacillus subtilis* on EtO sterilized medical devices. The percentage distribution of microorganisms on both medical devices is shown in (Figure 3a and 3b).

This study indicated the presence of *Staphylococcus epidermidis*, *Staphylococcus*

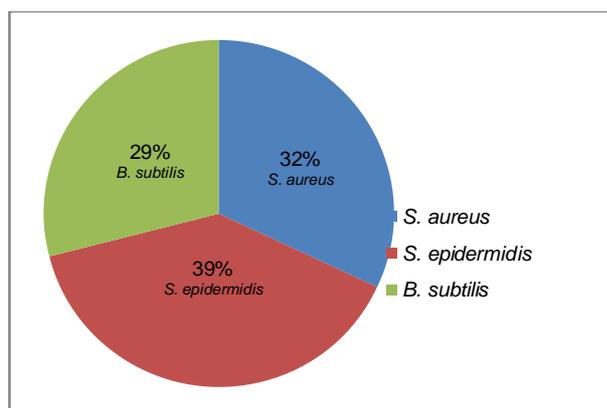


Fig 3a: Distribution of microorganisms over nasal oxygen cannula

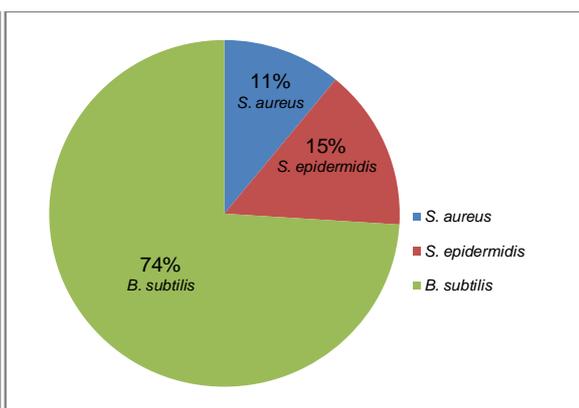


Fig 3b: Distribution of microorganisms over oxygen mask

Bacillus subtilis is an opportunistic pathogen that is widely dispersed in the hospital environment and its air borne spread can result in eye and sinus irritation, sore throat, fatigue and dizziness [14]. *B. subtilis* is resistant towards radiation and survived the standard dose 25 kGy. Therefore, dose required for complete sterilization of oxygen mask was 30 kGy as the percentage of *B. subtilis* was 74% of total microflora isolated from oxygen mask whereas, nasal oxygen cannula was sterilized at 25 kGy because *B. subtilis* was 29% of whole bioburden. *Staphylococcus epidermidis* is also included in the top five microorganisms responsible for causing nosocomial infections [15]. *Staphylococcus epidermidis* contributes towards number of diseases of which septicemia, endocarditis, endophthalmitis, urinary tract infections, vascular graft infections and shunt infections are common [16]. *Staphylococcus aureus*, an opportunistic pathogen is causing diverse diseases ranging from life-threatening diseases like sepsis to minor skin infections. It has been estimated that approximately 20% of humans are carriers of *Staphylococcus aureus* and results in number of infections like pneumonia, meningitis, bacteremia, endocarditis, skin and wound infection [17]. A research based study was carried out in

pediatric hospital of Karachi, Pakistan and 124 pure culture samples of *P. aeruginosa*, *S. aureus* and *Klebsiella species* were collected and results exhibited incidence rate greater than 24%. Nosocomial infections are major problem of developing countries like Pakistan because of unhygienic conditions and use of inadequately sterilized medical equipments [18]. Gamma radiation sterilization is preferred method because it ensures high sterility assurance level and due to high penetration power it can handle bulk products [9]. There are certain health hazards and ecological problems associated with EtO (ethylene oxide) sterilization e.g, it is carcinogenic and cause sore throat, dyspnea, nausea, dermal irritations or burns, headache, eye pain and vomiting [19].

CONCLUSION

The results obtained indicated the presence of *S. aureus*, *S. epidermidis* and *B. subtilis* on oxygen mask and nasal oxygen cannula which were already sterilized with ethylene oxide. Dose optimization exhibited that bioburden decreases with increase in radiation dose and 25 kGy and 30 kGy were appropriate doses for complete sterilization of nasal cannula and oxygen mask respectively. Radiation sterilization is proved to be the most safe, effective, reliable and

promising technology for healthcare products and medical devices and this will raise the standards of health and reduce the risk of nosocomial infections.

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