GAMMA IRRADIATION OF DISPOSABLE SYRINGES USING COBALT-60 SOURCE

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Abstract

Sterilization at the time of manufacturing is important step in production of disposable syringes. It removes harmful microbes present on disposable syringes. Dry heating, autoclave, ethylene oxide and wet heating are Conventional methods of sterilization and are not much effective as some organisms show resistance against them. Determination of the microbial load from disposable syringes and gamma radiation dose optimization was the objective of this study. Bio burden from disposable syringes was analyzed by direct plating method using specific media. Microbes isolated in present study were coagulase negative *Staphylococcus epidermidis, Bacillus subtilis*, *Micrococcus roseus*, *Staphylococcus aureus*. After bio burden detection samples were sent to Pakistan Radiation Service (PARAS) for γ-radiation dose optimization by cobalt-60. Samples were irradiated at 25 kGy, 30 kGy and 35 kGy. No microbial growth was seen at 35 kGy which shows absence of contamination and makes disposable syringes safe for use.

Introduction

Needles and syringes are important product of medical industry and it comes in direct contact with blood so there sterility must be assured otherwise it may cause blood borne disease (Battersby *et al.*, 1999).
Microbial load over the surface should be removed by Sterilization process (Siritientong *et al.*, 2011). In 1998, Pal and Chattopadhyay, subjected sterilized disposable needles and syringes to sterility testing of aerobic cultures and 56.3% of the samples tested were found contaminated showing failure of the sterilization process. In 1985, Ward and Mostafa reported different causes of contamination which may occur due to the puncturing of packs. No assurance of sterility can be granted, once the seal has been broken. Eighteen percent of the syringes were found damaged out of 308 syringes tested by performing ballooning test. The present study has been designed to check the sterility of disposable syringes and dose optimization by gamma radiation to sterilize them.

Materials and Methods

Survey: Several companies and pharmacies were visited to meet the distributors and manufacturers of disposable syringes in different areas of district of Lahore, Punjab. Pharmacies included were private pharmacies as well as hospital attached pharmacies. Detailed questionnaires were made about the disposable syringes available in Lahore and those were filled by distributors and manufacturers to gather relevant information.

Sampling: Samples were collected from various pharmacies. For the isolation of microbial flora from the samples, three types of media i.e. Nutrient agar, MacConkey agar and PDA were used. All Media were prepared by adding measured amount of media in distilled water and then sterilized by autoclave. Media were then poured in petri plates and pre-incubated petri plates for 24 hours to check the presence of contamination in prepared media. Contaminated petri plates were discarded after 24 hours and rests of the petri plates were used for culturing. Negative control for each sample was made by spreading normal saline on three plates of each media and then incubated at specific temperature. For one sample, 1 mL of sterilized normal saline was filled in barrel of the three syringes. Shake for 30 seconds and then move plunger up and down for 30 seconds. 0.1 mL normal saline from each syringe was directly poured onto the prepared media plate of each media. Same plating procedure was used for all other four samples. Spread sample solution by sterile spreader. Petri plates of MacConkey agar and nutrient agar were incubated at 37^{o} C for 24 hours and PDA plates were at 30^{o} C for 72 hours. Petri plates were checked for the microbial growth after incubation. Viable colonies on the petri plates were counted on the basis of morphology, color, shape, texture, number and elevation (Table 1).

γ-irradiated: After sterility testing samples on which bio burden was found were sent to PARAS for dose optimization by gamma radiation (25 k Gy, 30 k Gy, 35 k Gy). Expiry date at the time of testing was checked for samples. Possibilities of small punctures were eliminated by performing ballooning test. All samples were within expiry date and ballooning test showed negative results.

Microbial identification: Growth on selective media, gram staining and API strips techniques was used for identification of bio burden. Selective isolation and enumeration of *Staphylococcus* bacteria was done by culturing on Mannitol salt agar, while for biochemical identification of bacteria up to species level, API strips were used. 20E API has been particularly used in this study. It contains 20 separate compartment, all dehydrated. Streaking of each microbial colony was done to fulfill the requirement of fresh culture for API testing.

Statistical analysis: Statistical analysis was done with the help of (SPSS-24) and ANOVA was performed. For mean comparison the nonparametric test was used; p-values below 5% level were considered significant. \

Results and Discussion

All samples tested in this study were ethylene oxide sterilized and 60% were found to be contaminated. These samples were most commonly used among the patients.
B. subtilis (18%), coagulase negative *S. epidermidis* (73%), *S. aureus* (7%) and *M. roseus* (2 %) were isolated during present study *(*Figure 1), which were biochemically characterized by using Analytical Profile Index API strips (Figure 2).

Results obtained were almost similar to those of Pal and Chattopadhyay (1998), in their study, microorganisms isolated were *Bacilli* species (11%), *Micrococci* (6%), *S. epidermidis Sp. , S. marcescens Sp.* (2%), *P. aeruginosa Sp*.

B. subtilis is most commonly found on disposable syringes and is present in soil and is normal gut flora of humans and thought to be normal gut commensal (Hong *et al.*, 2009). *S. aureus* is a major cause of soft tissue, skin, respiratory, joint, bone, and endovascular disorders (Lowy, 1998). *S. epidermidis* a Gram-positive bacterium (International Association of Microbiologists International Committee on Bacteriological, 1951) is also a part of the normal human flora, typically found on skin, and less commonly in mucosal membrane. It effects mostly immunocompromised people and infections are generally hospital-acquired. It is also found to be usual contaminant of specimens sent for diagnostic laboratory (Otto, 2009). *M. roseus* also affect immunocompromised people, like people having HIV.

Sterility of disposable syringes by Gamma radiation (Co-60) is a new and most promising technique. All the contaminated samples were send to PARAS and irradiated at different Doses i.e. 25 k Gy, 30 k Gy and 35 k Gy and then optimized specific doses which effectively killed all microbes and then retesting of samples showed no microbial growth.

Conclusion and Recommendation: This study concludes that conventional methods of sterilization are not effective enough for some microbes. Medical devices should be gamma irradiated to ensure proper sterilization. No microbial growth was seen on samples irradiated at 35 k Gy, hence considered as an optimum dose for sterilization of disposable syringes.

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Table 1. Characteristics of microbes found in present study.

Bacteria	Colony morphology	Elevation	Opacity	Color	Gram characteristic
Bacillus subtilis	Round with growing margins	Flat	Translucent	White	Gram positive
Micrococcus roseus	Round with smooth margins	Slightly convex	Opaque	Pink	Gram positive
Staphylococcus epidermidis	Round with smooth margins	Slightly convex	Opaque	White	Gram positive
Staphylococcus aureus	Round with smooth margins	Slightly convex	Opaque	Golden yellow	Gram positive

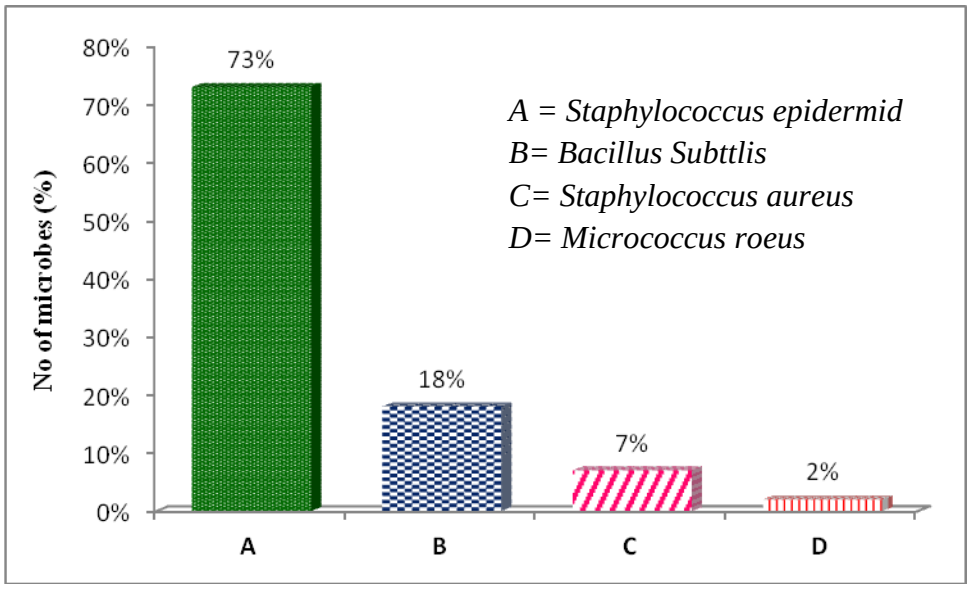

Fig. 1. Number of microbes present on sample before irradiation.

Figure 2. API strip showing positive biochemical tests for *Micrococcus roseus.*

References

Battersby, A., Feilden, R. and Nelson, C. (1999). Sterilizable syringes: excessive risk or cost-effective option. *Bulletin of the World Health Organization*, 77(10): 812-819.

Hong, H. A., Khaneja, R., Tam, N. M., Cazzato, A., Tan, S., Urdaci, M., Brisson, A., Gasbarrini, A., Barnes, I. and Cutting, S. M. (2009). *Bacillus subtilis* isolated from the human gastrointestinal tract. *Research in Microbiology*, 160(2): 134-143.

International Association of Microbiologists International Committee on Bacteriological, N. (1951). International journal of systematic bacteriology. *International Journal of Systematic Bacteriology*.

Lowy, F. D. (1998). *Staphylococcus aureus* infections. *New England Journal of Medicine*, 339(8): 520-532.

Otto, M. (2009). *Staphylococcus epidermidis*-the 'accidental' pathogen. *Nature reviews. Microbiology*, 7(8): 555-567.

Pal, D. and Chattopadhyay, U. K. (1998). Sterility testing of disposable syringes and needles marketed in Calcutta. *Indian Journal of Public Health*, 42(4): 131-132.

Siritientong, T., Srichana, T. and Aramwit, P. (2011). The effect of sterilization methods on the physical properties of silk sericin scaffolds. *American Association of Pharmaceutical Scientists*, 12(2): 771-781.

Ward, R. M., and Mostafa, S. M. (1985). Sterility of disposable syringes. *Anaesthesia*, 40(2): 201-202.