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Radiosterilization of cotton based product for the improvement of microbiological quality

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ABSTRACT

The study was designed to determine the bioburden of locally manufactured surgical gauzes. Seven surgical gauzes labelled as 'sterile' were cultured onto nutrient agar, MacConkey Agar, Mannitol Salt Agar and Sabourad Dextrose Agar for the determination of Total Viable Bacterial Counts (TVBC), Total Coliform Count (TCC), Total Staphylococcal Count (TSC) and Total Fungal Count (TFC), respectively. All samples were found to be contaminated at 10^1 to 10^5 CFU/g level with bacterial contaminants such as *Bacillus spp.*, *Streptococcus spp.*, *Micrococcus spp.*, and *Staphylococcus aureus*. For quality improvement, samples were subjected to a series of radiation doses of 2.0, 2.5, 5.0, 15 and 25 kGy. Among bacterial isolates, species of *Bacillus* showed highest level of resistance to radiation dose. A dose of 25 kGy was found suitable for the total elimination of contaminants rendering the product sterile.

KEYWORDS

Surgical gauze;
Radiation sterilization dose;
Contaminants.

INTRODUCTION

Sterile surgical products are a prerequisite for improving the public health service of any country. In many developing countries including Bangladesh, due to the unhygienic practice in production line, surgical products are occasionally found to be heavily contaminated with pathogenic microorganisms. The ill-equipped system and the use of unsterilized surgical products used during operation are the major routes of hospital infection and cross infection. This would eventually prolong the sufferings of a patient in hospital.

Microbiological quality control of different kinds of single use, prepacked medical or surgical products (e.g. syringes, catheters, surgical gauze, surgical gloves etc) is therefore an important aspect in public health issue. Sterilization is the final manufacturing operation that can significantly affect the safety and effectiveness of a finished product. There are several methods for sterilizing different kinds of medical products such as dry heat, moist heat, filtration, chemicals (e.g., ethylene oxide and formalin), radiation sterilization etc..

Among these sterilizing methods, radiation sterilization is a convenient and cost effective method for achieving a high level of sterility[1-3]. The ability of ionizing radiation to destroy microorganisms has been well documented, and has been commercially applied for surgical purposes during the last few decades. This

method has the ability to irradiate large volume of products at the same time. Moreover, being a cold process (where no or very negligible heat is generated), a wide array of medical products including thermolabile plastic based products can be easily and safely sterilized by this method[4]. Radiosterilization of healthcare products already sealed in containers ensure that, once an item is sterilized, it cannot be contaminated before use.

Bangladesh is a developing country which lacks sterilization facilities in most areas. Especially, when a product is cheap, the manufacturers are reluctant to perform radiation sterilization to avoid extra cost and often try to escape such practice. Moreover, most of the manufacturers do not comply with good manufacturing practice (GMP) and hazard analysis critical control point (HACCP) and other standard code of practices. Therefore, these locally made medical items available in the market are not beyond suspicion of having contamination with microorganisms including pathogens. Considering this point, an initiative was taken to investigate the microbiological quality of locally manufactured cotton-based medical products (surgical gauze) in and around Dhaka city. The study also aimed to achieve sterilization and quality improvement of these surgical products using gamma rays.

MATERIALS AND METHODS

The product sample employed was cotton gauze from seven different manufacturers. Samples were collected from different places of Dhaka city and its adjoining areas i.e. Nabinagar, Savar and Mitford road and were coded as S1, S2, S3, S4, S5, S6 and S7. Each sample (10g) was placed in a sterile conical flask containing 90 ml of sterile saline solution and was shaken at 30°C for 30 min. In the next step, the microbial distribution of each samples were analyzed by serial dilution using Nutrient Agar (NA), MacConkey Agar (Mac Agar), Mannitol Salt Agar (MSA) and Sabouraud Dextrose Agar (SDA) for the determination of Total Viable Bacterial Counts (TVBC), Total Coliform Count (TCC), Total Staphylococcal Count (TSC) and Total Fungal Count (TFC), respectively. Bacterial isolates from different surgical gauzes samples were identified based on morphological, cultural and biochemical characteristics following the "Bergey's Manual of determinative bacteriology" 8th edition[5].

In order to inactivate relatively sensitive microbial contaminants, all the samples were exposed to a screening radiation dose (i.e. 2.0, 2.5, 5.0, 15.0 and 25.0 kGy) by Co^{60} gamma radiation source (50,000 Ci) at the Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Savar, Dhaka, Bangladesh. At this time, total viable microbial counts were determined for the dosed samples. All resultant colonies were picked up, subcultured and maintained as stock cultures of isolates in nutrient agar slants at 4p C. Bacterial susceptibility to antimicrobial agent such as chloramphenicol (C), erythromycin (E), penicillin (P), tetracycline (TC) etc. were determined *in vitro* by Kirby Bauer method[6] by preparing a suspension of the isolate and spreading evenly onto Mueller-Hinton agar in a petri dish. Disks impregnated with various defined concentrations of different standard antibiotics are then placed onto the surface of the agar. After incubation, a clear circular zone of no growth in the immediate vicinity of a disk indicates susceptibility to that antimicrobial. The cut-off values in respect to susceptibility or resistance of the organism to each antibiotic tested were determined by comparing recorded zone diameters with the zone size interpretive chart given in the Clinical Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing: Seventeenth Informational Supplement[7] for strains assigned to *Staphylococcuse aureus* and *Streptococcus* sp. For *Micrococcus* sp. and *Bacillus spp.* strains, Kirby-Bauer zone interpretive chart was followed[6].

RESULTS AND DISCUSSION

Microbiological quality assessment of seven gauze samples was carried out both qualitatively and quantitatively. None of the products were found sterile and TVBC ranged from 8.0×10^3 to 1.1×10^5 CFU/g (TABLE 1). This value is considered very high when compared with the report of Yan et al.[8] and also Yan and Tallentire[9] where the bioburden of surgical gauzes swabs were in the range of 1.1×10^2-1.23×10^2 CFU/g microorganisms. TSC was nil for sample no. 5 which also showed lower TVBC. But for rest of the samples, TSC ranged from 4.0×10^3 to 1.5×10^4 CFU/g. However, there was no coliform and fungal count found in these samples.

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TABLE 1 : Quantitative analysis of microbial load of the surgical product (gauze).

Sample code	TVBC (CFU/g)	TSC (CFU/g)	TCC (CFU/g)	TFC (CFU/g)
S1	2.4×10^4	1.1×10^4	Nil	Nil
S2	3.3×10^4	1.3×10^4	Nil	Nil
S3	2.5×10^4	9.0×10^3	Nil	Nil
S4	8.0×10^3	4.0×10^3	Nil	Nil
S5	9.5×10^3	Nil	Nil	Nil
S6	1.1×10^5	1.5×10^4	Nil	Nil
S7	4.7×10^4	1.3×10^4	Nil	Nil

Isolates were studied for their cultural characteristics on nutrient agar to observe color, opacity, form (whether circular, rhizoidal filamentous, or irregular); elevation (whether flat, raised, convex or undulate) and margin (whether entire, undulate, filamentous or curled) of the colony. The biochemical tests included catalase, oxidase, indole, citrate utilization test, methyl red (MR), Voges-Proskauer (VP), nitrate reduction, urease test, starch hydrolysis, gelatin liquefaction and carbohydrate fermentation test with lactose, dextrose and sucrose (TABLE 2).

Depending on their morphological, cultural and biochemical characteristics, all gram positive isolates were provisionally identified as *Bacillus spp., Staphylococcus aureus, Micrococcus spp.* and *Streptococcus spp*. Yan et al.[8] also reported isolation of mostly spore forming bacteria followed by cocci and non-spore forming rods from similar medical products.

TABLE 2 : Results of biochemical tests for selected isolates found in surgical gauze samples.

Isolate Code	Fermentation			H_2S production	NO_3 reduction	Indole production	MR Reaction	VP Reaction	Citrate utilization	Urease activity	Caltalase activity	Oxidase activity	Gelatin liquefaction	Starch Hydrolysis	Provisionally identified as
	Lactose	Dextrose	Sucrose												
S1-1	-	A	A	-	+	-	-	-	-	-	+	-	+	+	*Bacillus sp*
S1-2	A	A	A	-	+	-	+	-	-	-	+	-	+	-	*Staphylococcus aureus*
S1-3	-	-	-	-	+	-	-	-	-	+	+	-	+	-	*Micrococcus sp*
S2-1	A	A	A	-	+	-	+	+	-	-	+	-	+	-	*Staphylococcus aureus*
S2-2	-	A	A	-	+	-	-	+	-	-	+	-	+	+	*Bacillus sp*
S2-3	-	-	-	-	+	-	-	-	-	+	+	-	+	-	*Micrococcus sp*
S2-4	-	-	-	-	-	+	+	-	-	-	-	+	+	-	Streptococcus sp
S3-1	-	A	A	-	+	-	-	-	-	-	+	-	+	+	*Bacillus sp*
S3-2	A	A	A	-	+	-	+	+	-	-	+	-	+	-	*Staphylococcus aureus*
S3-3	-	-	-	-	+	-	-	-	-	+	+	-	+	-	*Micrococcus sp*
S4-1	-	A	A	-	+	-	-	-	-	-	+	-	+	+	*Bacillus sp*
S4-2	A	A	A	-	+	-	+	-	-	-	+	-	+	-	*Staphylococcus aureus*
S5-1	-	-	-	-	+	-	-	-	-	+	+	-	+	-	*Micrococcus sp*
S5-2	-	A	A	-	+	-	-	-	-	-	+	-	+	+	*Bacillus sp*
S6-1	A	A	A	-	+	-	+	-	-	-	+	-	+	-	*Staphylococcus aureus*
S6-2	-	A	A	-	+	-	-	-	-	-	+	-	+	+	*Bacillus sp*
S6-3	-	-	-	-	+	-	-	-	-	+	+	-	+	-	*Micrococcus sp*
S7-1	-	A	A	-	+	-	-	+	-	-	+	-	+	+	*Bacillus sp*
S7-2	-	-	-	-	+	-	-	-	-	+	+	-	+	-	*Micrococcus sp*
S7-3	A	A	A	-	+	-	+	-	-	-	+	-	+	-	*Staphylococcus aureus*

A= acid production; + = positive result; - = negative result

A dose of 2.0 kGy was applied to the various surgical gauzes whose presterilization microbial count (CFU/g) was determined before (TABLE 1). A radiation dose of 2.0 kGy resulted in a 1 to 2 log reduction in the microbial count. The survivors after having substerilization dose of 2.0 kGy were isolated, purified and identified. Survivor included mostly *Bacillus sp.* and cocci. A dose of 5.0 kGy resulted in the elimination

of Gram positive non spore forming rods. At higher doses of radiation up to 10 kGy, the survival rate was greatly reduc ed. At the dose of 15 kGy, only few spore former bacilli were survived. When the gauzes were irradiated with 25 kGy, all kinds of viable microorganisms including spores were eliminated. TABLE 3 shows the reduced microbial load after irradiation dose of 2.0 and complete elimination after 25 kGy. These results are supported by Johnson and Johnson[10] and Mioara and Ene[11].

TABLE 3 : Bacterial counts of different surgical gauze at 2.0 kGy and 25 kGy radiation doses.

Sample Code	Microbial Count (CFU/g) at 2.0 kGy	Microbial count CFU/g at 25 kGy
S1	9.0×10^3	-
S2	8×10^3	-
S3	4×10^3	-
S4	8.4×10^1	-
S5	2.0×10^2	-
S6	2.0×10^3	-
S7	6.0×10^3	-

In this study, four types of standard antibiotic discs (i.e. chloramphenicol-30 µg, erythromycin-15µg, penicillin G-10 µg, tetracycline-30 µg) were used to determine the susceptibility of radio-resistant strains isolated from surgical gauze samples. Following the reference charts, results were recorded as whether the organism is susceptible (S), intermediately susceptible (I), or resistant (R) to that antibiotic. As shown in TABLE 4, all strains were found to be resistant to penicillin G (P). Except Penicillin, five strains of *Staphylococcus aureus* were found to be susceptible to chloramphenicol (C), tetracycline (TE) and erythromycin (E). *Streptococcus* strain was found resistant to tetracycline (TE), internmediate to chloramphenicol and susceptible to erythromycin (E). *Bacillus* strains were found susceptible to erythromycin (E) and tetracycline (TE) and were found intermediate to chloramphenicol (C). Four strains of *Micrococcus* showed resistance towards chloramphenicol (C) and penicillin G (P). So, on the basis of the data, majority of the isolates from gauze samples were highly susceptible to antimicrobial agents.

TABLE 4 : Susceptibility testing of strains isolated from surgical gauze samples.

Strains (No. of isolates)	Antibiotics (and disc potency)	Zone diameter interpretive standards (mm)			Inhibition zone Diameter (mm)	S/ I/R
		Resistant (mm or less)	Intermediate (mm)	Susceptible (mm or more)		
Staphylococcus aureus (5)	Chloramphenicol (30 µg)	12	13-17	18	26	S
	Erythromycin (15 µg)	13	14-22	23	25	S
	Penicillin G (10 µg)	28	-	29	18	R
	Tetracycline (30 µg)	14	15-18	19	21	S
Streptococcus sp (1)	Chloramphenicol (30 µg)	17	18-20	21	19	I
	Erythromycin (15 µg)	15	16-20	21	23	S
	Penicillin G (10 µg)	-	-	24	18	R
	Tetracycline (30 µg)	18	19-22	23	12	R
Bacillus sp (7)	Chloramphenicol (30 µg)	12	13-17	18	14	I
	Erythromycin (15 µg)	13	14-22	23	25	S
	Penicillin G (10 µg)	28	-	29	20	R
	Tetracycline (30 µg)	14	15-18	19	28	S
Micrococcus sp (4)	Chloramphenicol (30 µg)	12	13-17	18	11	R
	Erythromycin (15 µg)	13	14-22	23	25	S
	Penicillin G (10 µg)	28	-	29	10	R
	Tetracycline (30 µg)	14	15-18	19	22	S

CONCLUSION

The present investigation was undertaken to determine the microbiological load and major microflora associated with seven locally manufactured surgical gauze samples. Before an effective sterilization dose can be established for surgical gauze, it is necessary to determine the initial contamination level and resistance of the contaminants to radiation. The ultimate aim of this study was

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to achieve sterility of these surgical products using gamma rays. Although surgical products (gauzes) should necessarily be sterile, the microbiological counts (especially TVBC and TSC) in this study were very high, indicating the products not safe for use. Some pathogenic microorganisms e.g. *Staphylococcus aureus, Streptococcus spp.* were found in the gauze samples. There may be multiple reasons for the existence of high number of microorganisms including pathogens in the product. In Bangladesh, production and process control is not strongly adhered to HACCP or GMP. As a result, hygienic conditions are not always maintained in the production and processing site. Treatment of samples with a series of radiation doses gradually improved the quality of the products. 25 KGy gamma radiation was found to eliminate all spores and viable cells rendering the product sterile. From this study it is concluded that the available surgical gauzes are not safe for use without sterilization and irradiation process can significantly minimize the health risk or chance of infection of users of this surgical product (gauze).

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