Assessment of Efficacy of Steam Sterilization for Assembled Laparoscopic Instruments- A Microbiological Study

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ABSTRACT

BACKGROUND

The most commonly used infection control methods are disinfection and sterilization. Disinfection technique helps to reduce the chances of contamination but is less effective to pathogenic organisms as compared to sterilization. Hence, we wanted to assess the safety of steam sterilization of the assembled laparoscopic instrument with test infection.

METHODS

Two different types of re-usable laparoscopic instruments were selected as test instruments; trocar and dissection forceps. Biological indicator used in the present study was *Geobacillus stearothermophilus* ATCC-7953 in sporulated form. For the present study, three study groups were defined: an experiment group, a negative control and a positive control. The assessed results gave a total of 1080 sampling units. Individual packing of the instruments in the surgical grade paper was done followed by autoclaving in the pressured saturated steam. Seeding of the biological indicator was done in Tryptic Soy Broth (TSB) culture medium, followed by incubation at 56^{o} C for 21 days. The results were compiled in Microsoft Excel sheet and were analysed by SPSS software.

RESULTS

100 percent satisfactory growth was seen in the positive controls which confirmed the test in the present research. This also confirmed the viability of the culture media along with adequate efficacy of the incubation condition for spore germination.

CONCLUSIONS

It is safe to use pressurized saturated steam sterilization for assembled laparoscopic instruments. However; further studies in this regard are recommended for better exploration of results.

KEYWORDS

Laparoscopic, Steam, Sterilization

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BACKGROUND

The operative site infection is known as surgical site infection (SSI). SSIs are having numerous adverse effects on patients such as postoperative complications, need for additional treatment of SSI, prolonged hospital stay, and even mortality. Substantial research has been conducted to prevent SSI, and, as a result, recommendations have been published as guidelines for SSI.[1-3]

If medical devices & surgical instruments are not properly cleaned, and also if high-level disinfection or sterilization done, health care-associated infections can result, including surgical site infections (SSI).Infections with blood borne pathogens (e.g., hepatitis B and C, HIV), and ventilator- or catheter-associated infections. Therefore, it is critical that the HCWs who are responsible for processing instruments follow all steps carefully to provide adequately clean and apply high-level disinfection or sterilize instruments for patient care.

The most commonly used infection control methods are disinfection and sterilization. Disinfection reduces the chances of contamination but it is not effective like sterilization less effective to pathogenic microorganisms as compared to sterilization. Disinfection does not remove all vegetative spores. Sterilization, however, removes all forms of microorganisms including viruses, bacteria, fungi, and spores.[4,5] Steam sterilization rapidly heats & penetrates. Moist heat kill microorganisms & destroys its cellular components that is important for replication. Advantage of steam sterilization is that it's simple, rapid, safe and cost effective. Some sterilization methods require a 120°C operating temperature, which causes the degradation of thermolabile medical devices. Other limitations are the need for vacuum chambers in common plasma sterilization methods and the use of toxic gases, like formaldehyde or ethylene oxide.[6,7] Under the light of above mentioned results, we have planned the present study to assess the safety of steam sterilization of the assembled laparoscopic instrument with test infection. The classical recommendations state that the heat resistant surgical instruments such as laparoscopic instruments are to be open, disassembled and with the surfaces free for steam sterilization, including the laparoscopic trocars and forceps. But, there are other guidelines that do not emphasize this kind of care. There is no doubt that autoclaving of disassembled materials by the method of thermal conduction provides the best condition.

Among health professionals, there is a myth that to achieve the quality of sterilisation through the saturated pressure steam autoclave, direct contact of the steam with the instrument is necessary, and by this somehow totally abolishing the concept of physical principle of latent heat. The time has come to question these old age concepts by establishing the scientific facts against this type of concept.

As some instruments such as laparoscopic accessories are complexly design and to dismantle and sterilise due to age old concept of doing so, they present a problem for surgical team to correctly assemble in operating field. This has come to the notice also that some surgical paramedics are totally unaware of correct assembly and hence compromise functionality creating unnecessary surgical procedure more stressful and complex by disrupting its start.

The scientific literature does not provide a conclusive answer about the safety of saturated steam pressure sterilization, of the assembled laparoscopic instrument,[8-10] and it suggests conducting a new laboratory experimental test study.[11]

This research aimed to analyse the safety of steam sterilization, of the assembled laparoscopic instrument along with challenge infection, in order to bring to forth correct scientific evidence to support the decisions of the nurses that manage the SSC, focusing on the safety of the surgical patients.

METHODS

The present study was conducted in the department of Microbiology and the department of Surgery with the aim of microbiologically assessing the efficacy of the steam sterilization of assembled laparoscopic instruments. Ethical clearance taken from ethical committee of the institute 'with obtaining of written consent after explaining the whole research project in detail. Two different types of re-usable laparoscopic instruments were selected as test instruments; Trocar and dissection forceps. Biologic indicator used in the present study was *Geobacillus stearothermophilus* ATCC-7953 in speculated form, with population of microbes of 106 UFC/filter paper substrate. The self-contained biological indicator here is built on a substrate of paper of size 2.5x0.5cms with a lower limit of minimum 1 lakh spores which are calibrated *Geobacillus stearothermophilus* ATCC-7953. This microorganism was chosen as it carries the standard for biological monitoring of the effective control of autoclave cycle, as it has the property of humid heat resistance and low pathogenic conditions in normal conditions. For the present study, three study groups were defined: an experiment group, a negative control and a positive control.

Microbial culture results of a total of 185 assembled laparoscopic instruments were analysed of which 185 trocars and 185 forceps were taken. All these assessed bled results gave a total of 1080 sampling units. Analysis of 5 disassembled laparoscopic each total 10units was done as negative controls for a set of 30 sampling culture units. A set of 30 non-sterilized paper filter substrates were used as positive controls. Disassembling of the biologic indicator tubes was done using aseptic techniques, followed by separation of substrate papers with the *Geobacillus stearothermophilus*. During the process of assemblage, three units of paper substrate were placed inside of each laparoscopic instrument. Individual packing of the instruments in the surgical grade paper was done followed by autoclaving in the pressured saturated steam. Dissembling of the instruments was done after sterilization process inside the biologically protected cabinet. Seeding of

the biologic indicator was done in Tryptic Soy Broth (TSB) culture medium, followed by incubation at 56° C for 21 days. In cases of absence of microbial growth at the end of these 21 days, exposure of the tubes to the thermal shock for a time period of 20 minutes followed by re-incubating was done for analysing final readings. All the results were compiled in Microsoft excel sheet and were analysed by SPSS software.

RESULTS

The results of the present study are summarized in Table 1, Table 2 and Table 3. A 100 percent satisfactory growth was seen in the positive controls, which confirmed the test in the present research. This also confirmed the viability of the culture media along with adequate efficacy of the incubation condition for spore germination. Both the negative controls and the laparoscopic instruments sample cultures showed 100 percent negative growth.

Instrument Type	Biological Indicator Placement	Percentage of Positive Cultures
Dissection Forceps	First	0
	Second	0
	Third	0
Trocar	First	0
	Second	0
	Third	0

Table 1. Culture Analysis of Experimental Groups

Instrument Type	Biological Indicator Placement	Percentage Positive Cultures
Dissection forceps	First	0
	Second	0
	Third	0
Trocar	First	0
	Second	0
	Third	0

Table 2. Culture Analysis of Negative Groups

Group	Percentage Positive Cultures
Positive Control	100

Table 3. Culture Analysis of Positive Controls

Infection and Prevention Control: Module 6, Processing Surgical Instruments and Medical Devices.

DISCUSSION

Moist heat used for the sterilization by using steam at high temperature & constant pressure. In the autoclave latent heat changed in to the saturated stem, which kills the bacterium either in the vegetative or spore form completely. According to Boyle's law at high temperature and constant pressure steam change in saturated stem which killed the organism by denatured bacterial protein & nucleic acid. In sterilisation the pressurised steam is the main method for the heat resistant laparoscopic instrument as it not only brings the D value low and with high diffusivity and also penetration of the sterilising agent used but also carry speed, nontoxicity and lower cost. In this process of pressurised saturated steam heat in contact with the material in the autoclave through its cold surface went through condensation, which releases the latent heat of vaporising water and at the same time heating the water. This results in thermal coagulation of protein and leads to killing of the microbes; hence this steam sterilisation is based on heat exchange between the medium and the object which has to be sterilised. This phenomenon occurs in autoclave.

Sterilization of assembled laparoscopic instruments was studied previously,[10-13] concluding both positive and negative related to the practice of autoclaving assembled instruments, even when methodological issues arising from several of these papers.

This is widely used for sterilization of different type object like media for bacterial culture, glassware for different laboratory, surgical article and laparoscopic instrument. The efficacy of stem sterilised control by, both biological and chemical indicators, have gained widespread acceptance to validate autoclave cycles and to monitor the uniformity of processing conditions. Sterilization of assembled laparoscopic instruments was studied previously,[8-10] inferring both positive and negative related to the practice of autoclaving of assembled instruments, irrespective of methodological issues arising from several of these publications.

The first research[8] proposed the hypothesis that the assembled laparoscopic instrument that both assembled and disassembled laparoscopic instrument would have the same sterility using vegetative bacteria suspension (Serratia marcescens) and sporulated bacteria (Bacillus subtilis e Bacillus stearothermophilus as challenge contaminants of laparoscopic forceps and trocar two in number. The inoculation and retrieval was done by swab, retrieving the challenged microorganism in both assembled and dissembled sterilised laparoscopic instrument. Irrespective of the fact that swab methodology permits quantitative analysis, it has limitation in standardising the rolling resistance, during the procedure the degree of angulation and pressure is not able to control reproducibility and the result have vast degree of variability. Autoclave temperature and pressure gauges are insufficient for this purpose, since they do not detect air leaks or air pockets which result in lower temperatures and under processing. Sterility cannot be assured by any indicator since variables such as the initial microbial load, spore resistance, presence of protective substances, and the prior history of the microorganism cannot be controlled. Absolute sterility (100% kill) is theoretically unattainable due to the logarithmic nature of microbial death kinetics.[10,11-13]

Li XL et al analysed the straight contact among the bacterial load on surgical instrument and the time of holding before the disinfection procedure and further Comparison of disinfecting efficacy of hydrogen peroxide (H2O2) glutaraldehyde, and ethyl alcohol on contaminated surgical instruments. Out of the total of 120 in sterilisation pairs, 60

pairs were of tissue forceps and 60 pairs of DeBakey forceps were evaluated in their study. The four different inocula were prepared in the two different medium. The inocula of 5×103 CFU/ml of Staphylococcus aureus, Pseudomonas aeruginosa E. coli were inoculated on the sheep blood agar and the inocula of Bacillus subtilis and their spores were inoculated on the tripticase soy agar plates. Number of colonies were calculated and further compared with the initial time i.e. the time zero after the incubation. The pre-disinfection count of microorganisms were compared and calculated with post disinfection microbial count in every group. To suppress the growth of micro -organisms, nutrient agar used as a medium. In the initial 6h, the bacterial load did not show any change. It was absolutely the same as it was before the 6h. However, after the passage of 6 h, the bacterial load started increased immediately. They inferred that it would be obligatory to clean the stainless steel surgical instruments during the first 6 h after the surgery, so that the accurate and effective serialization of instrument can be achieved.[14]

Moriya G et al evaluated the maintenance of sterility in moist/wet material after being submitted to steam sterilization and stored for a period of 30 days. Total 1600 porcelain cylinders were attached to instruments of surgery for proving the sterility, which were used as carriers for incubation in culture medium. The surgical instruments were kept into the boxes following the standard surgical care practices. 40 surgical boxes packed in nonwoven cloth covering Spunbound, Metblouwn, Spunbound (SMS): half (the experimental group) were placed in an autoclave but the drying phase was interrupted, yielding moist/wet material and the second portion i.e. the other remaining part {negative control group} went through the complete cycle. Each of the surgical boxes were intentionally over contaminated with Serratia marcescens externally, and finally stored for one month. Difference in weight before and after autoclaving of surgical boxes confirms the moisture present in the surgical boxes. After storage, the boxes' contents were submitted to sterility tests and no microbiological growth was observed. The presence of moisture inside the boxes did not interrupt with maintaining their sterility after deliberated external contamination and 30-day storage.[15] A different research[9] used one of the parts of the laparoscopic instrument, a 12mm trocar with its lumen filled with organic material (hamburger meat) and microbial challenge contamination to assess the efficiency of sterilization using 132° C in conventional and flash cycles with exposures of 10 and 3 minutes respectively. All vegetative microorganisms were destroyed with conventional and flash cycles of sterilization. Filling of the lumen with organic material as usual showed resistance to direct contact of steam almost similar condition when sterilised with assembled laparoscopic instrument. In the similar conditions, with organic material as lumen filling[9] researchers tested commercial biologic indicators *Geobacillus stearothermophilus* ATCC 7953 in the trocar lumen without hamburger meat and different time exposures, 3, 4, 5 and 6 minutes. Only when time exposure was increased from 7 to 10 minutes the spores were fully destroyed. These results are in favour of the latent heat microbial killing, in spite of the hard scenario of challenge contamination and massive organic material.

As the standard parameters for pressurized saturated steam with pre-vacuum autoclave are increased to 134° C in 4 minutes the researchers' need[9] of extending the sterilization time to succeed in fully eliminating the test microorganisms may have connection to the higher concentration of the organic material used in filling of lumen of trocar. The present research used the same technique of microbiological challenge and succeeded in killing the spores *Geobacillus stearothermophilus* ATCC 7953 using pressurized saturated steam with pre-vacuum sterilization cycle at 134° C in 5 minutes.

CONCLUSIONS

It is safe to use pressurized saturated steam sterilization for assembled laparoscopic instruments. However, further studies in this filed are needed for better exploration of results. The possibility of sterilizing previously assembled laparoscopic instruments by means of steam under pressure undoubtedly made work easy for many hospitals. Laparoscopic instrument can be safely autoclaved by this methodology for achieving sterilization. Sterilization under pressurized saturated steam of assembled laparoscopic instruments is microbiologically safe, breaking along the paradigm of classic recommendations of autoclaving only disassembled material. Results of this research, under the experiment conditions, are a strong scientific evidence that supports a systematic review of this topic and gives inputs to the decision-making process related to the microbiological safety of pressurized saturated steam sterilization of the assembled laparoscopic instruments. Additionally, it is desirable that it may give inputs to lawmakers to formalize the possibility of sterilisation autoclaving of pre-assembled laparoscopic instruments as well.

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