



Evaluation of Third Generation Quaternary Ammonium Compounds for the Sterilisation of Operation Theatre

Kannan I^{1*}, Thenmozhivalli PR², Paul Sony³, C. Naga Prashanthi⁴

^{1,2,3,4} Department of Microbiology, Tagore Medical College and Hospital, Chennai-600127, INDIA

*Corresponding author's E-mail: microbiology@tagoremch.com

Article History	Abstract
Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 25 Nov 2023	<p><i>Sterilisation of operation theatre is the global method in the prevention of surgical wound infections. In recent years the first- and second-generation compounds are replaced by the third-generation quaternary ammonium compounds. The efficacy of these compounds is still doubtful. Aim: An attempt has been in the present study to evaluate the third-generation quaternary ammonium compounds for its efficacy in sterilisation of operation theatre. Methods: The samples were collected before and after fogging. Quaternary Ammonium Compound D-125 is used in the present study. The presence of bacteria in the indoor air was sampled by plate exposure method. The brain heart infusion agar plate was kept open for 15 minutes at the height of one metre from the level of ground. For the analysis of surface contamination, the sampling was done by swabbing the surface. The swabs were inoculated into Robertson cooked medium (HIMEDIA) and were incubated 37OC for seven days in anaerobic condition. Results: The results showed the complete removal of microorganism after 45 minutes of fogging. Conclusion: The present study has showed that the quaternary ammonium compounds are effective disinfectant and sterilisation agent in the sterilisation of operation theatre.</i></p>
CC License CC-BY-NC-SA 4.0	Keywords: Operation Theatre Sterilisation, Quaternary Ammonium Compounds, Spread Plate Methods, Surface Contamination

1. Introduction

The postoperative bacterial contamination and infection of the surgical wound is the major risk factor during the surgery [1]. Sterilisation of operation theatre is the global method in the prevention of surgical wound infections.

The increase in the incidence of Methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococcus* (VRE) in the hospital environment has raised lot of concern in the sterilisation of hospital environment especially operation theatre [2-5]. The operation theatre is a closed environment and has every chance of contamination during surgery by the patient and theatre staff members. The studies have shown that the predominant bacterial contamination is coagulase negative staphylococcus followed by *Staphylococcus aureus* [6]. It is also found that the nasopharyngeal shedding of the patient and theatre staff members are the main reasons for the contamination [7].

Conventional method of sterilisation of operation theatre

is fumigation by formaldehyde gas as it is effective in killing even the bacterial spores [8, 9]. However, the toxicity and carcinogenicity of formaldehyde gas have raised a lot of concern about the use of it [10]. In recent years the first and second generation compounds are replaced by the third generation quaternary ammonium compounds. The efficacy of these compounds is still doubtful. Hence an attempt has been in the present study to evaluate the third-generation quaternary ammonium compounds for its efficacy in sterilisation of operation theatre.

2. Materials And Methods

It is a cross sectional study conducted in operation theatres of a tertiary care hospital of 750 bed capacity in Chennai, India.

Fogging

The samples were collected before and after fogging. The sterilisation agent used is the combination of alkyl dimethyl benzyl ammonium chloride (2.37%) and alkyl dimethyl ethyl benzyl ammonium chloride (2.37%) produced under the name of DTcare-125 (Chemtex speciliaty limited). It is the mixture of quaternary ammonium compounds with other ingredients like organic silver. It has been recommended for both disinfection and sterilisation. The product is available as the concentrated stock solution that can be used to surface sterilise as well used to sterilise the indoor air.

Quaternary Ammonium Compound D-125 is used at dilution of 1:64 (15ml in 1ltr water) for critical area disinfection such as instruments, OT tables, lamps, critical contact surfaces etc. It is used at dilution of 1:128 (7.5ml in 1ltr water) for non-critical area disinfection such as floor mopping, furniture and wall wiping etc. For air disinfection make a dilution of 1:64 and fog with fogging machine at 1ltr per 1000cft (28.3 cum).

Sampling and Culture

The operation theatres selected for this study were general surgery, ENT, OBG, ophthalmology and orthopaedics. The samples were collected before and after fogging from indoor air and surfaces (OT table, OT light, instrument trolley, suction apparatus, IV stand, Boyle's apparatus, floor and wall) from eight operation theatres of the hospital.

The presence of bacteria in the indoor air was sampled by plate exposure method. The brain heart infusion agar plate was kept open for 15 minutes at the height of one metre from the level of ground. Then the plates were incubated at 37OC for 24 hours in aerobic condition. The number of colonies grown in the plates was counted and noted.

The sterilisation efficacy of surface was assessed by the removal of anaerobic bacterial spores which indicates the thorough removal of all forms of microorganisms. The sampling was done by swabbing the surface. The swabs were inoculated into Robertson cooked medium (HIMEDIA) and were incubated 37OC for seven days in anaerobic condition. After seven days the changes in the media like turbidity and discolouration were noted. Then the media is gram stained to observe the morphology of anaerobic bacilli.

Statistical Analysis

The paired student t test was done to find the significance in the difference in the total number of colonies before and after sterilisation with SPSS vs 20.0 software.

3. Results and Discussion

A total of five operation theatres were selected for the present study. Both the indoor air and the important surfaces were subjected for culture before fogging and after fogging.

TABLE 1. Results of plate exposure method showing the number of bacterial colonies before and after sterilisation.

Operation Theatre	Number of Colonies		P Value
	Before Sterilisation	After Sterilisation	
General surgery	4	0	p<0.001
ENT	3	0	
OBG	5	0	
Ophthalmology	4	0	
Orthopaedics	2	0	

The Table 1 shows the results of bacterial culture analysis of indoor air of five operation theatre by plate exposure method. Before fogging all the operation theatres showed the presence of bacteria in the indoor air. However, none of the operation theatres showed presence of bacteria in the indoor air after fogging. [Statistically significant, (p<0.001)].

TABLE 2. Results of plate exposure method showing the number of fungal colonies before and after sterilisation

Operation Theatre	Number of Colonies		P Value
	Before Sterilisation	After Sterilisation	
General surgery	8	0	p<0.001
ENT	3	0	
OBG	6	0	

Ophthalmology	9	0
Orthopaedics	18	0

The results of fungal culture before and after fogging by plate exposure method are shown in the Table 2. From the table it is evident that all the operation theatres showed the presence of fungal spores before sterilisation but after fogging none of the operation theatre showed the presence of fungal spores which is statistically significant ($p < 0.001$).

TABLE 3. Culture results of surface sampling from general surgery operation theatre

Site of Sample	Culture Result	
	Before Sterilisation	After Sterilisation
OT table	Clear	Clear
OT light	Clear	Clear
Instrument trolley	Clear	Clear
Suction apparatus	Turbid	Clear
IV stand	Turbid	Clear
Boyle's apparatus	Clear	Clear
Floor	Clear	Clear
Wall	Clear	Clear

As far as surfaces are concerned the swabs were analysed for the presence of bacterial spores, as the surfaces are more liable for the presence of bacterial spores. The Table 3 shows the culture results of surface sampling from general surgery operation theatre. Before fogging the IV stand and suction apparatus showed the presence of anaerobic spore bearers and all the other sites were free of any spores. However, after fogging, all the sites showed the absence of bacterial spores.

TABLE 4. Culture results of surface sampling from ENT operation theatre

Site of Sample	Culture Result	
	Before Sterilisation	After Sterilisation
OT table	Clear	Clear
OT light	Clear	Clear
Instrument trolley	Clear	Clear
Suction apparatus	Clear	Clear
IV stand	Clear	Clear
Boyle's apparatus	Clear	Clear
Floor	Turbid	Clear
Wall	Clear	Clear

The culture results of surface sampling from ENT operation theatre are shown in Table 4. The ENT operation theatre showed the presence of anaerobic spore bearer only on the floor. After sterilisation, all the sites showed negative for the presence of bacterial spores.

TABLE 5. Culture results of surface sampling from OBG operation theatre

Site of Sample	Culture Result	
	Before Sterilisation	After Sterilisation
OT table	Turbid	Clear
OT light	Clear	Clear
Instrument trolley	Clear	Clear
Suction apparatus	Clear	Clear
IV stand	Clear	Clear
Boyle's apparatus	Clear	Clear
Floor	Turbid	Clear
Wall	Clear	Clear

The Table 5 shows the culture results of surface sampling from OBG operation theatre. Before fogging the floor and OT table showed the presence of anaerobic spore bearers and all the other sites were free of any spores. However, after fogging, all the sites showed the absence of bacterial spores.

TABLE 6. Culture results of surface sampling from ophthalmology operation theatre

Site of Sample	Culture Result	
	Before Sterilisation	After Sterilisation
OT table	Clear	Clear
OT light	Turbid	Clear
Instrument trolley	Clear	Clear
Suction apparatus	Clear	Clear
IV stand	Clear	Clear
Boyle's apparatus	Clear	Clear
Floor	Turbid	Clear
Wall	Clear	Clear

The culture results of surface sampling from ophthalmology operation theatre are shown in Table 6. The operation theatre showed the presence of anaerobic spore bearers on the OT light and floor. After sterilisation, all the sites showed negative for the presence of bacterial spores.

TABLE 7. Culture results of surface sampling from orthopaedics operation

Site of Sample	Culture Result	
	Before Sterilisation	After Sterilisation
OT table	Clear	Clear
OT light	Clear	Clear
Instrument trolley	Clear	Clear
Suction apparatus	Clear	Clear
IV stand	Clear	Clear
Boyle's apparatus	Clear	Clear
Floor	Turbid	Clear
Wall	Clear	Clear

The Table 7 shows the culture results of surface sampling from orthopaedics operation theatre. Before fogging the floor showed the presence of anaerobic spore bearers and all the other sites were free of any spores. However, after fogging, all the sites showed the absence of bacterial spores.

In the present study an attempt has been made to evaluate the sterilisation potential of operation theatre. The results obtained are encouraging as the quaternary ammonium compounds could able to remove even fungal and bacterial spores from the environment. Many works have already done regarding the use of quaternary ammonium compounds as disinfectants and sterilants [11-13]. Quaternary ammonium compounds are amphoteric surfactants that are widely used for the control of bacterial growth in clinical and industrial environments [14]. Eventhough the quaternary ammonium compounds are considered to be an agent of disinfectant, the present study has showed that it could able to eliminate the bacterial spores. Probably this may be due to the reason that the bacterial spore contamination in the operation theatres selected for this study would have at lower level. Bacterial spores are common in many hospital environments including operation theatre [15]. Hence a good sterilisation agent should be effective sporicidal. Hence a study should be conducted to find its efficacy in removal of heavily spore contaminated surfaces.

The use of quaternary ammonium compounds is advantageous as it is non-irritant and surgeon can resume the routine in 45 minutes after the fogging. Hence the sterilisation of operation theatre can be performed after each surgery which cannot be possible with the conventional aldehyde compounds.

4. Conclusion

The present study has showed that the quaternary ammonium compounds are effective disinfectant and sterilisation agent in the sterilisation of operation theatre. Eventhough in the present study the bacterial spores are eliminated by the DT-125 in the operation theatre environment, the question whether they are effective sporicidal agent should be scientifically evaluated.

Funding: Nil

Conflict of interest: None initiated.

Permission from IRB: Yes.

References:

1. Whyte W, Hodgson R, Tinkler J. The importance of airborne bacterial contamination of wound. *J Hosp Infect* 1982; Jun;3(2):123-35.
2. Bonten MJM, Hayden MK, Nathan C, Voorhis JV, Matushek M, Slaughter S, et al. Epidemiology of colonisation of patient and environment with vancomycin-resistant enterococci. *Lancet* 1996; 348(9042):1615-19.
3. Boyce JM, Potter-Bynoe G, Chenevert C, King T. Environmental contamination due to methicillin-resistant *S.aureus*: possible infection control implications. *Infect Control Hosp Epidemiol* 1997; Sep;18(9):622-7.
4. Hayden MK, Bonten MJ, Blom DW, Lyle EA, van de Vijver, DA, Weinstein RA, et al. Reduction in acquisition of vancomycin resistant *Enterococcus* after enforcement of routine environment cleaning measures. *Clin Infect Dis* 2006; 42(11):1552-60.
5. Bhalla A, Drin D, Donskey CJ. *Staphylococcus aureus* intestinal colonization in associated with increased frequency of *S. aureus* on skin of hospitalized patients. *BMC Infect Dis* 2007; 7((105); 108-23
6. Edmiston C E Jr, Seabrook GR, Cambria RA, et al. Molecular epidemiology of microbial contamination in the operating room environment: Is there a risk for infection? *Surgery* 2005; Oct;138(4):573-9.
7. Suzuki A, Namba Y, Matsuura M, Horisawa A. Airborne contamination in an operating suite: report of a five year survey. *J Hyg (London)* 1984; 93(202): 567-573.
8. Vesley D, Lauer J, Hawley R. Decontamination, sterilization, disinfection, and antisepsis. In: Fleming DO, Hunt DL, editors. *Laboratory safety: Principles and practices*. 3rd ed. Washington DC: ASM Press; 2001. p. 383-402.
9. Fink R, Liberman DF, Murphy K, Lupo D, Israeli E. Biological safety cabinets, decontamination or sterilization with paraformaldehyde. *Am Ind Hyg Assoc J* 1988; 49(6):277-9.
10. Rutala WA. APIC guideline for selection and use of disinfectants. *Am J Infect Control* 1996; 24(4):313-42.
11. Chaidez C, Lopez J, Castro-del Campo N. Quaternary ammonium compounds: an alternative disinfection method for fresh produce wash water. *J Water Health*. 2007; 5(2):329-33.
12. Jimenez L, Chiang M. Virucidal activity of a quaternary ammonium compound disinfectant against feline calicivirus: a surrogate for norovirus. *Am J Infect Control*. 2006; 34(5):269-73.
13. Chitra Sanjeev Juwarkar. Cleaning and Sterilisation of Anaesthetic Equipment. *Indian J Anaesth*. 2013; 57(5): 541–550.
14. Brannon, D. K. *Cosmetic microbiology: a practical handbook*. CRC Press, Inc., 1997. Boca Raton, Fla.
15. Kannan I, Jessica Yolanda Jeevitha, Sambandam Cecilia, Jayalakshmi M, Premavathy RK , Shantha S. Evaluation of surface contamination of bacteria in various dental clinics with special reference to obligate and facultative anaerobic spore bearing bacilli. *Int J Med Res Health Sci.*, 2014; 3(3):554-559.