International Journal of Medical Research Professionals
P-ISSN: 2454-6356; E-ISSN: 2454-6364
DOI: 10.21276/ijmrp

IJMRP

Original Research Article

A Microbiological Study of Assessment of Various Antiseptic Formulations after Intentional Contamination of the Containers Used by Health Care Professionals

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ABSTRACT

Background: Antiseptics are the materials which are used for reducing the bacterial counts on the skin and mucousa. Povidone-iodine (PP) and chlorhexidine are commonly used antiseptic skin solution. Recent studies showed a lower rate of frequency of intravenous catheter-related bloodstream infection among patients in whom surface skin was disinfected with chlorhexidine gluconate (CX). Hence; we conducted the present study to assess the survival rates of micro-organisms within different formulations of CX and PP.

Material & Methods: The present study was carried out to assess the efficacy of different formulation of antiseptics. BHI (Brain Heart Infusion) culture was used and culture sample of Serratia marcescens was kept in it followed by incubation in the under-cultivation in Tryptone Soy Broth (TSB). Daily recording of the readings was done followed by recording of the final reading at the end of third week. Growth of negative and positive controls was observed for comparison. All the results were analyzed by SPSS software.

Results: Absence of the micro-organisms was demonstrated in the Group A and Group B as 100 percent of the culture media was kept limpid. After three weeks of the readings, the positive controls were kept limpid while in two days' time, positive growth characteristic of S. Marcescens was observed as red colour growth. In group A samples, the positive growth and negative growth pattern was exhibited as 0/630 and 630/630 respectively. Similar pattern of positive growth pattern of positive and negative control was exhibited by Group B samples.

Conclusion: For the repetitive use of containers, cleaning can be inferred as a minimal procedure in the distribution of antiseptic solutions.

Key Words: Chlorhexidine, Microbiology, Povidone-iodine.

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Article History:

Received: 19-04-2016, **Revised:** 07-05-2016, **Accepted:** 29-05-2016

Access this article online	
Website: www.ijmrp.com	Quick Response code
DOI: 10.21276/ijmrp.2016.2.3.057	

INTRODUCTION

The materials which are used for reducing the bacterial counts on the skin and mucousa because of their anti-microbial action are the antiseptics.[1] For the clinicians, lots of confusion and problems are created by growing various specimens in the contaminated blood cultures. Upto 6 percent, the contamination rates of the institution has been reported.[2] If only one positive culture is observed from the two or more blood culture obtained, the contamination by Coagulase negative Staphylococcus (CoNS), aerobic and anaerobic diphtheroids, Micrococcus spp., Bacillus spp., and viridians Streptococcus are considered to be positive.[3] Clinical interpretations can be misinterpreted due to false positive due to false positive blood culture reports. In such cases, additional diagnostic tools are required such as immuno assays chain reactions etc all of which increases the cost factor.[4,5]

Before taking out the blood culture samples, one of the common antiseptic skin solution used is Povidone-iodine (PP). Recent studies showed a lower rate of frequency of intravenous catheter-related bloodstream infection among patients in whom surface skin was disinfected with chlorhexidine gluconate (CX) in comparison to the subjects in which the surface skin was disinfected by PP. The most recent CDC guidelines for prevention of SSIs, published in 1999, recommend that surgical hand antiseptic agents display a broad spectrum of activity, be fast acting, and have a persistent effect. The elimination and reduction of transient and resident skin flora, respectively, should occur immediately; thereafter, persistent or residual activity combats proliferation of microorganisms on the gloved hand. Both immediate and residual activity limit the consequences of breaks

in aseptic technique, which occur in more than 30% of procedures and increase with the duration of surgery.[6-10] Hence, we conducted the present study to assess the survival rates of micro-organisms within different formulations of CX and PP.

MATERIALS & METHODS

The present study was carried out in the department of microbiology of the institution and included assessment of the efficacy of different formulation of antiseptics. BHI (Brain Heart Infusion) culture was used for the present study and culture sample of Serratia marcescens was kept in it followed by incubation in the under-cultivation in Tryptone Soy Broth (TSB). Ethical approval was taken from the institutional ethical committee and written consent was obtained after explaining them the entire research protocol. Intentional contamination of the containers was done with the material after the confirmation of the over 100 UFC / ml of the inoculum size were confirmed. Automated auto-mini-pipette was used for inoculating the containers. 1 ml of the material was collected in the pipette followed by uniform distribution on the full length of the internal walls of the container in rotation motion. 120 samples for both the formulations with 60 sample for each formulation was used for the testing the two study specimens giving a total of 360 containers. Two study groups were formed containing the two investigating antiseptic solution; CX and PP. Aqueous, detergent and alcoholic formulations were made and testing of the specimens was done at different time intervals;

Group A: Chlorhexidine.

Group B: Povidone-iodine (PP).

Intentional contamination of the 180 containers was done initially with 1 ml of 100 UFC/ml of S. marcescens suspension. Equal distribution of three different formulation of the CX antiseptic solution; alcoholic, detergent and aqueous, was done with distribution of 150 ml in each sample. Initial cleaning of all the specimens with ethylene oxide (EO) was done for ensuring the sterile and aseptic environment of starting of the experiment and study. Storing of the containers with specimens was done at room temperature for one week along with daily shaking of the container thrice a day. The containers were tightly closed with the lid so that proper shaking could be done. Thrice a day shaking was done thoroughly to ensure complete mixing. The incubation with subsequent change and mixing was done at room temperature in containers at the end of first, second and third week. Daily recording of the readings was done followed by recording of the final reading at the end of third week.

Positive control: To control the strain viability and equivalency to the prepared media of the two tubes, test micro-organisms were grown in 10 ml of TSB which served as the positive controls.

Negative control: Incubation of the two limpid TSB samples was done at twenty two degree centigrade for 72 hours which indicated the absence of micro-organisms and served as negative control.

All the results were analyzed by SPSS software. Univariate regression formulae were used for the assessment of level of significance.

RESULTS

Absence of the micro-organisms was demonstrated in the Group A and Group B as 100 percent of the culture media was kept limpid. After three weeks of the readings, the positive controls were kept limpid while in two days' time, positive growth characteristic of S. Marcescens was observed as red colour growth. Table 1 shows the microbiological culture growth after three weeks of incubation, of antiseptic tested in Group I. In group A samples, the positive growth and negative growth pattern was exhibited as 0/630 and 630/630 respectively. Table 2 highlights the microbiological culture growth after three weeks of incubation, of antiseptic tested in Group II. Similar pattern of positive growth pattern of positive and negative control was exhibited by Group B samples. Table 3 shows the microbiological culture growth after three weeks of incubation, of antiseptic tested in positive control. Table 4 highlights the microbiological culture growth after three weeks of incubation, of antiseptic tested in negative control.

Table 1: Microbiological culture growth after three weeks of incubation, of antiseptic tested in Group I

Results	Group A
Positive growth	0/630
Negative growth	630/630

Table 2: Microbiological culture growth after three weeks of incubation, of antiseptic tested in Group II

Results	Group B
Positive growth	0/630
Negative growth	630/630

Table 3: Microbiological culture growth after three weeks of incubation, of antiseptic tested in positive control

Results	Positive control
Positive growth	2/2
Negative growth	0/2

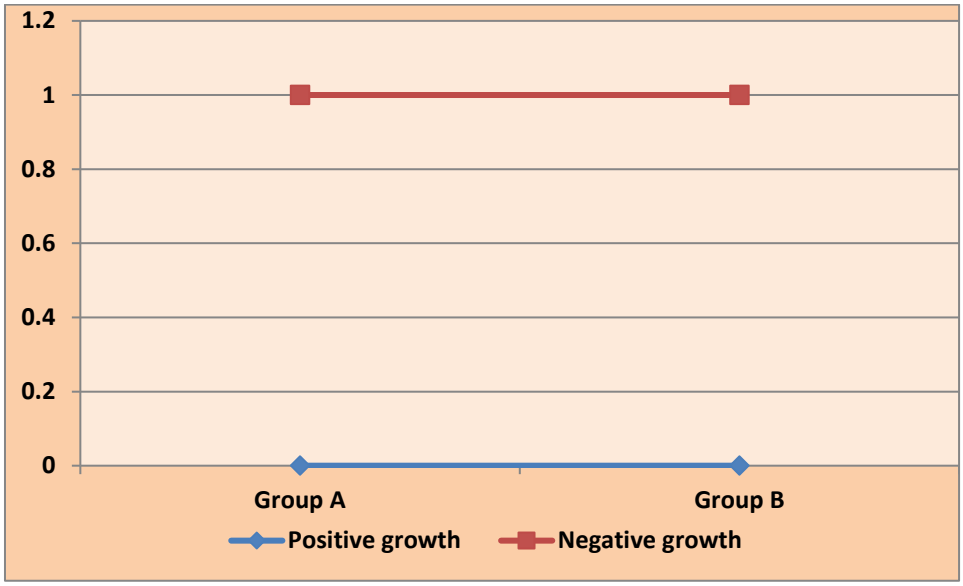
Table 4: Microbiological culture growth after three weeks of incubation, of antiseptic tested in negative control

Results	Negative control
Positive growth	0/2
Negative growth	2/2

DISCUSSION

Antiseptics are routinely used by health care professionals. The main use of these agents is the cleansing of hands, preparation of the site of surgery, to prevent infections, removal of infectious agents and germs from the hands of the surgical team etc. One of the major concerns for the health care professionals are the antiseptic products infected by microbiological contamination and is quoted numerous times in the past literature.[11,12] Uncertainty exist regarding the use of the biocides and the pattern of development of the microbiological resistance as the concentration of these agents is significantly higher than that of established Minimum Inhibitory Concentration (MIC) of bacteria which is required for the growth of these micro-organisms in the antiseptic solutions.[13] Exogenous cause is the main etiologic factor causing the contamination of these agents. Processing containers often get contaminated and this largely hampers the health care system operated by various professionals who are a part of this system.[14] Hence, we conducted the present study to assess the survival rates of micro-organisms within different formulations of CX and PP.

Graph 1: Positive and negative growth of various specimens



In the present study, when the results were extrapolated, before safe reuse of containers, cleansing was verified to be the minimum procedure required. This is due to the observation of the present study that the load of microorganisms present in the cleaned container would certainly be smaller than the inoculum challenge of 1 x 105 UFC/mL. Padovani CM et al analyzed the microbiological profile of different antiseptic solutions after intentional contamination of the containers. Their results suggested that micro-organisms are inactivated by the antiseptic solutions.[15] Suwanpimolkul G et al conducted a randomized trial of 2% chlorhexidine tincture compared with 10% aqueous povidone-iodine and assessed the effect on contamination rates of the blood culture. From the results, they concluded that in comparison with the 10% aqueous povidone-iodine, two percent alcoholic chlorhexidine is superior for venipuncture site disinfection before obtaining blood cultures.[16] Mimoz O et conducted a randomized trials to compare the affectivity of alcoholic chlorhexidine in comparison with aqueous povidone-iodine for obtaining specimens for blood cultures. They analyzed 403 adult patients who had at least one blood culture drawn through a peripheral vein. From the results, they suggested that skin preparation with aqueous povidone-iodine is less effective than skin preparation with alcoholic chlorhexidine in reducing contamination of blood cultures.[17] Caldeira D et al conducted a Meta-analysis with skin antiseptics for prevention of contamination in venous-puncture drawn blood cultures.from the results, they concluded that in comparison with aqueous povidone-iodine ,alcoholic chlorhexidine solutions reduced blood culture false positives.[18] Marlowe L et al retrospectively analyzed the blood culture contamination rates after skin antisepsis with chlorhexidine, compared with povidone-iodine. They assessed children aged 2-36 months with peripheral blood culture result and observed that the blood culture contamination rate decreased from 24.81 to 17.19 contaminated cultures per 1,000 cultures ($P < .05$) after implementation of chlorhexidine. From the results, the authors concluded that skin antisepsis with chlorhexidine significantly reduces the blood culture contamination rate among young children, as compared with povidone-iodine.[19]

A review of quantitative bacteriology by Krizek TJ et al commented that because all incisions are contaminated with bacteria, progression to infection is a result of the initial inoculum of bacteria defeating systemic and local defenses.[20] Those authors commented on several studies involving acute wounds, delayed incision closure, or clinical skin grafting in human beings, which all resulted in poor site outcomes when more than 105 bacteria/g were present at the time of intervention.

Nearly 20 years earlier, Elek SD et al provided data detailing the minimum pus forming dose for virulent staphylococci in human volunteers.[21] Anderson MJ et al analyzed the efficacy and toxicity of antiseptics and concluded that all antiseptics tested in the mucosal infection model reduced methicillin susceptible Staph. aureus.[22]

CONCLUSION

From the above results, the authors conclude that for the repetitive use of containers, cleaning can be inferred as a minimal procedure in the distribution of antiseptic solutions. However; future studies are required for better exploration of the field of sterilization in context to health care system.

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Source of Support: Nil. **Conflict of Interest:** None Declared.

Cite this article as: Praveen Kumar Prabhakar, Harihar Deep Bhardwaj. A Microbiological Study of Assessment of Various Antiseptic Formulations after Intentional Contamination of the Containers Used by Health Care Professionals. Int J Med Res Prof. 2016;2(3):259-62.