Original Article

The In Vitro Efficacy Testing Of Skin Disinfectants Against Nosocomial Pathogens

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ABSTRACT

Background: Nosocomial infections increase the morbidity among hospitalized patients and are a major cause of death. The national surveillance data and public health research have demonstrated that hospital- acquired infections (HAIs) take a major human toll on society. Disinfectants play a major role in reducing the hospital acquired infections (HAIs). There are many skin disinfectants which are commercially available and there has been a considerable recent interest in the bacterial adaptation and resistance to skin disinfectants.

Aim: To study the bactericidal activity of 0.5% chlorhexidine gluconate, 0.5% chlorhexidine gluconate in 80% ethanol, 5% povidone – iodine, 10% povidone – iodine and 10% 20%, 30%,40%, 60%, 80% and 99.5% ethanol against Methicillin resistant Staphylococcus aureus [MRSA], multi drug resistant [MDR] Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli - extended spectrum beta lactamase producers [ESBL] and Klebsiella pneumoniae [ESBL]. Each strain was evaluated in quadruplicate.

Methods: The testing was carried out by means of a suspension test. The pathogen was exposed to each of the disinfectants at various concentrations for 15, 30, 60,120 and 240 seconds at room temperature. After the exposure of the inocula to the disinfectants, the antimicrobial activity of the disinfectants in the suspensions was inactivated by neutralizers. Of the resulting suspensions, 100µl of each was transferred to nutrient agar plates in triplicates and these were incubated at 37°C for 72 hrs. The number of colonies in each plate was counted and tabulated.

Results: Povidone-iodine (10%) and 60% ethyl alcohol were found to be effective against 20 bacterial strains than 0.5% chlorhexidine gluconate, 0.5% chlorhexidine gluconate in 80% ethyl alcohol and 5% povidone-iodine. Statistical analysis was done by a nonparametric test. The differences in the percentage change in the colony counts between the 4 disinfectants were significant at 15 and 30 seconds of exposure [$P < 0.05$].

Conclusion: The results suggest that 10% povidone-iodine and 60% ethyl alcohol were superior and more potent as well as rapid against the common nosocomial pathogens.

Key Words : Hospital- acquired infections (HAIs), Nosocomial pathogens, Methicillin resistant Staphylococcus aureus [MRSA], Multi drug resistant (MDR), Extended Spectrum Beta Lactamase producers (ESBL), Disinfectants.

KEY MESSAGES:

1. Nosocomial infections increase the morbidity among hospitalized patients and are a major cause of death.
2. Disinfectants play a major role in reducing the hospital acquired infections (HAIs).
3. The disinfectants should be tested periodically to check their potency and also to determine whether the microorganisms have developed resistance against them or not .

INTRODUCTION

Nosocomial infections or hospital acquired infections (HAIs) are infections that develop in a hospitalized patient, that were not present or were in incubation at the time of admission. Hospital acquired infections are typically exogenous, the source being any part of the hospital ecosystem (hospital personnel, operative procedures, animate objects including medical devices, therapeutic pressure and environmental pressure such as food, water, and air). They may occur sporadically or as outbreaks. [1] Nosocomial infections increase the morbidity among hospitalized patients and are a major cause of death. According to a survey which was conducted under the auspices of the World Health Organization in 55 countries, a mean of 8.7% of the patients were found to have nosocomial infections and at any given time, 1.4 million people worldwide were found to suffer from hospital acquired infections (WHO, 2002). [2]

A significant proportion of nosocomial infections result from cross-contamination and from the transmission of microorganisms by the hands of health care workers (HCWs). [3]

Disinfectants play a major role in reducing the hospital acquired infections (HAIs). They are widely used in hospitals for various purposes such as hand-washing, skin preparation before epidural or spinal blocks, vascular catheter –site care, skin preparation before blood culture, etc. The function of the topical antiseptics is to quickly decrease a broad spectrum of resident and transient microbes to sub pathogenic levels and to prevent the rebound of growth. [4] The effectiveness of these products is usually investigated by in vitro techniques, as their activity on the human skin is difficult to assess. [5] The testing of disinfectants under laboratory conditions is very important with regards to the determination of their correct concentration and other aspects of their use. It forms the basis of the selection of the skin disinfectants. [6] Skin disinfectants used should

be effective against bacteria, yeasts, and enveloped viruses within a minimum period of exposure. [7]

The speed with which the bactericidal effect is achieved was studied by exposing the pathogens to common disinfectants for graded periods of duration and by then culturing the inocula from these suspensions after the antimicrobial activity to the disinfectants was antagonized by a neutralizer in a 1:1000 dilution. There are many skin disinfectants which are commercially available and there has been a considerable recent interest in the bacterial adaptation and resistance to the skin disinfectants. [8] The purpose of the study was to know the in vitro bactericidal activity of different disinfectants against the common bacterial strains which were associated with nosocomial infections and to suggest suitable disinfectants which could be used in our hospital environment.

MATERIALS AND METHODS

Methicillin resistant Staphylococcus aureus [MRSA], multi drug resistant [MDR] Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli - extended spectrum beta lactamase producers [ESBL] and Klebsiella pneumoniae [ESBL] were used in this study. The four strains from each of the above mentioned organisms were isolated from various clinical samples which were received in our laboratory. The skin disinfectants which were studied were 0.5% chlorhexidine gluconate, 0.5% chlorhexidine gluconate in 80% ethanol, 5% povidone – iodine, 10% povidone – iodine, 10% 20%, 30%, 40%, 60%, 80% and 99.5% ethanol.

The antimicrobial efficacy evaluations of the skin disinfectants involved the measurements of the microbial population reductions at a specific time point after the exposure to the tested product. Therefore, the antimicrobial action of the product had to be stopped by using inactivating agents such as 1% Tween 20 and 1% Tween 80 for 0.5% chlorhexidine gluconate and 0.5 % chlorhexidine gluconate in 80% ethanol, physiological saline for ethanol and 2% sodium thiosulfate for 5% and 10% povidone-iodine. [9]

Preparation of the test inoculum [10]:

Each strain was inoculated in 5ml of peptone water and incubated over night at 37°C. The suspension containing 109 CFU/ml was used as the test inoculum.

The suspension test [10]:

10µl of the test inoculum was added to 5ml of each of the disinfectant solutions and this was vortexed for 5 seconds to obtain an approximate bacterial density of 2×106 CFU/ml. The control suspension was prepared by adding the inoculum to 5ml of physiological saline for each strain. After the exposure of the inocula to the disinfectants for 15, 30, 60, 120 and 240 seconds at room temperature, the antimicrobial activity of the disinfectants in the suspensions was inactivated by diluting 10µl of each of the suspensions with 10ml of neutralizers.

Sodium thiosulphate (2.0%) was used to neutralize 5% and 10% povidone-iodine, Tween 80 was used to neutralize 0.5% chlorhexidine gluconate and 0.5% chlorhexidine gluconate in 80% ethanol and physiological saline was used to neutralize the effect of ethanol. Of the resulting suspensions, 100µl of each was transferred to nutrient agar plates in triplicates and these were incubated at 37°C for 72 hrs. The number of colonies in each plate was counted and tabulated.

Testing the validity of the inactivation of the antimicrobial activity of the disinfectants by 1:1000 dilution with neutralizers [10]:

Ten µl of the test inoculum was added to 10ml of physiological saline for control, and 10µl to 10ml of the mixture consisting of 10µl of a disinfectant and 10ml of the respective neutralizer. The inoculums were exposed to the mixture of disinfectant and neutralizer for 30 min at room temperature, 100µl of these suspensions were transferred to nutrient agar plates in duplicate and these were incubated at 37ºC for 72 hours for doing the colony counting.

The antimicrobial activity was considered to be inactive when the decrease in the colony count, as compared to the control, was less than 5%. The data were presented as a percentage change in the colony counts between the disinfectants at each period of exposure and these were analyzed by a nonparametric test.

RESULTS

The results obtained by the suspension test on 20 bacterial strains against the disinfectants such as 0.5% chlorhexidine gluconate, 0.5% chlorhexidine gluconate in 80% ethyl alcohol, 5% povidone-iodine and 10% povidone-iodine are presented in [Table/Fig-1], [Table/Fig-2], [Table/Fig-3] and [Table/Fig-4] respectively.

Almost all the strains grew colonies after 30 s exposure to 0.5% chlorhexidine gluconate. But only few strains grew colonies after 60 s exposure and no stains grew colonies after 120 s exposure.

Bacteria	No. of colonies in control	Exposure Time				
		15S (in %)	30S (in %)	60S (in %)	120S (in %)	240S (in %)
MRSA 1	800	4.5	1.2	0.25	0	0
MRSA 2	600	4.6	2.7	0.17	0	0
MRSA 3	170	5.8	4.7	0	0	0
MRSA 4	700	6.8	4.2	0	0	0
PA1	486	4.9	0	0	0	0
PA 2	930	6.4	1.3	0	0	0
PA 3	800	7.6	1.4	0	0	0
PA 4	726	5.5	1.6	0	0	0
E.COLI 1	150	13.3	7.3	0	0	0
E.COLI 2	190	7.3	0	0	0	0
E.COLI 3	400	20.5	7.5	0	0	0
E.COLI 4	340	18.5	3.5	0	0	0
AB 1	130	26.1	16.1	0	0	0
AB 2	332	7.2	0	0	0	0
AB 3	130	47.6	7.7	0	0	0
AB 4	400	11.5	0	0	0	0
KLEB 1	423	9.7	3.5	0	0	0
KLEB 2	357	5.9	2.5	0	0	0
KLEB 3	283	6.7	0	0	0	0
KLEB 4	408	7.6	3	0	0	0

[Table/Fig-1]: Control colony count per plate in various bacteria and percent change in the count after exposure to 0.5% chlorhexidine gluconate.

NOTE: MRSA - Methicillin resistant Staphylococcus aureus, PA- Pseudomonas aeruginosa [MDR], E.COLI- Escherichia coli [ESBL producer], AB- Acinetobacter baumanni, KLEB - Klebsiella pneumoniae [ESBL producer].

Very few strains grew colonies after 30 s exposure to 0.5% chlorhexidine gluconate in 80% ethyl alcohol. No strains grew colonies after 60 s exposure.

Bacteria	No. of colonies in control	Exposure Time				
		15S (in %)	30S (in %)	60S (in %)	120S (in %)	240S (in %)
MRSA 1	150	0.7	0	0	0	0
MRSA 2	400	0	1	0	0	0
MRSA 3	300	0.3	0	0	0	0
MRSA 4	100	0	0	0	0	0
PA 1	100	0	0	0	0	0
PA 2	120	0	0	0	0	0
PA 3	240	0	0	0	0	0
PA 4	86	0	0	0	0	0
E.COLI 1	90	0	0	0	0	0
E.COLI 2	160	0	0	0	0	0
E.COLI 3	82	0	0	0	0	0
E.COLI 4	320	0	0	0	0	0
AB 1	60	0	0	0	0	0
AB 2	100	0	0	0	0	0
AB 3	80	0	0	0	0	0
AB 4	90	0	0	0	0	0
KLEB 1	353	0.6	0	0	0	0
KLEB 2	284	0	0	0	0	0
KLEB 3	397	0	0	0	0	0
KLEB 4	413	0.2	0	0	0	0

[Table/Fig-2]: Control colony count per plate in various bacteria and percent change in the count after exposure to 0.5% chlorhexidine gluconate in 80% ethyl alcohol.

Most of the strains grew colonies after 15 s exposure to 5% povidone-iodine and no strains grew colonies after 30 s exposure.

A result obtained by testing 20 bacterial strains against various concentrations of ethyl alcohol is shown in [Table/Fig-5]. There were

Bacteria	No. of colonies in control	Exposure Time				
		15S (in %)	30S (in %)	60S (in %)	120S (in %)	240S (in %)
MRSA 1	450	1.5	0	0	0	0
MRSA 2	380	2.1	0	0	0	0
MRSA 3	170	4.7	0	0	0	0
MRSA 4	280	3.2	0	0	0	0
PA 1	360	0.5	0	0	0	0
PA 2	320	2.5	0	0	0	0
PA 3	198	2	0	0	0	0
PA 4	249	2	0	0	0	0
E.COLI 1	520	0.8	0	0	0	0
E.COLI 2	388	1.3	0	0	0	0
E.COLI 3	649	0	0	0	0	0
E.COLI 4	398	0.7	0	0	0	0
AB 1	442	1.3	0	0	0	0
AB 2	362	0	0	0	0	0
AB 3	482	1	0	0	0	0
AB 4	394	0	0	0	0	0
KLEB 1	460	0.9	0	0	0	0
KLEB 2	340	0	0	0	0	0
KLEB 3	495	0.4	0	0	0	0
KLEB 4	396	0	0	0	0	0

[Table/Fig-3]: Control colony count per plate in various organisms and percent change in the count after exposure to 5% povidone-iodine.

Bacteria	No. of colonies in control	Exposure Time				
		15S (in %)	30S (in %)	60S (in %)	120S (in %)	240S (in %)
MRSA 1	360	1.5	0	0	0	0
MRSA 2	300	0	0	0	0	0
MRSA 3	400	0	0	0	0	0
MRSA 4	390	0	0	0	0	0
PA 1	211	0	0	0	0	0
PA 2	182	0	0	0	0	0
PA 3	160	0	0	0	0	0
PA 4	385	0	0	0	0	0
E.COLI 1	501	0	0	0	0	0
E.COLI 2	240	0	0	0	0	0
E.COLI 3	350	0	0	0	0	0
E.COLI 4	498	0	0	0	0	0
AB 1	215	0	0	0	0	0
AB 2	160	0	0	0	0	0
AB 3	180	0	0	0	0	0
AB 4	120	0	0	0	0	0
KLEB 1	503	0	0	0	0	0
KLEB 2	482	0	0	0	0	0
KLEB 3	491	0	0	0	0	0
KLEB 4	367	0	0	0	0	0

[Table/Fig-4]: Control colony count per plate in various bacteria and percent change in the count after exposure to 10% povidone-iodine.

individual variations among the strains which were used in our study with regards to the susceptibility to various skin disinfectants. The statistical analysis was done by a nonparametric test. The differences in the percentage change in the colony counts between the 4 disinfectants were significant at 15 and 30 seconds of exposure [$P < 0.05$]. There was no percentage change in the colony count between the disinfectants after 60 seconds of exposure and no strains grew colonies after 120 seconds of exposure to the 4 disinfectants.

No strains grew colonies after 15 s exposure to 60%, 80% and 99.5% ethyl alcohol.

DISCUSSION

Nosocomial infections have been recognized for more than a century as a critical problem affecting the quality of the health care which is provided in hospitals. A significant proportion of the infections result from cross-contamination, and transmission of microorganisms by the hands of health care workers (HCWs) is the main route of spread. [11] Four categories of HAIs such as surgical site infections (SSI), catheter-associated bloodstream infections, ventilator- associated pneumonia and catheter-associated urinary tract infections are a major source of prolonged illness [12] which can be avoided by the proper usage of skin disinfectants.

Effective skin antiseptics are essential in preventing the increased incidence of infections during routine patient care, surgery and intramuscular, intravenous and intravascular catheter insertions. It has been suggested that the disinfectant should be effective against microorganisms within a minimum period of exposure and at optimal concentrations. [13]

Microorganisms have developed resistance to antiseptics and disinfectants and this has been less extensively studied. So, the

Bacteria	No. of colonies in control	Different Concentrations of Ethanol						
		10%	20%	30%	40%	60%	80%	99.5%
MRSA 1	1000	60	36	46	40	0	0	0
MRSA 2	820	58.5	48.8	48.7	7.1	0	0	0
MRSA 3	350	57.1	51.4	45.7	18	0	0	0
MRSA 4	390	25.6	41	15.4	1	0	0	0
PA 1	1190	63	33.6	26.9	0	0	0	0
PA 2	965	99	63.2	12.6	0	0	0	0
PA 3	200	80	75	6	0	0	0	0
PA 4	600	63.3	48.3	13.3	1.5	0	0	0
E.COLI 1	760	52.6	36.8	7.1	0	0	0	0
E.COLI 2	506	47.4	39.5	31.2	0	0	0	0
E.COLI 3	498	60.2	50.2	40.1	0	0	0	0
E.COLI 4	160	78.1	48.7	10	0	0	0	0
AB 1	622	39.9	29.2	19.6	1.6	0	0	0
AB 2	120	90.8	83.3	19.1	0	0	0	0
AB 3	200	50	30	8	0	0	0	0
AB 4	600	66.7	25	20	0	0	0	0
KLEB 1	685	41.1	29.3	21.7	3.6	0	0	0
KLEB 2	473	47.1	36.1	20.1	2.5	0	0	0
KLEB 3	509	47.7	38.7	31.4	3.5	0	0	0
KLEB 4	453	64.7	47	29.6	2.2	0	0	0

[Table/Fig-5]: Colony count per plate in various bacteria and percent change in count after 15 s exposure to different concentrations of ethanol.

disinfectants should be tested periodically to check their potency and also to determine whether the microorganisms have developed resistance against them or not.

The optimal disinfection regimen for avoiding the spread of nosocomial infections has not yet been defined. Many other antiseptics or their combinations are still being used and investigated and research efforts to identify improved antisepsis approaches are continuing. [14], [15] In our study, we compared the efficacy of 4 disinfectants which were commonly used in our hospital setup. It included 0.5% chlorhexidine gluconate, 0.5% chlorhexidine gluconate in 80% ethyl alcohol, 5% povidone-iodine and 10% povidone-iodine. In addition, we also compared the efficacy of different concentrations of ethyl alcohol. All these disinfectants were tested against common pathogens which are found to be associated with nosocomial infections.

In our in vitro test results, there were marked individual variations in the responses to the disinfectants between the strains. Among the 20 strains which were tested against 0.5% chlorhexidine gluconate, only two strains of MRSA grew colonies after 60 seconds of exposure and thereafter, there was no growth, whereas in a study which was performed by Sakuragi T et al, [10] on the bactericidal activity of skin disinfectants on Methicillin –resistant Staphylococcus aureus , one strain survived even after 240 seconds of exposure. The organism survival rate was less in our study as compared to their study. This discrepancy may be due to individual variations in the susceptibility of the strains to disinfectants.

On studying the efficacy of 0.5% chlorhexidine gluconate in 80% ethanol, we found that four strains grew colonies after 15 second exposures and there was no growth after 30 seconds, in contrast to the findings of the study by Sakuragi T et al., [10] where they observed that 0.5% chlorhexidine gluconate in 80% ethanol was very effective even at 15 seconds of exposure itself. So, the efficacy of 0.5% chlorhexidine gluconate was dramatically improved by the addition of 80% ethyl alcohol. The bactericidal activities of 5% povidone-iodine and 0.5% chlorhexidine gluconate in 80% ethanol were found to be similar after exposing the strains to them for 15 seconds.

The Povidone-iodine solution, at a concentration of 10%, had a greater in vitro microbicidal activity. After 15 seconds of exposure to 10% povidone-iodine, no organisms survived. Surprisingly, in another study which was performed by Haley et al., [16] the organisms survived even after 15 seconds of exposure. On comparing the efficacy, 10% povidone-iodine was found to have a greater invitro microbicidal activity than 5% povidone-iodine, 0.5% chlorhexidine gluconate and 0.5% chlorhexidine gluconate in 80% ethanol.
We also found that 60%, 80% and 99.5% of ethyl alcohol was effective against MRSA and this correlated well with the study which was done by Sakuragi T et al. [10] Moreover, all other strains showed sensitivity to 60%, 80% and 99.5% of ethyl alcohol.

The concentrations of the bacteria which were exposed to the disinfectants may be much higher in our study than the in vivo concentrations. As the number of organisms in the normal skin flora ranges widely, the disinfectants must be effective in a broad range of concentrations, thus securing a bacteria free state on the surface as well as in the deeper structures.

To conclude, the bactericidal effect on 20 bacterial strains against 10% povidone-iodine and 60% ethyl alcohol was found to be more rapid and potent than 0.5% chlorhexidine gluconate, 0.5% chlorhexidine gluconate in 80% ethyl alcohol and 5% povidone-iodine. This result suggests that 10% povidone-iodine is superior to other antiseptics for use before surgery, for vascular catheter site care and for wound dressing. But one drawback of povidone-iodine

is that it causes staining and so, it cannot be used for routine hand care in the patients. It can be replaced by ethyl alcohol which is considered to be the safest antiseptic and also, it generally has no toxic effects on the human skin. Moreover, it is less cytotoxic. So, for the routine hand care purposes and for use before intramuscular and subcutaneous injections, we suggest 60% ethyl alcohol as an ideal skin disinfectant.

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