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Effectiveness of commonly used antiseptics on bacteria causing nosocomial infections in tertiary hospital in Malaysia

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The antimicrobial inhibitory effects of five common antiseptics [Chlorhexidine (CHX), Hydrogen peroxide (H_2O_2), Iodine, Ethanol and Dettol] were investigated using agar well diffusion method. The organisms used included methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii, Escherichia coli*, *Klebsiella* species and *Pseudomonas aeruginosa*. The undiluted concentrations of the antiseptics showed variable zones of inhibition against the tested organisms, on MRSA it ranged from 25 mm (CHX) to 30 mm in other antiseptics, on *A. baumannii* 20 mm (CHX) to 34 mm Dettol, on *E. coli* 20 mm Dettol to 38 mm (H_2O_2), on *Klebsiella* spp. 20 mm Dettol to 24 mm (CHX), whereas on *P. aeruginosa* it ranged from 13 mm Iodine to 30 mm (H_2O_2). The minimal inhibitory concentration (MIC) of chlorhexidine concentration against MRSA and *P. aeruginosa* was 10%, while *A. baumannii* was 20%. All the study bacteria were resistant to ethanol by all concentrations. The result showed that H_2O_2 was the most effective antiseptics than the others followed by CHX. The study bacteria were found to be crucially susceptible to the routinely used antiseptics tested. Though, there is the need for continuous surveillance for the detection of emerging resistance pattern.

Key words: Antimicrobial, antiseptics, disinfectants, nosocomial.

INTRODUCTION

Nosocomial infections (NI) are referred to those infections occurring after 48 h of hospital admission, or 3 days of discharge (Kouchak and Askarian, 2012). About 10% of the hospital admitted persons will have NI, and it has been shown that NI is usually associated with prolong length of hospital stay, increased costs, and resulted in

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significant morbidity and mortality (Al-Talib et al., 2010; Raines and Rosen, 2016). Currently, NI has become a trend in healthcare setting globally including Malaysia. Nosocomial bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*, have become endemic in many health care centres. Infections with these organisms are often difficult to treat, owing to a reducing armamentarium of active antiseptic agents. Also, hospital associated infections involving these and other microorganisms are associated with considerable morbidity and mortality (Climo et al., 2013). Antiseptics and disinfectants had a fundamental role in infection control practices and help in the avoidance of NI. Antiseptics are used in sterilization of medical and surgical instruments and wards equipment. However, extensive using of different antiseptics might lead to the development of resistant pathogens that eventually makes the antiseptics become ineffective (Matthew et al., 2017). Different bacteria showed variable degrees of resistance to antiseptics (McDonnell and Russell, 1999); although, Gram-negative bacteria are commonly more resistant than Gram-positive bacteria to antiseptics (Russell, 1999). Antiseptics are mainly used to inhibit the growth of microorganisms or to interrupt the route of transmission of germs between the infection source and healthy subjects (Mbajiuka et al., 2015). Previous study by El-Mahmood and Doughari (2009) revealed that five frequently used antiseptics were contaminated with nosocomial Gram positive and negative bacteria, therefore, antiseptics used in hospitals and laboratories must be evaluated regularly to determine their potency validation to remove or inactivate known pathogens from inanimate objects (Sridhar, 2012). Chlorhexidine is an antiseptic with a broad-spectrum activity against many organisms, including *S. aureus* and *Enterococcus* species. Chlorhexidine is a cationic polybiguanide that has antibacterial effects and has been used as antiseptic in clinical practice (Mullany et al., 2006). Chlorhexidine salts dissociate and release the positively charged chlorhexidine cationic molecules which bind to negatively charged bacterial cell walls and causing bactericidal effect (Cheung et al., 2012). Using chlorhexidine at low concentrations resulted in a bacteriostatic effect while at high concentrations it can cause membrane disruption and cell death. Chlorhexidine lasts much longer than other antiseptics, therefore, it is often combine with alcohol in skin preparation to reduce microbial burden on patients' skin and prevent secondary bacterial infections (Climo et al., 2013). Previous studies have found that daily bathing with 2% chlorhexidine-impregnated washcloths reduced the incidence of NI infections by 60% (Climo et al., 2013; Vernon et al., 2006).

Hydrogen peroxide (H_2O_2) plays a central role in sterilization and disinfection of critical items in Malaysian hospitals. Also H_2O_2 is the most effective antiseptic used in hospitals since the 1920s because it kills bacteria cells by destroying their cell walls. H_2O_2 has "hydroxyl radicals" a potent oxidant, which react with macromolecules such as membrane lipids and DNA thus resulting in bacterial death (Shahriari et al., 2011). In its pure form, H_2O_2 is a colourless liquid, slightly more viscous than water. H_2O_2 is used in hospital and ICU in a vapour form to decontaminate rooms from multi-drug resistant, also used to sterile surfaces, including surgical tools (Lemmen et al., 2015).

Nowadays most of the hospital used Iodine (povidone iodine) which is a natural dark violet, non-metallic solution that considered among the most effective skin antiseptics and used widely in minor wound cleaner. Iodine has excellent bactericidal, fungicidal, tuberculocidal, virucidal and sporicidal properties (Bouaziz et al., 2016). Iodine can penetrate the cell wall of microorganisms quickly, and the lethal effects are believed to result from disruption of protein and nucleic acid structure and synthesis (McKeen, 2012). Although, povidone-iodine has a rapid bactericidal effect than chlorhexidine, but povidone-iodine has not been shown to have a persistent effect like chlorhexidine (Bigliardi et al., 2017).

Ethanol is used extensively in the homes, healthcare settings and laboratories. It consists of two water-soluble chemical compounds ethyl alcohol and isopropyl alcohol that have germicidal characteristics. Alcohols showed bactericidal rather than bacteriostatic activities against vegetative forms of bacteria but do not destroy bacterial spores. Hence, alcohol is not generally being used as sterilizing material instrument (Tuhina et al., 2013). Both ethanol and isopropanol have similar modes of action against different types of microorganisms, however isopropyl alcohol is likely to be more effective than ethanol against bacteria, while the reverse appears to be true for viruses (William et al., 2008). Dettol is another antiseptic, which is used in hospitals and homes; it is available in multi-forms like soap, spray, hand wash, surface wipes, mildew remover and a bathroom cleaner. The active ingredient in Dettol is para-chloro-meta-xylenol. Dettol has greater effects against Gram-positive bacteria and works by disruption of the cell wall and inhibiting the function of enzymes (Mahon et al., 2014).

The aim of this study was to evaluate the antimicrobial effects of some commonly used disinfectants and antiseptics against common bacteria that cause nosocomial infections in hospitals.

MATERIALS AND METHODS

Antiseptics

This study was conducted in microbiology laboratory at Institute of Medical Molecular Biotechnology, Faculty of Medicine, Universiti Teknologi MARA (UiTM) from February to August 2017. In this study, the same antiseptics which were already used by different wards and Operation Theater in UiTM Private Specialist Centre (PPP-UiTM), Sungai Buloh, Selangor, Malaysia were used. Five commonly used antiseptics and disinfectants were evaluated in this study including Heptin [Chlorhexidine Gluconate 0.5% in alcohol 70% Nanz Med Science Pharma, Himachal Pradesh, India)],

Hydrogen peroxide 6% w/v (Wellmex Sdn Bhd, Selangor, Malaysia), Iodine [Povidone Iodine 7.5% w/v (Thermalife, Pinang, Malaysia)], Alcohol [Ethanol 70% v/v Fisher, Loughborough, UK)] and Dettol [Chloroxylenol 4.8% w/v (Reckitt Benckiser, Hull, UK)].

Disinfectant dilution methods

A series of decreasing concentrations of the antiseptics were obtained using serial dilution method in which the original concentration of antiseptic was considered 100%, the subsequent concentration was prepared by adding 9 ml of antiseptic into a tube with 1 ml distilled water to give 90% concentration. Then the rest concentrations were prepared in descending same manner. The antiseptics concentrations used in this study range from 100 to 10%.

Cultivation of bacterial strains

Five bacterial types isolated from UiTM Private Specialist Centre were used in this study including methicillin-resistant *S. aureus* (MRSA), *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella* species and *P. aeruginosa*. Each bacterium was cultured in nutrient agar for 24 h at 37°C. All tested bacteria were maintained in nutrient broth at 4°C and subcultured on Luria Bertani agar plates 24 h prior to any antimicrobial test. Luria Bertani broth was used for all antibacterial testing.

Antimicrobial susceptibility assays

Well diffusion method

Agar well diffusion method was used to determine antimicrobial activity of different antiseptics. Two bacterial colonies were inoculated in Tryptic soy broth for 3 h at 37°C and turbidity was adjusted in phosphate buffered saline to 0.5 McFarland's scale. 100 µl of bacterial broth was spread on Muller-Hinton agar plates containing ten 6 mm wells. Thirty microliters of each different concentration (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100%) of each antiseptic was poured into each well and these plates were incubated at 37°C aerobically for 24 h. The diameter of the zone of growth inhibition around the wells were measured in millimeters and recorded. Wells containing antiseptics which showed no inhibition zones were considered as negative results. Antibiotic disc was used as positive control.

Minimal inhibitory concentration (MIC)

Broth dilution assay was used to determine the MIC of different antiseptics against bacteria causing nosocomial infections as recommended by the Clinical Laboratory Standards Institute (Wayne, 2012). The concentrations of the antiseptics tested ranged from 100 to 10%. This test was performed in sterile bijou bottles which were loaded with 100 µL of each antiseptic dilution into each bottle.

Bacterial inoculums (100 µL) containing 5×10^5 CFU of each microorganism were added to each bottle (European Society of Clinical Microbiology and Infectious Diseases, 2003). In each panel of the tested antiseptic, a positive control (without antiseptic) and negative control (no inoculum) were added. All bottles were aerobically incubated at 37°C. After incubation for 24 h, the bacterial growth was assayed by its visible turbidity. The highest dilution of the antiseptic which showed no visible bacterial growth and no turbidity in bijou bottle was considered as MIC. After 24 h of incubation, 100 µL of each mixture was pipetted and inoculated on blood agar and spread uniformly with the sterile spreader and again incubated for 24 h at 37°C. On the next day, all blood agars were examined and all bacterial colonies were counted and recorded.

Ethical approval

The study was conducted in accordance with the principles of the Declaration of Helsinki. All participants provided written informed consent.

RESULTS

The antibacterial effect of antiseptics on nosocomial bacteria was presented in Table 1 which showed the inhibitory effects of different antiseptics on nosocomial bacteria using different concentrations of antiseptics. Specific antibiotic discs were used as a positive control accordingly. H_2O_2 showed excellent inhibitory effects on all nosocomial bacteria even with lower concentrations (10%). However, ethanol did not show any inhibitory effects as shown in Figure 1a to c.

The MIC of chlorhexidine against MRSA and *P. aeruginosa* was 10%, while for *A. baumannii* was 20% (Table 2). The results showed that *Klebsiella* spp. was sensitive to H_2O_2 only with MIC of 10%, while *A. baumannii* was resistant to ethanol only. MRSA, *A. baumannii* and *E. coli* were sensitive to Iodine at various concentrations. *E. coli* was sensitive to H_2O_2 with MIC of 50%. Vancomycin disc was used as a positive control and showed inhibition in the growth of MRSA while polymyxin B disc was effective against *A. baumannii* and *P. aeruginosa.* Also, Imipenem showed inhibition of *E. coli* and *Klebsiella* spp.

Thus, H_2O_2 clearly shows effectiveness against all nosocomial bacteria since it has the largest zone of inhibition among all the antiseptics (Table 1).

Table 3 shows the inhibitory effects of the highest concentration of different antiseptics used on nosocomial bacteria after 10 min incubation. The results showed that H_2O_2 had excellent effect and all bacteria showed no growth on blood agar, while chlorhexidine and iodine had excellent effects on *E. coli* and *Klebsiella*. The next antiseptic in descending order of their effectiveness was Dettol since both *E. coli* and *Klebsiella* were able to survive. However, all studied bacteria showed full growth and not affected by ethanol.

DISCUSSION

This study showed that antiseptics used in PPP-UiTM still have considerable bactericidal effects on nosocomial bacteria. In 2010, Malaysia was estimated to have hundred thousand cases of nosocomial infection, amounting to 13.9% of the overall hospital admissions (Frost and Sullivan, 2011). This study revealed that different types of nosocomial bacteria vary in their response to different types of antiseptics.

Table 1. Bacterial inhibition zones by using different concentrations of antiseptics.

Antiseptic/Bacteria		Antiseptic concentrations (%)										Positive control
		100	90	80	70	60	50	40	30	20	10	
Chlorhexidine	MRSA	25	24	23	23	22	22	22	21	20	20	20[a]
	A. baumannii	20	18	17	17	17	16	16	16	15	14	15[b]
	E. coli	26	22	20	20	18	18	18	17	17	16	30[c]
	Klebsiella spp.	24	24	22	22	21	20	20	18	17	16	30[c]
	P. aeruginosa	22	21	21	21	20	19	19	18	18	17	16b
H_2O_2	MRSA	30	30	30	30	30	30	30	30	30	30	20[a]
	A. baumannii	30	30	29	29	28	28	27	27	22	20	15[b]
	E. coli	38	38	38	38	38	36	25	23	20	18	30[c]
	Klebsiella spp.	34	34	33	33	32	32	31	31	30	30	30[c]
	P. aeruginosa	30	30	28	28	26	26	25	24	20	18	15[b]
Iodine	MRSA	30	28	26	25	24	24	23	22	18	17	20[a]
	A. baumannii	21	21	20	20	19	18	15	14	12	10	15[b]
	E. coli	35	33	31	26	12	10	8	0	0	0	31[c]
	Klebsiella spp.	24	22	22	20	20	12	10	8	8	0	30[c]
	P. aeruginosa	13	12	12	11	10	9	8	7	0	0	15[b]
Ethanol	MRSA	0	0	0	0	0	0	0	0	0	0	20[a]
	A. baumannii	0	0	0	0	0	0	0	0	0	0	14[b]
	E. coli	0	0	0	0	0	0	0	0	0	0	30[c]
	Klebsiella spp.	0	0	0	0	0	0	0	0	0	0	30[c]
	P. aeruginosa	0	0	0	0	0	0	0	0	0	0	14[b]
Dettol	MRSA	30	29	28	27	26	25	24	23	22	21	20[a]
	A. baumannii	34	32	24	24	23	22	22	20	20	20	16[b]
	E. coli	20	18	18	16	16	14	14	14	14	12	30[c]
	Klebsiella spp.	20	18	18	16	15	14	14	14	13	12	30[c]
	P. aeruginosa	0	0	0	0	0	0	0	0	0	0	15[b]

a: Vancomycin, b: Polymyxin B, c: Imipenem.

Table 2. Minimal inhibitory concentration (MIC) of antiseptics against nosocomial bacteria.

Antiseptics	MRSA (%)	*A. baumannii* (%)	*E. coli* (%)	*Klebsiella spp.* (%)	*P. aeruginosa* (%)
Chlorhexidine	10	20	++	++	10
HPX	10	10	50	10	10
Iodine	30	40	80	++	++
Ethanol	++	++	++	++	++
Dettol	10	10	++	++	++

++ Full growth of bacteria seen on bijou bottles and blood agar plates.

Table 3. Antibacterial effect of highest concentration of antiseptics on nosocomial bacterial on blood agar.

Antiseptics	Bacteria				
	MRSA	*A. baumannii*	*E. coli*	*Klebsiella*	*P. aeruginosa*
Chlorhexidine	No growth	Moderate growth	No growth	No growth	No growth
Hydrogen peroxide	No growth	No growth	No growth	No growth	No growth
Iodine	No growth	No growth	No growth	Moderate growth	Moderate growth
Ethanol	Full growth	Full growth	Full growth	Full growth	Full growth
Dettol	No growth	No growth	Moderate growth	Moderate growth	Full growth

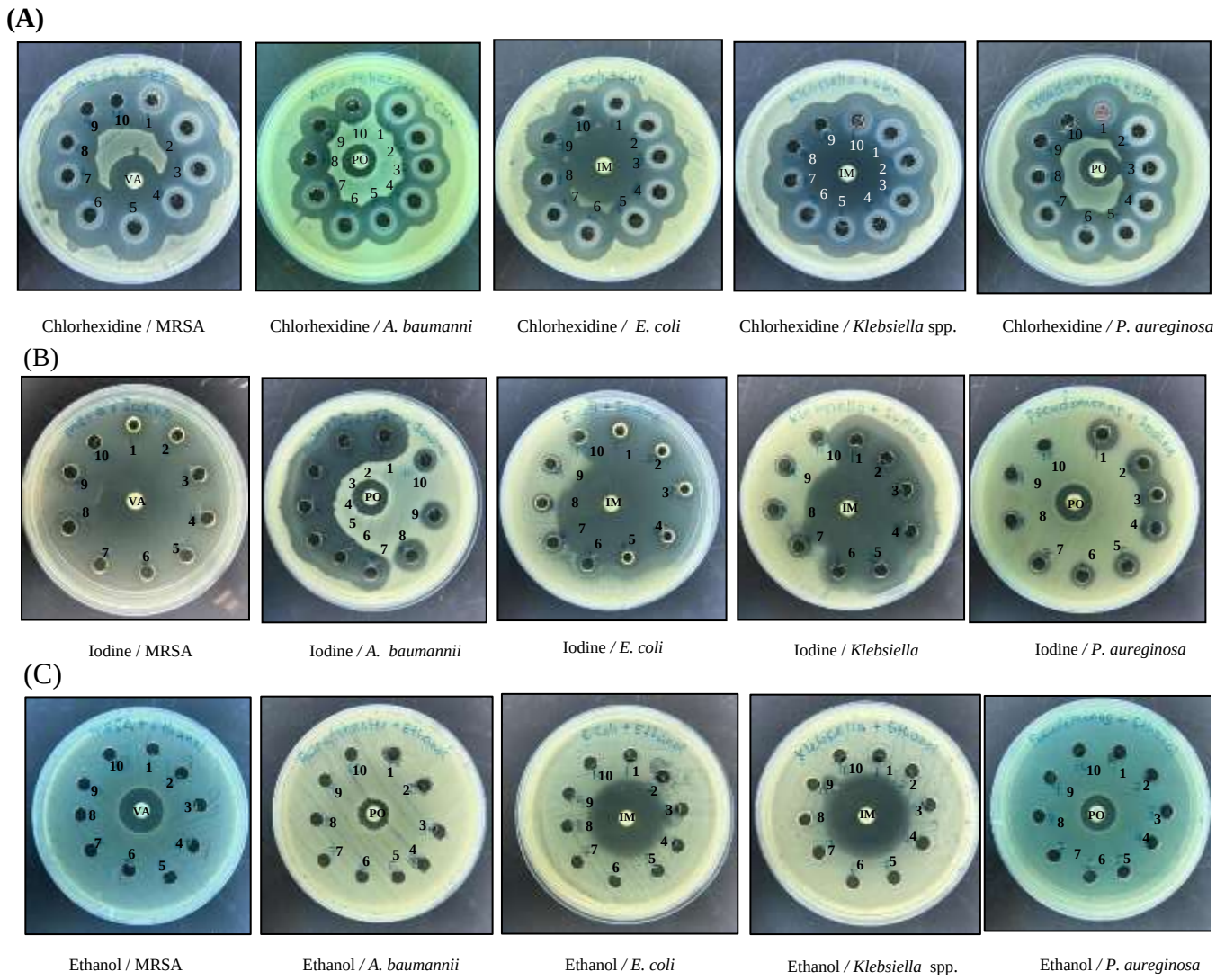

Figure 1. Susceptibility testing of A. Chlorhexidine B. Iodine and C. Ethanol against nosocomial bacteria.

Chlorhexidine is a broad spectrum bactericidal antiseptic that is widely used as in dental, surgical settings and also used in handwashing. Same as previously reported, this study showed excellent inhibitory effects of chlorhexidine against MRSA, *A. baumannii* and *P. aeruginosa* even with low concentrations (Ekizoglu et al., 2016; Lanjri et al., 2017); while on *E. coli* and *Klebsiella* it was ineffective with lesser inhibitory zones compared to Imipenem as a consequent of the outer membrane which acts as a selective permeability barrier in limiting the entry of many harmful chemical compounds into the bacterial cell (Russell et al., 1998). Chlorhexidine is working on the cytoplasmic membrane and its cationic nature helps in connection with the anionic group (phosphate groups of teichoic acids in Gram-positive bacteria and lipopolysaccharide in Gram negative bacteria) on the bacterial surface with resulting modification of membrane permeability. The effect is mainly due to electrostatic interaction of the chlorhexidine with the acid phospholipids in the cytoplasmic membrane which implies actual absorption onto the cytoplasmic membrane of Gram positive and Gram negative bacteria and leading to a destructive effect. Using chlorhexidine at low concentrations resulted in a bacteriostatic effect while at high concentrations; it can cause membrane disruption and cell death due to coagulation of the cytoplasm (Estrela et al., 2003). Chlorhexidine lasts much longer than other antiseptics; therefore, it is often combined with alcohol in a newer skin preparation composed of 2% chlorhexidine gluconate and 70% isopropyl alcohol (Mangram et al., 1999). It is reported to have a rapid onset of action and has persistent activity to reduce microbial burden on patients' skin and prevent secondary bacterial infections (Climo et al., 2013). Adaptation and resistance to chlorhexidine has been reported previously among MRSA and many other Gram-negative bacteria

including *P. aeruginosa* and *E. coli* (Kampf and Kramer, 2004).

Hydrogen peroxide has a broad-spectrum effect against bacteria, bacterial spores, viruses and yeasts (Brudzynski, 2006). This study showed excellent inhibitory effects of H_2O_2 against both Gram-positive and negative bacteria even with lowest concentration of H_2O_2 due to a potent oxidant which produce a hydroxyl radicals which in turn will attack cell membrane, lipids, DNA, and other essential cell components (Mai-Prochnow et al., 2008). The results of the present study were in agreement with previous report by Lemmen et al. (2015) who deduced that H_2O_2 was effective against nosocomial pathogens such as MRSA and multidrug-resistant *A. baumannii* in hospital settings. Previous study by Kenar et al. (2007) concluded that higher concentrations of H_2O_2 (10 to 30%) and prolonged interaction are required for sporicidal activity, unfortunately the effect of H_2O_2 on fungus was not included in this study. Although H_2O_2 showed inhibitory effect on *E. coli* after 50% dilution but it is still effective in reducing the expression of all the virulence factors of *E. coli* by oxidative stress of H_2O_2 (Hegde et al., 2008). Thus, H_2O_2 clearly shows effectiveness against all nosocomial bacteria since it has the largest zone of inhibition among all the antiseptics.

The results of this study showed that iodine had comparable effects to chlorhexidine but less than H_2O_2. These results however were not in agreement with previous finding that chlorhexidine are more effective than iodine in reducing nosocomial infections (Nishimura, 2006). The results reveal variations of the effect of Iodine on different bacteria with different dilutions. The best inhibitory effect of iodine seen against MRSA, *A. baumannii* and *E. coli* with dilutions of 30, 40 and 80%, respectively. Hence the more dilution of iodine might weaken the iodine linkage to the carrier polymer with an accompanying increase of free iodine in solution. Therefore, iodine must be diluted according to the supplier's directions to achieve antimicrobial activity. Based on the aforementioned results, we recommended to use iodine in lower concentrations to avoid skin irritation as previously reported (Murthy and Krishnamurthy, 2009). Due to its rapid, effective and broad-spectrum antimicrobial effects, povidone iodine is likely to remain a highly effective in preventing nosocomial infections in the foreseeable future. A previous clinical trials revealed that iodine was significantly superior to other antiseptic agents such as silver sulfadiazine cream and non-antiseptic dressings, but had lesser effect than rifampicin local cream. Therefore, iodine should be considered among the modern antiseptic agents. In contrast, iodine has many cellular targets, including fatty acids, nucleotides and the free sulfur amino acids cysteine and methionine in proteins 63. This makes the development of resistance unlikely.

Ethanol has a rapid broad-spectrum antimicrobial effect against bacteria, viruses and fungi; however, it is not sporicidal, therefore it is not recommended for sterilization, yet ethanol is used as antiseptics for both hard-surface and skin (McDonnell and Russell, 1999). This study demonstrates that all bacteria were resistant to ethanol at various concentrations. These results were in agreement with recent report by Pidot et al. (2018) who stated that the multidrug-resistant bacterium has become gradually tolerant to the ethanol in widely used hospital disinfectants such as hand rub solutions. Although ethanol performs a multifunctional inhibitory effect on bacterial cells, however the resistant bacteria can overcome the denaturation of proteins, inhibition of DNA, RNA, protein, and peptidoglycan synthesis by ethanol. Researchers have found out that drug-resistant bacteria that commonly cause hospital infections have the chance to develop resistance to ethanol (Cariz, 2018).

Dettol had broad spectrum activity as it inhibited the growth of Gram positive and Gram negative bacteria. Dettol is working through the penetration into the cell and action at the target site through intra-cellular mechanism. Both MRSA and *A. baumannii* were most susceptible to Dettol at different concentrations even at lowest concentration of 10%. It still showed the highest inhibition zone at 30 and 34 mm for MRSA and *A. baumannii*, respectively, however *P. aeruginosa* was resistant even with 100% concentration. Also, both *E. coli* and *Klebsiella* were resistant at 100% concentration. Previous studies showed variations for the effects of Dettol on different pathogens due to difference in the species or strains of the organisms or the techniques used (Rutala et al., 2000).

Conclusions

This study has confirmed that H_2O_2 was the strongest antiseptic against nosocomial bacteria followed by chlorhexidine, whereas ethanol was the weakest one. Determination of antimicrobial efficiency of antiseptics regularly is crucial to reduce NI which also could be reduced by using a proper antiseptic with adequate dilutions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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