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ORIGINAL ARTICLE

Evaluation of Chloroxylenol, Ethanol, Chlorine, and Povidone-iodine against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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ABSTRACT

Background: *Healthcare settings require effective antiseptics and disinfectants to combat Methicillin-resistant Staphylococcus aureus (MRSA) since it demonstrates resistance to beta-lactam antibiotics at the global level.* ***Objective:*** *The current study aims to assess in vitro action of the widely-used commercial antiseptics on methicillin-Resistant Staphylococcus aureus (MRSA).* ***Methodology:*** *Out of 50 samples, 5 methicillin-resistant Staphylococcus aureus strains isolated identified in cases of Acne vulgaris, and the efficiency of four frequently used disinfecting agents include ethyl alcohol, sodium hypochlorite (chlorine), iodine (povidone), and chloroxylenol (Dettol) was assessed using the suspension test against each isolate at different contact periods.* ***Results:*** *When using the spread plate method, it was discerned how many bacterial cells the antiseptics are capable of inhibiting. They also came to realize that the MRSA were highly sensitive to the ethyl alcohol with minimal inhibitory concentrations (MICs) of 70% in 30 seconds and 1 minute. MICs of 1 were found for both iodine and chloroxylenol; for iodine, its concentration was fixed at 5% and chloroxylenol at 1%. With 5% addition of chloroxylenol, no increasing antibacterial activities against the tested strains were recorded. MRSA has been proved sensitive to iodine and chloroxylenol, with MICs of 3% and 6%, respectively. Chlorine was able to kill MRSA at dilutions of 1%, 2%, and 3% within 10 minutes, which pointed to the fact that the addition of chlorine did not improve the bactericidal effectiveness of the agents.* ***Conclusion:*** *The results of the carried-out investigation support the importance of disinfection as one of the targets for MRSA control and elimination.*

INTRODUCTION

The increase in antimicrobial resistance (AMR) rate and the stagnation of antimicrobial development is one of the most worrisome issues facing current health care systems. Thus, it is important to embrace strategies that can effectively introduce mechanisms enabling control of bacterial density and spread of infection and excluding the use of antibiotics. Antiseptics are important in preventing infections in numerous areas of treatment and healthcare with various types of wound care, burn care, and surgical site-infected patients[1,2]. The major function of antiseptic is exerted by multiple mechanisms of action, that is why the appearance of resistance to antiseptics is hardly probable[3]. In selecting an acceptable antiseptic for a certain indication, attention should be given to the physicochemical properties of the chosen agent and its therapeutic indication[4].

In general, it is easier to discuss antiseptic properties in comparison with antibiotic ones: the former has a higher antimicrobial activity and antimicrobial spectrum index, and there is also a significantly lower risk of bacteria's resistance to them. On this account, antiseptics are better substitutes to antibiotics for treatment of localized simple dermatoses. The antimicrobial activity of antiseptic depends on concentration, time, and temperature and that the evolution of the latter effect may be quite intricate. Taking into consideration the size of the antibacterial constituent, the antiseptic preparations can be grouped into two categories. For molecules of the chlorhexidine size and larger, they cannot penetrate bacterial membrane channels (porins) and therefore have to be adsorbed on the microbial membrane for activity to occur. However, the small ions accessible to porins, such as di iodine or free iodine from povidone iodine, go through porin and oxidize the proteins in the bacterial cytoplasm[5,6]. Porins are present in the gram-positive bacteria plasma membrane, while in addition to the plasma membrane, the gram-negative bacteria outer membrane contains porins[7]. At present, one of the very common opportunistic pathogens is gram-positive bacteria, *Staphylococcus aureus* (*S. aureus*), which can affect the immunocompromised cases. The impetus to consider *S. aureus* a threat to public health is that people act as natural carriers to this pathogen, or reservoirs[8,9]. *S. aureus*, when entering the skin superficially, can cause folliculitis, which is a mild skin infection[10]. Thus, *S. aureus* continues to be a serious pathogen in humans; as it invades deeper layers of tissues. It may cause systemic infection, septicemia, sepsis, and infective endocarditis with a high rate of morbidity and mortality even in the era of antibiotic use. This leads to an extended length of stay for the patient and also a burden, in terms of physical and psychological to both the patient's family as well as the caregivers, not forgetting the economic implication this puts on the nation[11,12].

Methicillin-resistant *Staphylococcus aureus* initially emerged in the early 1960s when *S. aureus* acquired the ability to resist new antimicrobials shortly after methicillin was used for the first time as an antibiotic. Since then, MRSA has remained the world's no. 1 nosocomial pathogen and has remained the subject of focus among nosocomial researchers in the health community[13,14]. In particular, the increase in frequency in the hospital setting and the length of phases of treatment is primarily due to the dissemination of multi-drug-resistant (MDR) *S. aureus* strains. Its spread, as seen earlier, has remained high. One of the best known is methicillin-resistant *S. aureus* (MRSA). MRSA accounts for more than half of the Healthcare-Associated *S. aureus* isolates in the majority of countries. It is assumed that roughly 50 million people in the world are infected with MRSA[15]. The deaths attributable to *Staphylococcus aureus* (MRSA) and antibiotic resistance in the same year (2019) were more than a hundred thousand. The MRSA isolation rate of over fifty percent was noted in the ICUs of the US in 2011 and Asia has the highest incidence of MRSA in the world[16].

MRSA is transmitted from one patient to another through the airborne pneumonia and the poor washing of the health care givers hands. The wound MRSA of surgical site is the key reason for bacteremia. Meanwhile, there are no clear guidelines on MRSA wound care. MRSA is controlled through antiseptics, but the history of using antibiotics results in the formation of antiseptic-resistant bacteria. Subsequently, the discovery of new antibiotics and enhancing awareness about them have not resulted in the reduction of MRSA as one of the most significant pathogens, representing high mortality rates[17,18].

The aim of this study is to determine the in vitro antimicrobial effectiveness of commonly used antiseptics, including povidone iodine, ethanol, chloroxylenol, and chlorine, toward MRSA isolated from acne vulgaris cases.

METHODOLOGY

Out of total 50 samples, 5 methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were isolated from acne vulgaris**.** Among these 5 isolates, one isolate was selected that is different from the others as it is resistant to a variety of antibiotics such as doxycycline, ampicillin, gentamicin, erythromycin, ceftriaxone, oxacillin, and methicillin. Also, during the clinical examination, the selected isolate resulted in severe damage to the face of the patient, ranging from inflammatory and abscess formation to deformity.

S. aureus MRSA was conventionally isolated according to El-Gohary[19]. Typical colonies on Staphylococcus Medium No.110 (Oxoid, UK) were picked up, transferred to Brain Heart Infusion (BHI) broth and incubated at 35-37°C / 24 hr. Gram staining, oxidase, catalase and coagulase tube test were used for biochemical identification. Identification of bacterial isolates was conducted using the Advanced Expert System of the VITEK 2 system to identify the MRSA, and the diagnosis has been confirmed by using RT-qPCR to detect *mecA* gene (111bp) [20].

Antiseptics:

Four different types of used antiseptic available in the local market, namely chloroxylenol, ethanol, chlorine, and povidone-iodine, were chosen in order to assess and compare the efficiency of these materials against MRSA. Using sterile distilled water, three different concentrations of the following antiseptics: chloroxylenol 1%, 5%, 3%, 6%; chlorine 1%, 2%, 3%; and povidone iodine 1%. Ethyl alcohol at 70% and 99% concentrations were tested, while all other antiseptic were tested at 5%, 3%, and 6% (Table 1).

Table 1: Type, composition, concentration, and dilution of the disinfectant used.

Antiseptic	Concentration	Tested dilutions
Chloroxylenol - Dettol	5%	1.5%, 3%, 6%
Ethanol	100% and 70%	70%, 99%
Chlorine	5%	1%, 2%, 3%
Povidone-iodine	7.5%	1.5%, 3%, 6%

Preparation of inoculum

Two to five colonies of MRSA were streaked into 4-5 ml of nutrient broth, and incubated for 24 hours at 35-37 °C according to Reller [21].

Quantitative suspension test:

The bactericidal efficacy of the selected disinfectant concentrations was evaluated using a quantitative suspension test over three exposure times (5, 10, and 15 minutes), with the exception of ethyl alcohol, which was evaluated at 30 seconds and 1 minute **Table 2**. Briefly, the processed bacterial inoculum standardized with McFarland was incorporated with 3 dilutions of the prepared disinfectant. For appropriate reaction times, to inactivate the disinfectant, 1 ml of the disinfectant-bacterial suspension was added to 0.5 ml of which a solution of tween 80 was used to neutralize the solution. In all nine determinations of each disinfectant sample, the procedures were done three times on each tested dilution. Consequently, 0.01 ml aliquots of the disinfectant-bacteria-neutralizer mixture were spread out on nutrient agar (Oxoid, UK) and spread evenly over the surface. By the end of every incubation time in the plates, the existence of colonies was assessed at 24 hours, 37 °C[19].

Table 2: Bacterial activity (expressed as presence or absence of colonies) of different disinfectant concentrations and contact times on MRSA isolate.

Disinfectant	Concentration	Contact times		
		5	10	15
Iodine	1.5% 3% 6%	+ - -	+ - -	+ - -
Dettol	1.5% 3% 6%	+ - -	+ - -	+ - -
Chlorine	1% 2% 3%	+ + +	- - -	- - -
Ethyl alcohol	70%	30sec.	1min.	
		-	-	
Ethyl alcohol	99%	+	+	

Since this test has three parts —an agent, dilution, and contact time —an evaluation score was derived for the purpose of the antiseptic comparison. The evaluation's score varied from good to inadequate depending on whether colonies were found inside the plate and how much the disinfectant was diluted: ++++ suggests that there is no growth score for the colonies at the lowest concentration, while the score increases with an increase in the concentration. In **Table 3**, no colonies were observed up to optical densities of 1:4 dilutions in ++++ = excellent; at a dilution of 1:2 in +++ = very good; and in the stock solution in ++ = good; in the measured MIC commercial concentration in + = poor[22].

Table 3: Evaluation of selected disinfectants based on progressive effects ranging from excellent to no effect depending on detection of colonies.

Antiseptic	Score	Grade
Ethyl alcohol 70%	+++++	Excellent
Iodine	++++	Very good
Dettol	++++	Very good
Chlorine	+++	Good
Ethyl alcohol 99%	+	No effect

RESULTS

In our study, ethyl alcohol was applied in two concentrations: 70% and 99%, and at contact times of 30 and 60 seconds. The present study highlighted that there was a high level of bactericidal effect of ethyl alcohol against the MRSA isolates without formation of colonies in the agar plates at 70% of its concentration and different contact times. The most viewed numbers of time intervals were 30 seconds and 1 minute. Ethyl alcohol 99 was less effective in bactericidal activity in MRSA isolates, and this was evident where colonies were observed at the agar plates on the tested concentrations and contact times (Figure,1).

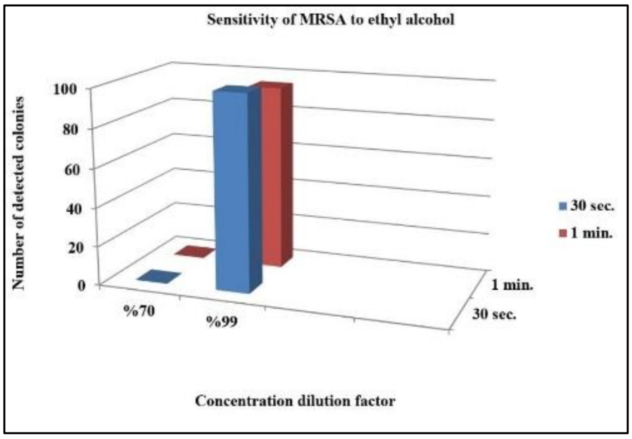

Fig.1. Bactericidal effects of ethyl alcohol against MRSA

The present study showed high bactericidal efficacy of Povidone-iodine against MRSA isolates with non-detectable colonies in the concentrations of 3% and 6% at varied contact times of 5, 10, and 15 microorganisms, while MRSA colonies were grown at 1% and 5% of iodine at the exposure times of 5, 10, and 15 minutes (Figure 2).

The information gathered on Dettol evaluation indicated that it possesses a very good bactericidal effectiveness against MRSA isolate; colonies were not detected until 3% concentration at contact durations of 5, 10, and 15 minutes. However, no appreciable inhibitory effect at tested concentration was observed at the contact times of 5, 10, and 15 minutes. 5% (Figure 3).

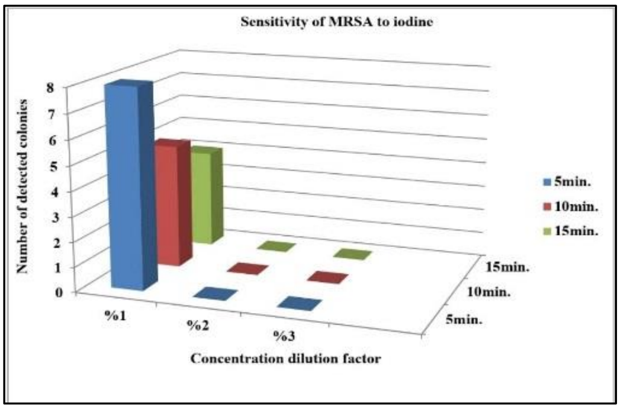

Fig. 2. Bactericidal effects of iodine against MRSA

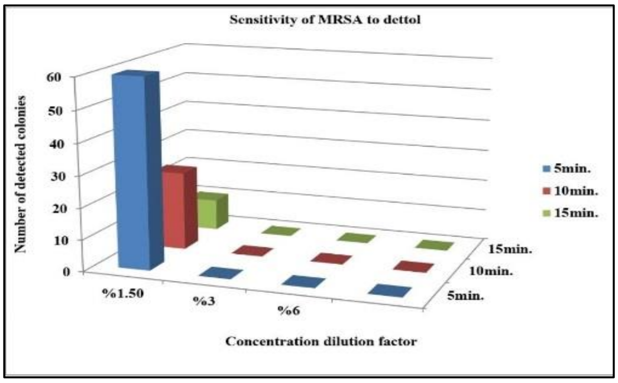

Fig. 3. Bactericidal effects of Dettol against MRSA

In the current research, chlorine was effective against MRSA isolate at commercial concentrations of (1%, 2%, and 3%); no colonies were obtained for the tested contact times (10 and 15 minutes), but MRSA colonies were observed at chlorine concentrations of (1%, 2%, and 3%) with an exposure time of 5 minutes (Figure 4).

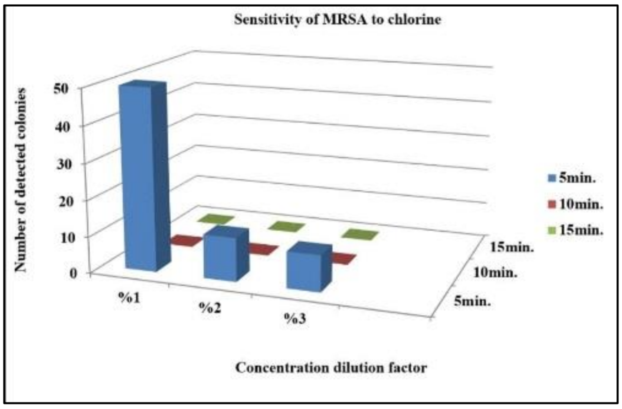

Fig. 4. Bactericidal effects of chlorine against MRSA

DISCUSSION

S. aureus, particularly MRSA, is accountable for well-known nosocomial-gained infection well known. Staphylococcus infections are endogenous and exogenous[23]. The purpose of using chemicals is to minimize the presence of the transient flora on the hands and eradicate the transfer of the antibiotic-resistant and pathogenic bacterial strains that cause infection, such as MRSA, before the infection develops. Measures directed toward MRSA eradication mainly rely on three key strategies: preventative measures also include isolation methods, antibiotics, and fumigation[24]. Disinfection has been regarded as the most effective control measure in minimization or eradication of any infectious disease, and more so with the current evidence indicating that MRSA possesses the ability to develop resistance to these chemicals while the risk of these microbes towards antiseptic agents remains unknown.

Due to such consideration, the effectiveness of the antiseptic agents used in undertaking this study is iodine, sodium hypochlorite, ethyl alcohol, and chloroxylenol on MRSA.

Classically, recommendation for removing surface contamination involves washing the area before use of a microbicidal agent, for instance, with seventy percent alcohol what makes the ethanol used is because of its cheap price as well as its availability in the health care services. Ethyl alcohol destroys bacteria by changing the form or shape of the proteins of the bacterial cell wall damaging bacterial cell membranes, but it does not destroy spores[25].

Lower concentration of 70% ethanol exerts an inactivating effect on MRSA, whereas 99% was not sufficiently an inactivating concentration. This may be due to the fact that a high concentration of ethanol is incapable of passing through the bacterial membrane. These results are in concordance with the studies done by that ethanol has potential application as an antiseptic for MRSA[26]. These results are in contrast with findings on the same aspect of studies. Specifically, ethyl alcohol 70% demonstrated that it had insufficient bactericidal effects on MRSA isolates in which colonies were recoverable in all concentrations and contact times[19]. The same goes for ethyl alcohol 99%, which was reported by the same study to have inadequate bactericidal effects on MRSA isolates. Results coincide with a study showed that ethanol with concentrations greater than 90% and greater than 100% exhibits no bactericidal or virucidal safety[27].

Povidone-iodine (PVP-I) is one of the most active ingredients in antiseptic or antiseptic products that are widely used in the medical field and in the public health domain to control the transmission of infectious agents[28]. On the other hand, PVP-I is an iodophor that is soluble in water and comprises a complex of iodine and polymer polyvinylpyrrolidone (povidone/ PVP). Upon dissolution in water, the iodine is released in the form of free iodine I2, which can effectively infiltrate microorganisms and induce oxidation of proteins, nucleotides (with a particular emphasis on cysteine), and fatty acids. This process results in a reduction of protein synthesis and damage to the cell membrane or wall, ultimately leading to cell death[16]. Iodine is proved to be a bactericidal, fungicidal, virucidal, and sporicidal; because iodine can enter the cellular wall, it kills rapidly; it reacts with cell contents; free sulfur amino acids; cysteine and methionine; nucleotides; and fatty acids[23].

In our work, the antibacterial activities of iodine, with MICs of 1.5% for iodine, was unable to potentiate the antibacterial activities against strains tested, at three exposure times, however, indicated high sensitivity of MRSA to Iodine with MICs 3% and 6% (iodine) at three exposure times. These findings are therefore in concordance with several studies[23,25, 29], which observed that iodine has a profound ability to eradicate MRSA at 10%, 7%, and 5%, but our results disagree with them at concentrations of 1% and 0.5%. Low concentration exposure of biocides can prompt bacterial adaptive response or resistance to antimicrobial agents in bacterial cultures.

Dettol refers to several hygienic products that are widely used at homes as well as in hospitals and other institutions for various purposes of cleaning and disinfection of skin, and objects, and equipment, as well as environmental surfaces[30]. In this case, the use of Dettol application reduces the incidents of microbial colonization of the skin and surfaces by a very big margin. In the bactericidal activity of chloroxylenol there is rapid destruction of the membrane structure and function with a general leakage of cytoplasmic constituents out of the cell; such membrane damage is irreversible, and hence the cell cannot avoid the loss of essential[31,32].

Our data regarding Dettol evaluation elucidated that it has a very good bactericidal power on MRSA isolate. Our observations align with a study, which indicates that subtoxic concentrations of antibacterial biocides promote the activation of horizontal gene transfer mechanisms that allow the transfer of antimicrobial resistance genes among bacteria in a petri dish[33]. Contrary to the existence of studies done by researchers which indicated that commercial concentrations and diluted Dettol hinder MRSA to a moderate level[32,33]. Researchers were also able to establish the fact that Dettol was active and potent against different microorganisms, especially where organic matter is negligible or relatively low[34,35].

Sodium hypochlorite (NaOCl) (commercially used as chlorine) is a powerful oxidizing agent that possesses broadband microbial activity against gram-positive, gram-negative, aerobic, and anaerobic bacteria, including MDR bacteria[36]. NaOCl is an active sodium that is available in different places in the world, has a positive ratio of cost and effectiveness, and has many applications; for instance, surface disinfection, washing, or preparing drinking water[37]. Sodium hypochlorite, known as household bleach, which is a chlorine-releasing agent used for the disinfection process, affects proteins, DNA, and cell membranes, as has been researched by Aboualizadeh[38]. In the present study, chlorine showed a good inhibitory activity on MRSA isolate. Our result was in agreement with a study, which observed that diluted sodium hypochlorite was found to take longer time to counteract the MRSA than the concentrated form[40,41]. Inappropriate use of surface disinfectant (for example, incorrect concentration or excessive sub-inhibitory biocide residues on surfaces) may constitute a selective pressure contributing to the development of tolerant or resistant strains.

CONCLUSION

In conclusion, choosing a proper disinfectant or antiseptic is looked at as the foundation to prevent the appearance of antibiotic-resistant strains of bacteria like MRSA, and so is a crucial step in any sort of bio-security program. Seventy percent ethyl alcohol was very effective in inhibiting the growth of MRSA. Povodine-iodine and Dettol have low concentration antibacterial activity; nevertheless, they enhance the activities against the following MRSA strains at high concentration. In addition, no high activity of chlorine in all the concentrations was observed at short exposure times. The similar antibacterial activities as those of the other samples have been observed, while there were better results at longer exposure times. Ethyl alcohol 99.9% did not show any form of antibacterial activity in the tested isolate.

Conflict Of Interest

There is no conflict of interest to declare.

Ethical Approval

Not applicable

Consent for publication:

Not applicable

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