



TURKISH REPUBLIC OF NORTHERN CYPRUS

NEAR EAST UNIVERSITY

HEALTH SCIENCES INSTITUTE

***In vitro* ACTIVITY OF THREE DISINFECTANTS AGAINST  
MRSA AND MSSA STRAINS**

YASMIN ALI SALIM ABUANIZA

MASTER OF SCIENCE THESIS

MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY DEPARTMENT

NICOSIA, 2020

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ADVISOR

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NICOSIA, 2020

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## DECLARATION

I hereby declare that the work in this thesis entitled “**In vitro Activity of Three Disinfectants Against MRSA and MSSA Strains**” is the product of my own research efforts undertaken under the supervision of Assoc. Prof. Dr. Meryem Güvenir. No part of this thesis was previously presented for another degree or diploma in any university elsewhere, and all information in this document has been obtained and presented in accordance with academic ethical conduct and rules. All materials and results that are not original to this work have been duly acknowledged, fully cited and referenced.

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## **ABSTRACT**

**Yasmin Ali Salim Abuaniza. *In vitro* Activity of Three Disinfectants Against MRSA and MSSA Strains. Near East University, Institute of Health Sciences, Medical Microbiology and Clinical Microbiology Program, M.Sc. Thesis, Nicosia, 2020**

In our study, it was aimed to investigate the effect and duration of action of three antiseptic and disinfectants on MRSA and MSSA isolates. A total of 40 *S.aureus* isolates including 20 MRSA and 20 MSSA were included in our study. In our study, *S. aureus* ATCC 25923 standard strain was used as control strain. Sodium hypochlorite (pure, 1/10, 1/100), ethyl alcohol (95%, 70%, 50%) and povidin-iodine (pure, 1 / 2.1 / 4) antiseptic and disinfectants prepared in different concentrations were used. Bacteria were tested using the qualitative suppression test method. Antiseptic-disinfectant and bacterial contact was investigated for 1,2,5,10 and 30 minutes in the duration of the effect. As a result of our study, povidin-iodine; except for two MRSA and two MSSA strains, reproductive inhibitory effect was observed even in one minute contacts at all concentrations (pure, ½, 1/4). Sodium hypochlorite (pure) MRSA and MSSA strains had an anti-reproductive effect at all minutes (1,2,5,10 and 30 minutes). Growth was observed in three strains (2 MRSA and 1 MSSA) at a concentration of 95% of ethyl alcohol. Growth was observed in seven MRSA and 12 MSSA strains in 70% concentration of ethyl alcohol. Growth was observed in a total of 13 MRSA and 13 MSSA strains at 50% concentration of ethyl alcohol. Sodium hypochlorite (pure) and Povidin-iodine (pure) have been identified as the most effective substance in MRSA and MSSA isolates.

**Key words:** Antiseptic, disinfectant, MRSA, MSSA

## ÖZET

**Yasmin Ali Salim Abuaniza. MRSA ve MSSA Suşlarına Karşı Üç Farklı Dezenfektanın In vitro Aktivitesinin Araştırılması. Yakın Doğu Üniversitesi, Sağlık Bilimleri Enstitüsü, Tıbbi Mikrobiyoloji ve Klinik Mikrobiyoloji Programı, Yüksek Lisans Tezi, Lefkoşa, 2020**

Çalışmamızda, üç adet antiseptik ve dezenfektanın, MRSA ve MSSA izolatları üzerine etkisi ve etki süresinin incelenmesi amaçlanmıştır. 20 adet MRSA ve 20 adet MSSA olmak üzere toplam 40 *S.aureus* izolatı çalışmamıza dahil edilmiştir. Çalışmamızda kontrol suşu olarak *S. aureus* ATCC 25923 standart suşu kullanılmıştır. Farklı konsantrasyonlarda hazırlanan sodyum hipoklorit (saf, 1/10, 1/100), etil alkol (%95, %70,%50) ve povidin-iyot (saf, 1/2,1/4) antieptik ve dezenfektanlar kullanılmıştır. Bakteriler kalitatif süsoansiyon test yöntemi kullanılarak test edilmiştir. Etki süresinin araştırılmasında, 1,2,5,10 ve 30 dakikalık sürelerde, antiseptik-dezenfektan ve bakteri teması araştırılmıştır. Çalışmamız sonucunda Povidin-iyot; iki adet MRSA ve iki adet MSSA suşları haricinde tüm konsantrasyonlarda (saf, 1/2,1/4) bir dakikalık temaslarda bile üremeyi durdurucu etki gözlenmiştir. Sodyum hipoklorit (saf) MRSA ve MSSA suşlarında tüm dakikalarda (1,2,5,10 ve 30 dk) üremeyi durdurucu etki gözlenmiştir. Etil alkolün %95'lik konsantrasyonunda toplam 3 suşta (2 MRSA ve 1 MSSA) üreme gözlenmiştir. Etil alkolün %70'lik konsantrasyonunda toplam yedi MRSA ve 12 MSSA suşunda üreme gözlenmiştir. Etil alkolün %50'lik konsantrasyonunda toplam 13 MRSA ve 13 MSSA suşunda üreme gözlenmiştir. Sodyum hipoklorit (saf) ve Povidin-iyot (saf)'un MRSA ve MSSA izolatlarında en etkili madde olarak belirlenmiştir.

**Anahtar kelimeler:** Antiseptik, dezenfektan, MRSA, MSSA

# TABLE OF CONTENTS

## 1. INTRODUCTION

1.1. <i>Stahylococcus spp.</i>	1
1.2. <i>Staphylococcus aureus</i>	1
1.2.1. Morphology	3
1.2.2. Culture	3
1.2.3. Biochemical Properties	4
1.2.4. Cell Structure	5
1.2.4.1. Capsule	5
1.2.4.2. Cell Wall	6
1.2.5. Virulence Factors	6
1.2.6. Enzymes	7
1.2.6.1. Catalase	7
1.2.6.2. Coagulase	8
1.2.6.3. Lipase	9
1.2.6.4. Hyaluronidase	10
1.2.6.5. Staphylokinase	10
1.2.6.6. Deoksiribonuclease (DNase)	11
1.2.6.7. Penisilinase (Beta-lactamase)	12
1.2.7. Toxsins	13
1.2.8. <i>S.aureus</i> Infections	14
1.2.9. Metisillin Resistance	15
1.2.10. Antiseptic and Disinfectans	16
1.2.10.1. Sodium Hypochlorite	17
1.2.10.2. Ethyl alcohol	17
1.2.10.3. Povidone-Iodine	18



## **2. MATERIAL AND METHOD**

2.1. Material	19
2.1.1. Devices and Tools	20
2.1.2. Software Programs	20
2.2. Bacterial Strains Collection	20
2.3. Preperation of the Blood Agar	21
2.4.Preperation of the Mueller Hinton Agar	22
2.5.Catalase Test Analysis	22
2.6. Coagulase Test Analysis	23
2.7. Analysis of the Metisillin Resistance	23
2.8. Preparation of the Disinfectans	24
2.8.1. Preparation of the 5% Sodium Hydrochloride	24
2.8.2. Preparation of the 1/10 Sodium Hydrochloride	24
2.8.3. Preparation of the 1/100 Sodium Hydrochloride	24
2.8.4. Preparation of the 10% Povidine Iodene	24
2.8.5. Preparation of the ½ Povidine Iodene	24
2.8.6. Preparation of the 1/4 Povidine Iodene	24
2.8.7. Preparation of the 95% Ethyl Alcohol	24
2.8.8. Preparation of the 70% Ethyl Alcohol	24
2.8.9. Preparation of the 50% Ethyl Alcohol	24
2.9. Analysis of the Disinfectans Activity Against MRSA and MSSA strains	24

## **3. RESULTS**

3.1. Results of the Standard Strain of the <i>S. aureus</i> against Different Disinfectans	26
3.2. Results of the MRSA strains against Different Disinfectans	27
3.3. Results of the MSSA strains against Different Disinfectans	46
3.4. Comparision of the Results	65

**CHAPTER 4. DISCUSSION**

4.1 Discussion 67

**CHAPTER 5. CONCLUSION** 71

**CHAPTER 6. RECOMMENDATION** 71

References 72

**CURRICULUM VITAE**

76

## **LIST OF FIGURES**

<b>Figure 1.</b> Coagulase Positive Test Result	22
<b>Figure 2.</b> Analysis of ATCC 25923 Metisillin Resistance	23
<b>Figure 3.</b> Preparation of the 0.5 McFarland Bacteria Suspension	25

## LIST OF TABLES

<b>Table 1.</b> Results of the ATCC 25923 <i>S.aureus</i> against Different Disinfectans	26
<b>Table 2.</b> Results of the 20 MRSA strains against Different Disinfectans	27
<b>Table 3.</b> Results of the 20 MSSA strains against Different Disinfectants	46

## LIST OF ABBREVIATIONS

CoNS	Coagulase Negative <i>Staphylococcus</i>
TAT	Turnaround time
H <sub>2</sub> O <sub>2</sub>	Hydrogen peroxide
HA	Hyaluronic acid
Pc	Penicillin G
MHC	Major histocompatibility complex
MRSA	Methicillin-resistant <i>S. aureus</i>
HAIs	Healthcare associated infections
PVP-I	Povidone–iodine
MSSA	Methicillin-sensitive <i>S. aureus</i>

## SECTION ONE: INTRODUCTION

### 1.1. *Staphylococcus spp*

The bacterial *Staphylococcus* is one of the Gram-positive bacteria, the size of the diameter measured to be around 0.5 – 1.5 micrometer, the bacteria are recognized to be arranged in cocci which belong to the family of *Staphylococcaceae* also *Bacillales* pertaining in order, whatever bacteria under the medical microscope are look like spherical it termed microbiologically as (cocci), moreover arrangement of bacteria will be form grape-like clusters, Also *Staphylococcus* are facultative anaerobic with are non-motile, non-spore forming.. So the name was created by Alexander Ogston in 1880 he was bacteriologist, moreover the *Staphylococcus* have 40 species nine of them have two subspecies many of which preferentially colonise the human body, however *Staphylococcus aureus* (*S.aureus*) and *Staphylococcus epidermidis* (*S.epidermidis*) are the two most studied strains, inside of pathogenicity most of them are harmless it mean do not cause disease and it will pertaining on human flora in skin and mucousal in addition to other organisms, moreover it found worldwide which they are a small element of soil microbial flora. Moreover the *Staphylococci* species have a comparative as a complex nourishing obligation it require an organic foundation of nitrogen supplied by 5 to 12 essential amino acids. In addition members of this genus have ability to make catalase-positive and oxidase-negative which gave good idea in order to differentiating between *Streptococci* and *Staphylococcus* which catalase-negative pertains different cell wall composition to *Staphylococci*. (Harris, L., et al, 2002; Taylor, T. A., & Unakal, C. G., 2019; Rocchetti, T. T., et al, 2018)

### 1.2. *Staphylococcus aureus*

The *S. aureus* is one of the main pathogen of importance due to the rise in

antibiotic resistance to human and it is considered to be one of the main antimicrobial resistance agents to various types of antibacterials, moreover it is dissimilar from other types of the Coagulase Negative *Staphylococcus* (CoNS) for example *S. epidermidis* and more virulent in spite of their phylogenetic resemblances. The *S. aureus* species named turn to ability of bacteria to produce golden color at time when the bacterial colonies are developed in solid media, and other species of CoNS create white colonies when grown in media, whatever the composition of the cell wall of *S. aureus* are strong defensive coat barriers which are comparatively shapeless in appearance with approximately 2040 nm in thickness. As we have knowledge from previous researches that in the cell wall is the cytoplasm that is surrounded by the cytoplasmic membrane, the one of the most important layers in peptidoglycans are main constituents in pertaining of bacterial cell wall and it consists of up to 50% of the cell wall.

The one of the fundamental formations of the layered cell wall is to survive bacterial life from the high internal osmotic heaviness, moreover the extra cell wall component is phosphate-containing polymers known as teichoic acids. These are containing whole about 40% of total cell wall build, also it is divided into 2 types of teichoic acids in cell wall which are bound covalently to the peptidoglycan. The component of teichoic acids give a negative charge to *Staphylococcal* bacterial external layer also have role in the gaining and localisation of metal ions in addition to activities of autolytic enzymes, also both structural (peptidoglycan and teichoic acid) containing approximately 90% of the weight of the bacterial cell wall other are the rest is collected of surface proteins, exoproteins and peptidoglycan hydrolases, moreover of these structural components role are involving in bacterial virulence contributing factor and surface attachment to target agent. Also bacteria have ability to produce capsular structure about 90% of *S. aureus* clinical strains, polysaccharides capsule have role to escape from phagocytosis and to improve *S. aureus* virulence to bacteraemia therefore biofilm forming (Harris, L. G., et al, 2002; Taylor, T. A., & Unakal, C. G., 2019).

### 1.2.1. Morphology

*S. aureus* is a Gram-positive coccus it is diameters about 1 micrometer which look like grape shape under microscopic viewing it may resulting from these incomplete separations of the three planar with nonspore forming. The structural peptidoglycan is consider to main component of the *S. aureus* cell wall which contain N-acetylglucosamine  $\beta$ -1,4 structural unit that connected to the N-acetylmuramic acid act as a restating units: the ribitol teichoic structure of the cell wall are stick to the muramyl-6-phosphate through `N-acetylmannosaminyl- $\beta$ -1,4-N- acetylglucosamine`. Also the structure of virulence factor which known as protein A are connected peptidoglycan which have facility lead bacteria escape to immune response by host cell by sticking to the Fc region of the mammalian cell compound which may result in autoagglutination condition. Moreover, the other *Staphylococci* species do not have or/and absence in cell wall the structural A protein therefor they have not autoagglutination possessions, the organism is often haemolytic in blood agar due to production of four types of haemolysins (Bhunia, A. K., 2018; Gnanamani, A., et al, 2017).

### 1.2.2. Culture

The most usable and fast documentation of *S. aureus* bacteria to be in group of the positive when inoculated on the blood cultures which are amin way in diagnostic infection by *S. aureus* in addition to provides important idea about therapeutic. Moreover, mainstream of coagulase negative *Staphylococcus aureus* (CoNS), which are microbiological not important and it is usually may be contaminants. And microbiological culture are usually used in order to recognized pathogenic type of *Staphylococci* by blood cultures in order to choose the current antibiotic, moreover, and in order to diagnostic patient with bacterial *Staphylococcal* infection following procedure while cultured on blood agar also need the subculture for 24 hr then identification the bacterial colonies, nowadays the microbiological



methods are developed there are some method that given the result and difference of staphylococci in material of hours which lead to decrease the turnaround time (TAT) when comparing to previous method. Whatever there are many steps lead to distinguished bacteria characteristics easy by means of standards of direct microbiological Gram stain in addition to the microscopist knowledgeable which lead to recognized *S. aureus* from other *Staphylococci* type. Moreover, the blood culture method are helpful in side of reduced the time and rapid detection of bacteria in case of positive sample, following in case of these positive samples and recognized the bacterial shape by current direct Gram stain by injected blood culture broth, the specific shape appearance clusters in the method of gram stain under microscope give best idea toward recognition of *Staphylococcus* bacterial species, and processes need incubation additional 18–24 hours in order to distinguished between the *S. aureus* and other CoNS when cultured made on solid media and . it is significant to assumed the modifications in virulence, comparatively occurrence of coagulase negative *Staphylococci* are isolated as contaminants (Murdoch, D. R., & Greenlees, R. L., 2004; Chapin, K., & Musgnug, M., 2003).

### **1.2.3. Biochemical Properties**

*S. aureus* is one of the most harmful species of *Staphylococci* encountered, and dependent on its production of exoproteins and toxins (Karmakar, A., et al, 2016).

Whatever in side of biochemical property also there are some biochemical of bacterial characteristic which gave good idea to recognize the type of *Staphylococcus* bacteria in fresh cultures of the presumptive colonies from plates were Gram-stained in order to handpicked for detection of Gram-positive cocci, following the Gram-positive separates were sub-cultured and incubated aerobically overnight at 37 °C then it is gona be best subjected to conformist biochemical tests characterization which including (catalase positive, coagulase positive, acetoin production positive, methyl red positive , indole production negative, urease production positive, oxidase and sugar fermentation positive) as a defined previously to differentiate *S. aureus*, and

there are many of other biochemical property that *S.aureus* have (Table 1) (Mamza, S. A., et al, 2016).

#### **1.2.4.1. Capsul**

It can not be seen by ordinary microscope it need electron microscopy in order to visualize and it is different from other capsules because they are visualized by ordinary microscopy also the bacteria could be recognized in infections states with these polysaccharide but until now the complete function of the capsule are debatable but scantest thought it may involve in escape from immune, also capsule have vary type some are associated with endocarditis and it may result in injured heart valves, Also the invasive microbes that cause infection to the extracellular with bacterial virulence factor thought interpretation the bacterium resistant mechanism in aim to survive from host immune response, moreover the capsule manufacture described by scantest Gilbert in 1931 which recognized by mucoid colonies on culture media, Despite the rarity of capsulate strains the finding of antibodies in the serum of many healthy adults to the Smith capsular antigen has led to the hypothesis that ordinary *Staphylococci* may become capsulate *in vivo*. There is no evidence to support this hypothesis but there is evidence for a 'life cycle' in *S.aureus* in an animal host involving capsular antigen production (pseudocapsulation) rather than true capsule formation noted a change in antigen type during infection. Conversion from compact to diffuse growth was observed during experimental abscess formation, and non-capsulate strains of *S.aureus* were shown to be capable of producing capsular substance that was quantitatively different from strain to strain of life cycle may be closely related to the pseudocapsulation described under clumping factor, Generally most *Staphylococci* produce microcapsules 11 serotypes identified related to different serotype and also these are associated greatest MRSA separates especially 5 serotype (Lee, J. C.,1996; Anderson, J. C., 1976; O'Riordan, K., & Lee, J. C., 2004).

#### **1.2.4.2. Cell Wall**

In the cell composition of the *Staphylococcal* there are 50 % as a weight of the peptidoglycan and it is build up from many of the irregular subunit such as <sup>3</sup>1,4- $\beta$  linkages with *N*-acetylmuramic acid in addition to *N*-acetylglucosa<sup>3</sup> The composition of the chwmical chain of structural peptidoglycan are made by linkage of the <sup>3</sup>tetrapeptide when linked with *N*-acetylmuramic acid<sup>3</sup> and by a pentaglycine. The *s.aureus* have endotoxin similar action in their cell wall peptidoglycan structure which lead the host immune response to secrets of the macrophages by the cytokines later on make the beginning the processes of complement mediation then combination of the host platelets. (Lowy, F. D., 1998; Kessler, C. M., et al, 1991).

#### **1.2.5. Virulence Factors**

The term of the sepsis deal with the upsetting disease development and it is recognized by the inflammation of the whole body of host cells which later on result with etiologic mediator of sepsis to various deep tissue and bacteremia, also *S. aureus* consider one of these bacteria that have very strong virulence influences in order to stick the cell and make an infection and processing escape of host immune response intruder to continues the damaging of the host cell, moreover, inside the human bloodstream these features that have been associated with causing damage and inflammation mediation are harm immune response cells activity, also bacteria have ability of alteration to the coagulation factories cooperation with vascular, also there are many of these information and discuss about the reviewing the key of superficial attached factories that have been performed the and need for *S. aureus* infection aggressive host situation of blood, moreover and in order to understanding the highlighting machine-like of virulence role of the ability of bacteria toward the

causing pathogenicity communication on of superficial enzymatic factor appearance through the identifying octapeptide density and these protein involving in attachment mechanism are principally synthesized in the bacterial life cycle stage termed as exponential growth phase later on in the stage of stationary phase the excretion of these attachment are predominate which consider to be first step before infected host cell and the sequential appearance of these genes are very important for identification clinically, furthermore, the *S. aureus* activates pathophysiologic disorders and it is recognized by additional improved inflammatory of host and these infraction are vary according to dissimilar phases of *Staphylococcal* appear and it is involve altered pieces of virulence factor throughout the early phases of *Staphylococcus* infection appearance superficial proteins which are slicked to the extracellular surface that have favoritisms to make colonization processes on cell (Lowy, F. D., 1998; Cheung, A. L., et al, 1994; Powers, M. E., & Wardenburg, J. B., 2014).

### **1.2.6. Enzymes**

#### **1.2.6.1. Catalase**

Catalase enzyme is one of these enzyme that *S.aureus* have ability to secret it which is very important for hydrolyze and separate molecular containing water hydrogen peroxide  $H_2O_2$  to separate oxygen  $O_2$  molecular and  $H_2O$ , as a weight the molecular weight of the catalase was 250 kDa, moreover, it is involves hemoprotein groups which counted to be four in quantity. There are large group of creature in both family of prokaryotic and eukaryotic which are associated with secret catalase enzyme during life cycle as a an intracellular composition enzyme which mostly found on group of the bacteria that have facultative anaerobes characteristic and the catalase enzyme are consider to be the second maximum plentiful enzymatic which are antioxidant after superoxide dismutase which weakens reactive oxygen degree that universally attend pathological complaints. The bacteria are escape from impairment by the oxidative of  $H_2O_2$ , by these mechanism the bacteria will survive

theme self from defense system, also there are some other bacteria that have catalase secretion in their cell wall in order to facilitates cellular detoxification. Moreover this enzyme are neutralizes the bactericidal properties of hydrogen peroxide which concentration has been connected with pathogenicity. The catalase test facilitates the detection of the enzyme catalase in bacteria it is important for splitting catalase positive microorganism as of other catalase microorganism which useful in distinguishing among staphylococcus bacteria from *Streptococcus* and many of their types (Hadwan, M. H., 2018; Reiner, K., 2010).

#### **1.2.6.2. Coagulase**

Coagulase in one of these enzyme associated with *S. aureus* infection, moreover also it is a protein created by many other microorganisms that enable to change of blood fibrinogen to fibrin. It is used in the laboratory to distinguish between different staphylococcal isolates. More importantly, *S. aureus* is usually a positive coagulant, which means that a positive clotting test will show the presence of *S. aureus*. A negative clotting test will instead indicate the presence of negative clotting organisms, Moreover the coagulase basically are do not consider to be an enzyme chemically because it is protein play role in blood aggregation when bind to the prothrombin factor of host and the processes are sometime called as staphylothrombin, following the thrombin is stimulated by enzymatic protease in order to forming the complex later on this complex are causing fibrinogen transformation to the fibrin, also there are some method perform in order to distinguished the coagulase causative agent such as tube coagulase test which principally based on clotting formation when drop of host infected plasma are mixed with the *S. aureus* species later on resulting the procedure by clot formation, this is easy method an reliable technique to identification infection clinically with *S. aureus* in microbiology laboratory, also the gram stain method of bacterial growth colony must be done behind of the both catalase and coagulase test, identification positive result of method tell us the causative agent is *S. aureus*, Also there are thermostable

deoxyribonuclease method used microbiological in order to identification infection with *S. aureus*. Moreover the long-established bacteria colonies with agglutination latex method useful also when fibrinogen are bind with the host immunoglobulin G then stucked to the A protein amonge the clumping factor on bacterial surface, , also coagulase is observed when bacterial cell superficial respond to the prothrombin.

The mechanism action of the coagulase occur when the clotting factor fibrinogen and thrombin aggregated outside the cell (extracellular). Also there are many study mention as a genetically and it is obviously exposed the separation entities of both clumping factor and coagulase in addition it has been clarify that the occurrence of any mutation will cause the clotting causative action and maintain coagulase clumping while it is direct express normally. The pathogenic gene which associated with *Staphylococcus* in human body are conventionally separated according to the capability of clot formation of plasma in to two groups and the most causative agent formation of the clotting to host are *S. aureus* but other *Staphylococci* are consider tobe the CoNS which less causative pathogenicity to the skin and certain species able to forming inflammation when comparing to the *S. aureus*, there for the bacterial *Staphylococcus* microbiological separate in to coagulase +ve and -ve (Foster, T., 1996; Kateete, D. P., et al, 2010; Subramanian, A., et al, 2017).

### **1.2.6.3. Lipase**

Usually the hydrolysis of lipid substance conduct by mechanical action of lipase and it is subclass of the esterases, moreover it is consider to be water soluble substance that modifiable ester bonds in inexplicable acylglycerols border and lipases élite acyl group from glycerides creating lipase-acyl complex then transfers o OH group of water. *Staphylococcal* lipases isolated in the latent biotechnology field, the lipase enzyme are used in biocatalys in order to split the transesterification, alcoholysis, and alcohols esterification in non-aqueous media, moreover, the activity of the catalysis and lamentation of hydrolysis lipids action relative with the high

temperature in addition to PH range, whatever, each of these virulence enzymatic have some specific characteristic relate to the bacteria such as biocatalytic possessions associated to specificity, steadiness, temperature, and pH. Nowadays the lipase enzyme that associated with bacterial activity are recently have great position adaptability which lead it more intresting in medical field currently in order to study of extremophiles because it is charactrestic tohave high ability to make resistance to the mesophilic homologs inaddition to chemical agents and PH range (Chauhan, M., et al, 2013; Bacha, A. B., et al, 2018).

#### **1.2.6.4. Hyaluronidase**

The bacterial hyaluronidases are a class of enzymes that degrade hyaluronic acid (HA). (Abdelkader, S. A., et al 2018; Ji, H., et al, 2016). The mechanical action of the Hyaluronidase by the bacterial *S. aureus* has been reviewed previously and mention the production of hyaluronidase enzyme associated with HA nanocapsules activation which contain polyhexanide in addition it is associated with HA amoxicillin-loaded mesoporous silica nanoparticles coated, it may possibly to split when face hyaluronidase. This enzyme have specialty to attach very strongly to the broad spectrum antibiotic possessions with negatively charged and negatively charged help bacteria to unlock the surface membrane by electrostatic repulsion, in addition the HA have can accelerate healing process of the wound by stimulating initial inflammation. (Sutrisno, L., et al, 2018).

#### **1.2.6.5. Staphylokinase**

The enzyme are sometime mention in different term such as staphylococcal fibrinolysin or Müller's factor and it is created by *S. aureus* which have 136 amino acid with 15kDa as a molecular weight, moreover it positively controlled by bacterial gene manager which triggers plasminogen to plasmin, later on act as fibrin clots digests. Numerous type of the *S. aureus* have staphylokinase which are derived from

activation of the plasminogen, it cause alteration and slices immunoglobulin G and C3b, inhibiting phagocytosis. Whatever, the staphylokinase gene are demonstrate to be associated with lysogenic bacteriophages, moreover the staphylokinase and plasminogen together able to make plasmin proteolytic multifaceted result in ending activity of the fibrin clots (Foster, T. 1996).

#### **1.2.6.6. Deoxyribonucleases**

There are some of bacteria contain DNase which acting among material of nucleic acid hydrolyse in order to oligonucleotides production, moreover the mechanism action of the this enzymes are described in the works for numerous periods. At the same time as these enzymes are involve in diagnostic bacteria that have ability to produce these type of enzymes such as specific extracellular DNase countenance characteristic, like *S. aureus*, since there are some of the clinical study perform in order to recognized the function of Dnase enzyme but until now the issues of this protein are r continued to be indistinct. DNase production by certain bacteria have some physiological characters which involve in deliverance of nucleotides that have benefit in an improvement deliberate development in addition the Dnases have come feature toward the decreases of the infected exudate viscosity, also later potentially allow moving bacteria toward cells in order to invade to inside tissue of the host then bacterial distribution, and it may cause in risen neutrophil extracellular tras later on stopping promoting development of bacteria and later on result in murder bacteria. Moreover the DNase extracellular enzyme lead bacteria to escape and survive it self from antibacterial therapeutic agent and it mae bacteria more stable toward causing wide infection to host cells (Tetz, V. V., & Tetz, G. V. 2010; Palmer, L. J., et al, 2012).



### 1.2.6.7. Penicillinase (Beta-lactamase)

$\beta$ -lactamase antibiotics have the ability to deactivate Penicillin drugs. Since the  $\beta$ -lactam antibiotic ring is hydrolyzed by a serine enzyme, whatever the percentage of MSSA continues toward penicillin, moreover, MRSA demonstrates resistance against all penicillin drugs in addition to cephalosporins and these bacterial genes which are responsible for making resistance known as *mec* which encode and translate the binding site of the penicillin 2a and it is the *mec* gene of the *Staphylococci* created dissimilar type of species, also numerous MRSA strains look like offspring of an imperfect quantity of clones but there are particular others that seem as a multiclonal, the resistance genes are suggesting to be derived originally from transmission of *mec* DNA horizontally in addition to other types of *Staphylococcal* responsible resistance genes such as *bla* which have activity toward the  $\beta$ -lactamase group of drug resistance. In addition, the *fem* gene also has factors essential for methicillin resistance which is responsible for resistance appearance and these resistance genes appear as heterogeneous, furthermore, the bacterial resistance percentage and its inhabitants' phenotype differs, conferring by the ecological circumstances and this phenotype of bacterial resistance is recognized by the modified test now known as antimicrobial-sensitivity method (Lowy, F. D., 1998; Archer, G. L., & Niemeyer, D. M., 1994; Takayama, Y., et al, 2018). Other than penicillin, also there are other types of drug resistance that have been recognized such as vancomycin-resistant *S. aureus* (VRSA) and it is according to various reports mentioned it has been widely spread worldwide in addition it is found that the causative agent of VRSA are not only happen with *S.aureus*, there are other species such as *Staphylococcus haemolyticus* associated with VRSA, moreover, it has coagulase negative properties. The genes responsible to make resistance to vancomycin are normally found in bacterial plasmids of the enterococcal and it is a plasmid received by the *S.aureus* through conjugation mechanism in vitro, moreover, and it is according to research experimental on VRSA which conducted in United States in addition to Japan, they mention the clinical separation of the bacteria documented the MIC as 8  $\mu$ g /ml there for

it consider to be an intermediate sensitivity to vancomycin and Bacteria continue in production of the cell wall with modifications in order to keep it self from catching by vancomycin (Hiramatsu, K., et al, 1997; Lowy, F. D., 1998; Tenover, F. C., et al, 1998; Sieradzki, K., & Tomasz, A., 1997; Martin, R., & Wilcox, K. R., 1997)

### **1.2.7. Toxins**

The bacteria have ability to produce various type of toxin to host cell and it is categorized according to the achievement of the mechanisms, the immune response which termed as cytotoxins (33-kd) alpha protein is one of the weak reason to development in order alter the host cell proinflammatory, also it may later cause sepsis condition when continued bacteria in processes of cellular injury contribute to appearances of the disease (Walev, I., et al, 1995). There another toxin substances associated with *S. aureus* cell wall production which participate as a virulence factor to the host cell called pyrogenic toxin and it is one of these specific toxins termed superantigens structurally homology distribution numerous units of amino acid sequence, moreover it have role in attachment to the class II proteins of the major histocompatibility complex (MHC), later on it may major reason in widespread specific immune T-cell and more secretion of cytokine. Moreover *S. aureus* can cause various disease type such as toxic shock syndrome which cause buy predominate of enterotoxin in addition it cause food poisoning, the toxin have ability to change pH of stomach, also the toxic shock syndrome toxin 1 (TSST-1) have the same composition when comparing to the type B and C of enterotoxins because there as some homology of the amino acid sequence, in addition there gene specifically related to TSST-1 in *S. aureus*, Also the *S. aureus* secrete another pathogenic toxin termed as exfoliative toxins, this toxin have two epidermolytic toxin containing A and B which have significant role in erythema of the host superficial skin which terminology recognized as staphylococcal scalded skin syndrome clinically abbreviated as SSSS, the disease begin when these toxin are attached to skin or/and enter to host body but until nowadays the accomplishment mechanism of these toxins are rests debatable. There

are another toxin called Panton–Valentine leukocidin PVL and it is present in the majority CA-MRSA which associated with leukocytolytic infections of the cutaneous which creates holes in the membranes of infected cells (Lowy, F. D., 1998; Cribier, B., et al, 1992).

#### **1.2.8. *S. aureus* Infections**

The *S. aureus* are consider wide spread bacteria which cause various type of disease to human on skin, internal disease, and urinary tract infection everywhere in different geographic area (Lowy, 1998), moreover it is available temperately in various part of body and cause infection such bloodstream infections, endocarditis, osteomyelitis, lung infection, and skin infection, these infection if not treated in current time it may cause dramatic life-threatening necrotizing fasciitis or necrotizing disorder, the *S. aureus* infection can be recognized by these situation type and it can be defined as a virulence cactore of bacteria that. And the major argument clinically about treating of the *S.aureus* which associated with antibiotic resistance main problematic to the physicians nowadays (Barber, M.,and Rozwadowska-Dowzenko, 1948).

The *S. aureus* resistance to the penicillin was discovered for the first time in 1942, later on and according to many researches experiment it was found that bacteria automatically have ability produce specific enzyme which called penicillinase that have characteristic to resist against penicillin drug and this obsevation information was concluded before use penicillin drug in uses of the medical practice, moreover, the penicillinase enzyme have ability to change the ring composition of beta-lactam component representative that consider as a feature of the beta-lactam antibiotics (penicillin) derivatives during 1950s previously, and nowadays the resistance produce by the *S. aureus* involve all type of bacterial strains that's way it is consider as a pandemic state in public especially in hospitals, because of these reason nowadays

currently the maximum separates of *S. aureus* infectious are resistant to penicillin drug (Otto, M. (2012); Roundtree and Freeman, 1956).

Moreover, to keep patients away from the *S. aureus* resistance the pharmacist developed synthetic artificial the drug for the methicillin resistance which are developed from previous penicillin drug in order to inhibit the  $\beta$ -lactamase activity and keep patient safe away from methicillin-resistant *S. aureus* MRSA, moreover and for the first time the resistance of *S. aureus* toward penicillin was discovered in 1959 by Beecham, also there are many cases was recognized to be resistance by the *S. aureus* in United Kingdom in 1960 but there was some differences was notification noted in these cases unlike to methicillin-resistant *S. aureus* later of they found that the fundamental mechanism of the resistance which lead bacteria keep a safe against many type of these class of beta lactam antibiotic such as carbapenems penicillins in addition cephalosporins(Jevons *et al.*, 1963; Otto, M. 2012).

Also there are some epidemics produced by *S. aureus* species among antibiotic resistance for example the first penicillin resistant epidemic was happen in UK which spread only in European country in 1980 and it was the original families of MRSA appeared which nowadays in is consider as a pandemic worldwide distribution and continues, moreover, there are many countries experiment that summarized there are around 25 to 50% of whole population was suffering methicillin-resistant strains but there are some country like Netherland historically have very rare cases <1% when comparing to other area, and Japanese research study mention the uses a lot of antibiotic also fact to get MRSA (Chambers and DeLeo, 2009; Diekema *et al.*, 2001; Otto, M., 2012; Nakamura *et al.*, 2012).

### **1.2.9. Metisillin Resistance**

The Patricia Jevons previously defined MRSA since after two years of initial uses of methicillin drug production, the *S. aureus* in half century spread in every country around the world this lead the scientist to thinking more about it there for

many experimental study conducted about *S. aureus* in order to understand the causative disease and the mechanism of it is resistance in order to healthy take care about is and prevent complication of bacteria, Also the bacteria many specific characteristics that help physician more easy to understand and remarkable causative by *S. aureus* to human among distinguishing mechanisms of infection and it is virulence factors. The variation in gene composition of bacteria allow it to get new exogenous genes which resulting in change genetic sequence of bacteria, the causative that help bacteria to alter the genetic composition are environmental variation control pathogenicity. Sometime the bacteria have ability to live inside host cell without sharing any symptoms in this situation the patients termed as a carriage in this situation bacteria spread and distribution within host. The *S. aureus* recognized to have ability to resist some of antibiotic therapy such as group of Beta-lactam antibiotic and this property make the bacteria spread through worldwide (Moellering Jr, R. C. 2011; Jevons, M. P. 1961). Methicillin was introduced in 1959 when *S. aureus* make resistance to penicillin therapeutic but later on in UK the researcher found there was also *S. aureus* resistance to the in 1961 and later on clinically termed as MRSA and it mention methicillin resistance rapidly recurrent among European countries in addition nowadays it is consider the major problematic in hospitals in various countries such as United States of America, Australia, Japan (Hussain, F., et al.,(2000). The *mecA* encodes a methicillin-resistant penicillin-binding protein (Hiramatsu, K., et al, 2001). The gene *mecA* act as mobile transmission component and packaging occur of chromosome in form of SCCmec gene which have different in diameter, and genetic arrangement among various MRSA and susceptible to vancomycin because *S. aureus* have usceptibility glycopeptides which later cause excessive health worry (Enright, M. C., et al, 2002).

#### **1.2.10. Antiseptic and Disinfectans**

The antiseptic and disinfectants widely used as a antimicrobial in various field as a way to prevent bacterial contamination in addition cure growth of bacteria and it

is infectious especially in medical field assistance to avoid healthcare associated infections (HAIs), and its role especially in preventing extracellular biofilm formation on surfaces, also its activity against *S. aureus* (MRSA) (Lineback, C. B., et al, 2018).

#### **1.2.10.1. Sodium Hypochlorite**

The worldwide use of sodium hypochlorite as a root canal irrigating solution is due mainly to its efficacy for pulpal dissolution and antimicrobial activity. It was proved to be a powerful germicide, effective against a wide spectrum of microorganisms. Also, the sodium hypochlorite has both in vitro and in vivo antimicrobial activity against *S. aureus*, including MRSA. The antimicrobial effectiveness of sodium hypochlorite, based on its high pH. The high pH of sodium hypochlorite interferes with the cytoplasmic membrane integrity with an irreversible enzymatic inhibition, biosynthetic alterations in cellular metabolism and phospholipid degradation observed in lipid peroxidation. (Wong, S. M., et al, 2013; Estrela, C., et al, 2003; Estrela, C., et al, 2002).

#### **1.2.10.2. Ethyl alcohol**

The most and wide antimicrobial usage is ethyl alcohol with different concentrations which are active against various types of microorganisms in order to eliminate the development of bacteria in addition to preventing the disease caused by these bacteria such as *S. aureus*, moreover, the uses of high ethyl alcohol concentrations act as bactericidal against microorganisms, also it is used for cleaning objects, surfaces and is widely used in hospitals and the preferable level of these disinfectants is intermediate 70% because it is more safe than other concentrations, and it is not hard to get this type of disinfectants, cheap and it can work on non-sporing also, the *S. aureus* significantly plays a role in the formation of the biofilm and if it increased, the ethyl alcohol disinfectants will destroy or/and inhibit the formation of biofilm on

surface when it is frequently used but mention the ethyl alcohol in low concentration below 50 was in active against various microorganism especially in biofilm creation, also it is used in catheter prevention bloodstream infections, in addition to propolis inhibits drug multi-resistant bacteria, MRSA, Also it is recommended in many country regularly used in aimed to hand washing in addition to environmental hygienic of hospital (Hernandes, S. E. D., et al, 2004; Luther, M. K., et al, 2015; Tiwari, S., et al, 2018).

### **1.2.10.3. Povidone-Iodine**

In the early 1980s, Povidone-iodine (PVP-I) is consider as one of these antimicrobials have characteristic of broad-spectrum activity in aim of prevention or/and treat microorganism infection to human body also used in sterilization surfaces and it have overall antimicrobial action even affect those bacteria that have resistance to many type of bacteria such as *S. aureus*. PVP-I representative have ability to affected the gram positive bacteria as an in vitro in addition to gram negative bacteria and some bacteria that have ability to create spore, more over there are some other research experiment that have done in vivo on human hand fingertip pollution before and it is result shown PVP-I disinfectant has more powerful in removing bacterial contamination The PVP-I used in treatment of skin surface infection bay any of causative bacteria locally, the advantage of PVP-I have broad spectrum characteristic that do not allow any resistance occur and it is affective in minimum period of time, moreover the PVP-I direct affected ton the cell wall component and analysis the bacteria also it have ability to effect on the virulence eatures (destroying tissue enzymes, exotoxins, endotoxins) (Eggers, M., et al, 2018; Reimer, K., et al, 2002; Junka, A., et al, 2013).

## **SECTION TWO: MATERIALS AND METHODS**

### **2.1. Material**

#### **2.1.1. Devices and Tools**

- Pipette
- Yellow Tip
- Blue Tip
- Timer
- Incubator
- Autoclave
- Microscope
- Sterile Cup
- Slide
- Cover Slip
- Petri dish
- Blood agar Base
- Ethyl alcohol
- Povidone ioden
- Sodium Hypochlorite
- Swap
- MacFarland Measurement
- Hydrogen peroxide
- Cefoxitin antibiotic disk



### **2.1.2. Software Programs**

In our study the Microsoft office Excel application used in order to manipulate the each result parameters with other data. The Excel is a widely used program for different statistical analysis in social science. It is also used by many of other fields include health researchers, It can handling complex data manipulations and analyses them very easy and within minutes.

### **2.2. Bacterial Strains Collection**

This study was done in Near East hospital Microbiology Laboratory in Nicosia – Cyprus. Different sample type collected who identify as a *S. aureus* (40 was selected as MSSA and 40 as MRSA). Also, we used ATCC 25923 standard strain will used for the control in our study.

### **2.3. Preparation of the Blood Agar**

Composition of the blood agar;

- 5% Peptone
- 0.3% beef extract/yeast extract
- 1.5% agar
- 0.5% NaCl
- Distilled water
- Blood of the Sheep 5%
- pH between 7.2 - 7.6

### **Preparation of Blood Agar;**

1. Put blood agar 28 gm in 1 liter of D.W.
2. Then put it on the heater until fully dissolve.
3. Autoclave at 121 °C for 15 min.
4. Autoclaved blood agar later on let it to get cool without solidify.
5. Waite to get cool, Add defibrinated 5% of blood and mix.
6. Prevent bubbles.
7. Hand out to plates.

### **2.4. Preparation of the Mueller Hinton Agar**

#### **2.4.1. Composition of the Mueller Hinton Agar**

1. 2.0 g beef extract
2. 17.5 g casein hydrolysate
3. 1.5 g starch
4. 17.0 g agar
5. 1 liter of distilled water.
6. pH adjusted to neutral at 25 °C

#### **2.4.2. Preparation of Mueller Hinton Agar**

1. Suspend 38 gm of nutrient agar powder in 1 liter of distilled water.
2. Heat this mixture while stirring to fully dissolve all components.
3. Autoclave the dissolved mixture at 121 °C for 15 minutes.
4. Once the Mueller Hinton agar has been autoclaved, allow it to cool but not solidify.
5. Without air foams.
6. Distribute inside plates.

## 2.5. Catalase Test Analysis

- Put drops from hydrogen peroxide mixed that with smear of bacterial growth from the cultured
- If we see papules this test mean positive revers is negative
- Positive: *Staphylococcus spp.*

## 2.6. Coagulase Test Analysis

- Prepare the 1-2 mL plasma in tube
- Added suspended colonies into the tube by swap
- Put the tube in incubator (35 °C) until 4-6 hours.
- If we can see clotting the test is positive; remain liquid is negative
- Positive: *S.aureus*
- Negative: CoNS



Figure 1. Coagulase Positive Test Result

## 2.7. Analysis of the Metisillin Resistance

- Prepared 0.5 McF the test bacteria
  - Distributed bacteria on Mueller Hinton Agar petri dish
  - Put the cefoxitin antibiotic disk in middle of petri dish
  - Put the petri dish in incubator (35 °C) at 24 hours.
  - if the zoon more than 22 mm\*\* the bacteria is sensitive to antibiotic it is MSSA
  - If the zoon lease than 22 mm\*\* the bacteria is resistant it is MRSA
- \*\* EUCAST criteria used in this study for cefoxitin test results

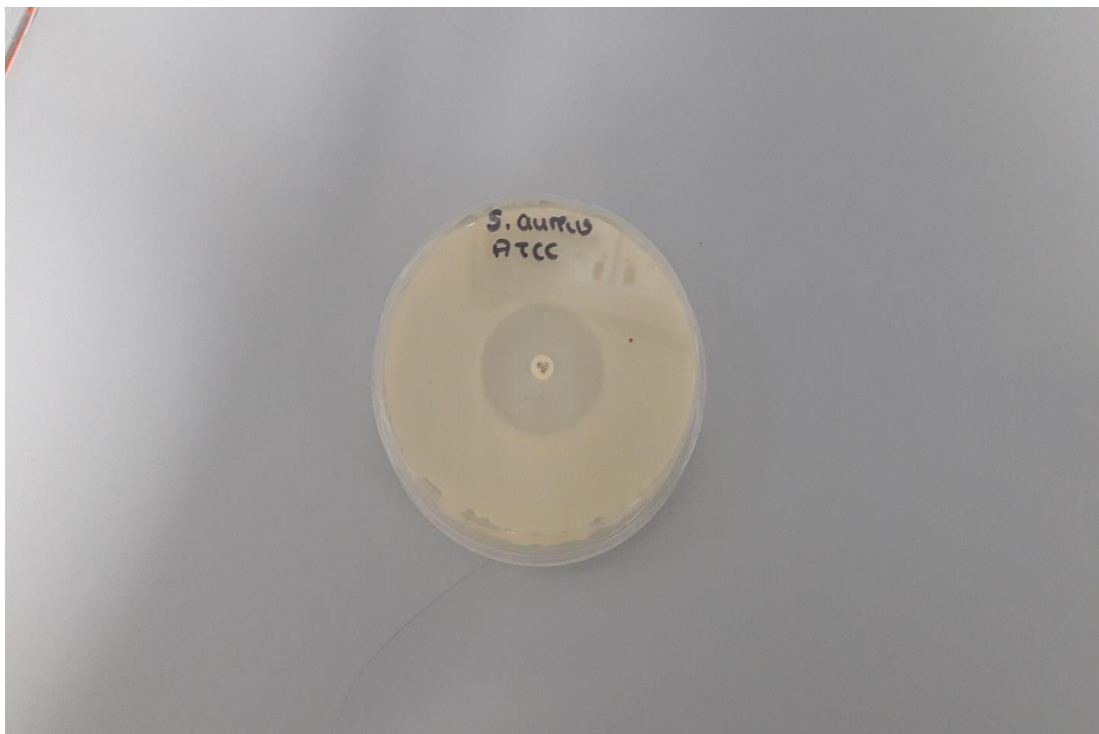


Figure 2. Analysis of ATCC 25923 Metisillin Resistance

## **2.8. Preparation of the Disinfectans**

### 2.8.1. Preparation of the 5% Sodium Hydrochloride

- 45 ml distile water
- 5 ml Sodium Hydrochloride

### 2.8.2. Preparation of the 1/10 Sodium Hydrochloride

- 9 ml of distile water
- 1 ml Sodium Hydrochloride

### 2.8.3. Preparation of the 1/100 Sodium Hydrochloride

- 49.5 ml of distile water
- 0.5 ml Sodium Hydrochloride

### 2.8.4. Preparation of the %10 Povidine Iodene

- 45 ml of distile water
- 5 ml Povidine Iodene

### 2.8.5. Preparation of the ½ Povidine Iodene

- 25 ml of distile water
- 25 ml Povidine Iodene

### 2.8.6. Preparation of the 1/4 Povidine Iodene

- 12.5 ml of distile water
- 37.5 ml Povidine Iodene

### 2.8.7. Preparation of the 95% Ethyl Alcohol

- 2.5 ml Ethyl Alcohol
- 47.5 ml distile water

### 2.8.8. Preparation of the 70% Ethyl Alcohol

- 15 ml Ethyl Alcohol
- 35 ml distile water

### 2.8.9. Preparation of the 50% Ethyl Alcohol

- 25 ml Ethyl Alcohol
- 25 ml distile water

## 2.9. Analysis of the Disinfectans Activity Against MRSA and MSSA strains

- Prepared suspension 0.5 McFarland ( $1.5 \times 10^8$  CFU/mL) of the test bacteria
- Take 1000 mL from every diluted disinfected and added in the tube
- Take 100 mL from suspension 0,5 McF bacteria added onto the 1000 mL from the test disinfectans
- Mixed the tube and start the stopwatch
- After 1 minute, culture the bacteria onto the blood agar
- After 2 minute, culture the bacteria onto the blood agar
- After 5 minute, culture the bacteria onto the blood agar
- After 10 minute, culture the bacteria onto the blood agar
- After 30 minute, culture the bacteria onto the blood agar
- Incubate all the plates in the incubator (35 °C) at 24 hours
- Record the colony numbers of the plates



Figure 3. Preparation of the 0.5 McFarland Bacteria Suspension

## SECTION THREE: RESULTS

### 3.1. Results of the Standard Strain of the *S. aureus* against Different Disinfectans

Table 1 show the results of the ATCC 25923 *S.aureus*.

Table 1. Results of the ATCC 25923 *S.aureus* against Different Disinfectans

Sodium hypochloride (5%)					
ATCC	1 min.	2 min	5 min	10 min	30 min
25923	Negative	Negative	Negative	Negative	Negative
Sodium hypochloride (1/10)					
ATCC	1 min.	2 min	5 min	10 min	30 min
25923	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative
Sodium hypochloride (1/100)					
ATCC	1 min.	2 min	5 min	10 min	30 min
25923	Negative	Negative	Negative	Negative	Negative
Ethyl Alcohol (95%)					
ATCC	1 min.	2 min	5 min	10 min	30 min
25923	Negative	Negative	Negative	Negative	Negative
Ethyl Alcohol (70%)					
ATCC	1 min.	2 min	5 min	10 min	30 min
25923	Negative	Negative	Negative	Negative	Negative
Ethyl Alcohol (50%)					
ATCC	1 min.	2 min	5 min	10 min	30 min
25923	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml
Povidine-Iodine (10%)					
ATCC	1 min.	2 min	5 min	10 min	30 min
25923	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/2)					
ATCC	1 min.	2 min	5 min	10 min	30 min
25923	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/4)					
ATCC	1 min.	2 min	5 min	10 min	30 min
25923	Negative	Negative	Negative	Negative	Negative

### 3.2. Results of the MRSA strains against Different Disinfectans

Table 2 show the results of the 20 MRSA strains.

Table 2. Results of the 20 MRSA strains against Different Disinfectans

MRSA-1	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/4)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative



MRSA-2	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/4)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	

MRSA-3	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/4)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	

MRSA-4	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^2$ cfu/ml	$10^2$ cfu/ml
MRSA-4	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^2$ cfu/ml	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/4)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	

MRSA-5	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min

MRSA-5	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^2$ cfu/ml	$10^2$ cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/4)				
	1 min	2min	5 min	10 min	30 min
Negative	Negative	Negative	Negative	Negative	

Sodium hypochloride (5%)				
1 min	2min	5 min	10 min	30 min
Negative	Negative	Negative	Negative	Negative
Sodium hypochloride (1/10)				

MRSA-6	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/4)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	

Sodium hypochloride (5%)				
1 min	2min	5 min	10 min	30 min
Negative	Negative	Negative	Negative	Negative
Sodium hypochloride (1/10)				
1 min	2min	5 min	10 min	30 min
10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative

MRSA-7	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/4)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

Sodium hypochloride (5%)				
1 min	2min	5 min	10 min	30 min
Negative	Negative	Negative	Negative	Negative
Sodium hypochloride (1/10)				
1 min	2min	5 min	10 min	30 min
Negative	Negative	Negative	Negative	Negative
Sodium hypochloride (1/100)				
1 min	2min	5 min	10 min	30 min
10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml

MRSA-8					
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/4)				
	1 min	2min	5 min	10 min	30 min
Negative	Negative	Negative	Negative	Negative	

MRSA-9	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				

MRSA-9	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/4)				
	1 min	2min	5 min	10 min	30 min
Negative	Negative	Negative	Negative	Negative	

MRSA-10	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
MRSA-10	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
Ethyl Alcohol (70%)					



	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
Ethyl Alcohol (50%)					
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (10%)					
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/2)					
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/4)					
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

MRSA-11	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^2$ cfu/ml	$10^2$ cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

Ethyl Alcohol (50%)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	
Povidine-Iodine (10%)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	
Povidine-Iodine (1/2)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	
Povidine-Iodine (1/4)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	

MRSA-12	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^2$ cfu/ml	$10^2$ cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	Negative	Negative
Ethyl Alcohol (50%)					

	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
Povidine-Iodine (10%)					
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/2)					
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/4)					
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

MRSA-13	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

Povidine-Iodine (10%)					
1 min	2min	5 min	10 min	30 min	
10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	
Povidine-Iodine (1/2)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	
Povidine-Iodine (1/4)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	

MRSA-14	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
MRSA-14	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative

	Povidine-Iodine (10%)					
	1 min	2min	5 min	10 min	30 min	
	Negative	Negative	Negative	Negative	Negative	
	Povidine-Iodine (1/2)					
	1 min	2min	5 min	10 min	30 min	
	Negative	Negative	Negative	Negative	Negative	
	Povidine-Iodine (1/4)					
	1 min	2min	5 min	10 min	30 min	
	Negative	Negative	Negative	Negative	Negative	
	MRSA-15	Sodium hypochloride (5%)				
		1 min	2min	5 min	10 min	30 min
		Negative	Negative	Negative	Negative	Negative
		Sodium hypochloride (1/10)				
		1 min	2min	5 min	10 min	30 min
		10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
Sodium hypochloride (1/100)						
1 min		2min	5 min	10 min	30 min	
10 <sup>3</sup> cfu/ml		10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml	
Ethyl Alcohol (95%)						
1 min		2min	5 min	10 min	30 min	
Negative		Negative	Negative	Negative	Negative	
Ethyl Alcohol (70%)						
1 min		2min	5 min	10 min	30 min	
Negative		Negative	Negative	Negative	Negative	
Ethyl Alcohol (50%)						
1 min	2min	5 min	10 min	30 min		
10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative		
Povidine-Iodine (10%)						
1 min	2min	5 min	10 min	30 min		
Negative	Negative	Negative	Negative	Negative		

MRSA-16	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/4)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
Negative	Negative	Negative	Negative	Negative	
Ethyl Alcohol (50%)					
1 min	2min	5 min	10 min	30 min	
10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	
Povidine-Iodine (10%)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	
Povidine-Iodine (1/2)					

	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/4)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
MRSA-17	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative

	Povidine-Iodine (1/4)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
MRSA-18	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^2$ cfu/ml	$10^2$ cfu/ml
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^2$ cfu/ml	$10^2$ cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^2$ cfu/ml	$10^2$ cfu/ml
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/4)					
1 min	2min	5 min	10 min	30 min	



	Negative	Negative	Negative	Negative	Negative
MRSA-19	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative
Povidine-Iodine (1/4)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	
Sodium hypochloride (5%)					
1 min	2min	5 min	10 min	30 min	

MRSA-20	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	10 <sup>3</sup> cfu/ml	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/4)				
	1 min	2min	5 min	10 min	30 min
Negative	Negative	Negative	Negative	Negative	

### 3.3. Results of the MSSA strains against Different Disinfectans

Table 3 show the results of the 20 MSSA strains.

Table 3. Results of the 20 MSSA strains against Different Disinfectant

MSSA-1	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^2$ cfu/ml	$10^2$ cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/4)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	

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MSSA-2	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/4)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	

MSSA-3	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^2$ cfu/ml	$10^2$ cfu/ml
MSSA-3	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	Negative	Negative	Negative	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/4)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	

	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

MSSA-4					
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	
Povidine-Iodine (1/4)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	

Sodium hypochloride (5%)				
1 min	2min	5 min	10 min	30 min
Negative	Negative	Negative	Negative	Negative
Sodium hypochloride (1/10)				
1 min	2min	5 min	10 min	30 min

MSSA-5	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/4)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

Sodium hypochloride (5%)				
1 min	2min	5 min	10 min	30 min
Negative	Negative	Negative	Negative	Negative
Sodium hypochloride (1/10)				
1 min	2min	5 min	10 min	30 min
10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative
Sodium hypochloride (1/100)				

MSSA-6	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/4)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

MSSA-7	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml



MSSA-7	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/4)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

Sodium hypochloride (5%)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	Negative
Sodium hypochloride (1/10)					
1 min	2min	5 min	10 min	30 min	
$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^2$ cfu/ml	$10^2$ cfu/ml	
Sodium hypochloride (1/100)					
1 min	2min	5 min	10 min	30 min	
$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^2$ cfu/ml	$10^2$ cfu/ml	
Ethyl Alcohol (95%)					
1 min	2min	5 min	10 min	30 min	

MSSA-8	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/4)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

Sodium hypochloride (5%)				
1 min	2min	5 min	10 min	30 min
Negative	Negative	Negative	Negative	Negative
Sodium hypochloride (1/10)				
1 min	2min	5 min	10 min	30 min
10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
Sodium hypochloride (1/100)				
1 min	2min	5 min	10 min	30 min
10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
Ethyl Alcohol (95%)				

MSSA-9	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/4)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

MSSA-10	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml

Ethyl Alcohol (95%)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	Negative
Ethyl Alcohol (70%)					
1 min	2min	5 min	10 min	30 min	
10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative
Ethyl Alcohol (50%)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (10%)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/2)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/4)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	Negative

MSSA-11	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
MSSA-11	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

Ethyl Alcohol (70%)					
1 min	2min	5 min	10 min	30 min	
10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	
Ethyl Alcohol (50%)					
1 min	2min	5 min	10 min	30 min	
10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	
Povidine-Iodine (10%)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	
Povidine-Iodine (1/2)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	
Povidine-Iodine (1/4)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	

MSSA-12	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

Ethyl Alcohol (70%)					
1 min	2min	5 min	10 min	30 min	
$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	Negative	
Ethyl Alcohol (50%)					
1 min	2min	5 min	10 min	30 min	
$10^3$ cfu/ml	$10^3$ cfu/ml	Negative	Negative	Negative	
Povidine-Iodine (10%)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	
Povidine-Iodine (1/2)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	
Povidine-Iodine (1/4)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	

MSSA-13	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^2$ cfu/ml	$10^2$ cfu/ml
MSSA-13	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^2$ cfu/ml	$10^2$ cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

Ethyl Alcohol (70%)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	
Ethyl Alcohol (50%)					
1 min	2min	5 min	10 min	30 min	
10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative	
Povidine-Iodine (10%)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	
Povidine-Iodine (1/2)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	
Povidine-Iodine (1/4)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	

MSSA-14	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

Ethyl Alcohol (70%)					
1 min	2min	5 min	10 min	30 min	
10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	
Ethyl Alcohol (50%)					
1 min	2min	5 min	10 min	30 min	
10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	
Povidine-Iodine (10%)					
1 min	2min	5 min	10 min	30 min	
10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	
Povidine-Iodine (1/2)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	
Povidine-Iodine (1/4)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	

MSSA-15	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative



Ethyl Alcohol (50%)					
1 min	2min	5 min	10 min	30 min	
10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	
Povidine-Iodine (10%)					
1 min	2min	5 min	10 min	30 min	
10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative	
Povidine-Iodine (1/2)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	
Povidine-Iodine (1/4)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	

MSSA-16	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml
	Ethyl Alcohol (50%)				

	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
Povidine-Iodine (10%)					
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/2)					
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/4)					
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	Negative	Negative	Negative	Negative

MSSA-17	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	Negative	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative

	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/4)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
MSSA-18	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	Negative	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/4)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
MSSA-19	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>2</sup> cfu/ml	10 <sup>2</sup> cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	Negative	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	10 <sup>3</sup> cfu/ml	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
Povidine-Iodine (1/2)					
1 min	2min	5 min	10 min	30 min	
Negative	Negative	Negative	Negative	Negative	

	Povidine-Iodine (1/4)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

MSSA-20	Sodium hypochloride (5%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Sodium hypochloride (1/10)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^2$ cfu/ml	$10^2$ cfu/ml
	Sodium hypochloride (1/100)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^2$ cfu/ml	$10^2$ cfu/ml
	Ethyl Alcohol (95%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Ethyl Alcohol (70%)				
	1 min	2min	5 min	10 min	30 min
	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	Negative	Negative
	Ethyl Alcohol (50%)				
	1 min	2min	5 min	10 min	30 min
	Negative	$10^3$ cfu/ml	$10^3$ cfu/ml	$10^3$ cfu/ml	Negative
	Povidine-Iodine (10%)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

	Povidine-Iodine (1/2)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative
	Povidine-Iodine (1/4)				
	1 min	2min	5 min	10 min	30 min
	Negative	Negative	Negative	Negative	Negative

### 3.4. Comparison of the Results

- ✓ In our study, povidine-iodine (pure, 1/2, 1/4) showed an inhibitory effect except for two MRSA and three MSSA strains even in one minute contact.
- ✓ Sodium hypochlorite (pure) showed an inhibitory effect both MRSA and MSSA strains all time contact (1, 2, 5, 10 and 30 minutes).
- ✓ In 1/10 concentration of the sodium hypochlorite, it was grown two MSSA isolate in the one minute; 14 MSSA isolates in the 1, 2, 5, 10 and 30 minutes. According to MRSA strains, grown one isolate in the one minute, one isolate in 1. and 2. minutes and seven isolates in the 1., 2., 5., 10. and 30 minutes.
- ✓ It was observed that 1/100 concentration of the sodium hypochlorite was not found to be effective to MRSA and MSSA isolates at all times.
- ✓ It was observed that 95% concentration of ethyl alcohol was totally three isolates was grown. Two MRSA isolates grown in the 1. and 2. minutes and one MSSA isolate grown in the 1. minute.
- ✓ According to the 70% concentration of ethyl alcohol, totally seven MRSA isolates were grown. Two MRSA isolates were grown in 1. minute; two MRSA strains in 1. and 2. minutes; two MRSA strains in 1., 2. and 5. minutes; one MRSA strains in 1, 2, 5, 10 minutes. Two MSSA isolates were grown in 1. minute; Two MSSA isolates in 1. and 2. minutes; six MSSA in 1., 2. and 5. minutes and two MSSA

isolates in all time contact. Totally, 12 MSSA isolates were grown.

- ✓ In 50% concentration of the ethyl alcohol, totally 13 MRSA isolates were grown. Eight MRSA isolates were grown in 1. minute; three MRSA strains in 1. and 2. Minutes; one MRSA strains in 1., 2. and 5. Minutes and one MRSA isolates in all time contact. Three MSSA isolates were grown in 1. Minute; Three MSSA isolates in 1. and 2. Minutes; One MSSA in 1.,2., and 5. Minutes; Four MSSA in 1.,2. ve 5. Minutes; four MSSA isolates in 2.5. and 10. Minutes and two isolates were grown in all grown contact. Totally, 13 MSSA isolates were grown.

## SECTION FOUR: DISCUSSION

Nowadays the common objective of the public health system hygiene approaches is to prevent diseases before their occurrence, moreover, it is a common economic understanding that prevention is always better than cure, the investigated of the disinfectants on the MSSA and MRSA strains are very important in order to prevent the current pathogenic agent spread between people through the medical staff in hospital unit which may lead to nosocomial eruptions and might be significantly reduced by the use of disinfectants. The College of Nurses of Ontario emphasizes that mention in specific experiment on the hand hygienic "hand washing" is the single most important infection control practice, moreover, there are several studies that demonstrate that efforts to control MRSA and MSSA are cost-effective (Boykin, A., et al, 2001; College of Nurses of Ontario, 2000)

Most of the agents that use in aimed to remove or prevent the microbial disinfection and it is widely uses is alcohol in different concentration and some of the other disinfectants in order to eliminate the development of wide range of pathogenic agent and decrease the chance to get ill, moreover the preferable disinfectant agent to have the rapid activity against the various type of microorganism in addition to have the outstanding microbiocidal features, and have a privation of appearance of resistance to microbial agent especially those accosted with biofilm, moreover, these are very useful in hyagen of the sink cleaning, remote zone, inanimate object, towels and the most important thing is used continuously in keeping hand cleaning over the time, moreover, to decrease the costs. The clean hand comparatively gonna be less achievement for aproximanently 20 minutes, but there are complete effect for 1 to 2 hours (WHO., 2006; World Health Organization., 2005; Baddour, M. M., et al 2008). In the present study, Baddour (Baddour, 2008), the NaCL was quite effective with acceptable bactericidal activity against the *S.aureus* strains (Baddour, M. M., et al,



2008). Moreover, the 10 min of immersion in 1% NaCl solution reduced 100% of the MRSA viability on acrylic specimens when compared to the positive control (Altieri, K. T., et al, 2013). But in some other paper mention that dentures disinfected by 1% sodium hypochlorite and it is result show of the observation of turbidity according to methodology of experiment (Altieri, K. T., et al, 2012). Moreover there are a research experiment design based on three different time 5, 30, 180 min of introducing the sodium hypochlorite on strain of MRSA, and all bacteria when introduced to sodium hypochlorite at all three time exposure conclude lower bacterial development in concentration and the sodium hypochlorite also demonstrate that have activity significantly reduced biological living substance depended on the exposure times (Liu, Q., et al, 2009).

In our experimental study and according to result observation we found that the sodium hypochlorite (pure) showed an inhibitory effect both MRSA and MSSA strains all time contact (1,2,5,10 and 30 minutes). Moreover, in 1/10 concentration of the sodium hypochlorite, it was grown two MSSA isolate in the two minute (MSSA-6, MSSA-4); 14 MSSA (MSSA-8, MSSA-9, MSSA-10, MSSA-11, MSSA-12, MSSA-13, MSSA-14, MSSA-15, MSSA-16, MSSA-17, MSSA-18, MSSA-19, MSSA-20) and isolates in the 1,2,5,10 and 30 minutes in table 3.3. According to MRSA strains, grown one minute (MRSA-7) in table 3.2, and isolate in the 1. and 2. Minutes (MRSA-13) in table 3.2. and seven isolates in the 1.,2.,5.,10. and 30 minutes (MRSA-14, MRSA-15, MRSA-16, MRSA-17, MRSA-18, MRSA-19, MRSA-20). Finally, It was observed that 1/100 concentration of the sodium hypochlorite was not found to be effective to MRSA and MSSA isolates at an all times.

The Penna et al. (Penna et al 2001) and Falagas et. al. (Falagas, et al, 2011).., experiment the one disinfectant choice applied was povidone-iodine in which the obtained result showed sensitivity of bacteria to the *S. aureus* according to method that used in experiment the MIC was 18750 mg/l and 9375 mg/l against MRSA and MSSA bacteria population and it is consider as a sensitive to both type odd *S. aureus*,

respectively. This result more or less agreed with (Penna, T. C., et al, 2001; Falagas, M. E., et al, 2011).

Also in another research which conducted by Sakuragi et. al. (Sakuragi, T., et al, 1995), and Haley et. al. (Haley, C. E., et al, 1985) the bacteria are exposure to the disinfectant 10% povidone iodine there are only 0.07% of ten hundred strain of bacteria MRSA are continued but others are eliminated but in the MSSA there are all of the hundred strain of bacteria are eliminated (Sakuragi, T., et al, 1995; Haley, C. E., et al, 1985). In our study result show the povidine-iodine disinfectants in different concentration as (pure, ½, 1/4) showed an inhibitory effect to all *S.aureus* strain except for two MRSA (MRSA 13 and MRSA-17), also two MSSA (MSSA-14, MSSA-15 and MSSA-16) strains even in one minute contact. In the meantime of the ethanol at 60% dilution of concentration and other 70% 75% and above more concentration have complet ability to eliminate the development of the *S. aureus* rapidly, Alcohol hand disinfectant seems to have real activity inorder to prevent MRSA spread, in a former study by Lai et. al. (Lai k., et al, 2006) the incidence of MRSA colonization or infection dwindled from 1.26 patient before the application of alcohol based disinfectant it seems reduce to 0.75 patient after the interference (Lai, K. K., et al, 2006). In many countries the disinfection (alcohol-based) used in hand hygienic have need individual around 30 seconds in aimed to act in appropriate way, for the reason that a distributor could be obtainable for following patient's on the bed and/or a pocket bottle may presented, therefor running away of different patient are essential (Kotb, S., & Sayed, M., 2015). The result observation about effect of 95% concentration of ethyl alcohol was totally three isolates was grown. Two MRSA isolates grown in the 1. and 2 (MRSA-3, MRSA-17) minutes and one MSSA (MSSA-4 ), isolate grown in the 1. minute. According to the 70% concentration of ethyl alcohol, totally seven MRSA isolates were grown. Two MRSA isolates were grown in 1 minute (MRSA-1, MRSA-2) ; two MRSA strains in 1. and 2 minutes; two MRSA strains in 1., 2. and 5 minutes; one MRSA strains in 1,2,5,10 minutes. One MSSA isolates were grown in 1 minute (MSSA-2); one MSSA isolates in 1. and 2 minutes (MSSA-9); seven MSSA in 1.,2. and 5. minutes (MSSA-6, MSSA-10 , MSSA-11,

MSSA- 17, MSSA-18, MSSA-19, MSSA-20) and three MSSA isolates in 10 min (MSSA-12, MSSA-14, MSSA-15) time and (MSSA-16 ) begin sensitivity in 5 minute to 10 minute then to 30 min. But in 50% concentration of the ethyl alcohol, totally 13 MRSA isolates were grown. Seven MRSA isolates were grown in 1 minute (MRSA-3, MRSA-6, MRSA-8, MRSA-9, MRSA-15, MRSA-17, MRSA-19); one MRSA strains isolated in 1. and 2 minutes (MRSA-14); one MRSA strains in 1., 2. and 5. minutes (MRSA-16) and two MRSA isolates in all time contact (MRSA-4, MRSA-20). Two MSSA isolates were grown in 1 minute (MSSA-3, MSSA-13); five MSSA isolates in 1. and 2 minutes (MSSA-5, MSSA-8, MSSA-9, MSSA-11, MSSA-12); Two MSSA in 1.,2., and 5. minutes (MSSA-14, MSSA-15); four MSSA found in 2, 5, and 10 minute only (MSSA-17 , MSSA-18, MSSA-19, MSSA-20) minutes and one MSSA was isolates grown in all minutes contact (MSSA-16 ).

Generally our experimental result was agree with Penna et al. (Penna et al 2001). Also, most likely our our data statistical analysis was nearly approved by Guilhermetti (Guilhermetti et al 2001) declared 10% of the PVP-I in addition alcohol disinfectant 70% concentration are greatest operative on hand for eliminating MRSA activity. Also, the ethyl alcohol hand disinfectants appears to be effective in controlling the transmission of MRSA. In a previous study the incidence of MRSA colonization or infection in 1.26 patient before starting application of this disinfectant based hand rub reduced to 0.75 patient after the interference Sayed, M., 2015; Guilhermetti M, et al, 2001).

## **SECTION FIVE: CONCLUSION AND RECOMMENDATION**

### **5.1. CONCLUSION**

The application of disinfectants had a remarkable effect against MSSA as well to the MRSA strain with less or more sensitivity of MSSA than MRSA. Moreover, this study highlighted a strong effect of the disinfectants used for environmental and equipment surfaces (except for isopropanol) which were found to exert a recognizable bactericidal effect without an evident difference between their effects on methicillin sensitive or resistant *S. aureus*.

### **5.2. RECOMMENDATION**

- 1.** Disinfection protocols are necessary to prevent and eliminate the source and spread of the disease
- 2.** It is necessary to all individual to use disinfectant to keep their hand clean.

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## CURRICULUM VITAE

### PERSONAL INFORMATION

<b>Name</b>	<b>Yasmin Ali Salem</b>	<b>Surname</b>	<b>Abuaniza</b>
<b>Place Of Birth</b>	<b>Libya</b>	<b>Date Of Birth</b>	<b>7-10-1987</b>
<b>Nationality</b>	<b>Libyan</b>	<b>Tel</b>	<b>(+90) 488894597</b>
<b>E-Mail</b>	<b>Abuaniza493@Gmail.Com</b>		

### EDUCATIONAL LEVEL

<b>University</b>	<b>Institution</b>	<b>Graduation Year</b>
<b>Al-Merqab University</b>	<b>Health science</b>	<b>2008-2009</b>

### JOB EXPERIENCE

<b>Duty</b>	<b>Institution</b>	<b>Duration</b>
<b>Assist Teacher</b>	<b>Al-Merqab University</b>	<b>2013- 2016</b>

### LANGUAGE SKILL

<b>Languages</b>	<b>Reading Comprehension</b>	<b>Speaking*</b>	<b>Writing*</b>
<b>Arabic</b>	<b>Excellent</b>	<b>Excellent</b>	<b>Excellent</b>
<b>English</b>	<b>Good</b>	<b>Good</b>	<b>Good</b>

### COMPUTER KNOWLEDGE

<b>Microsoft Office Word</b>	<b>Good</b>
<b>Microsoft Office Power Point</b>	<b>Good</b>
<b>Internet Using</b>	<b>Good</b>

## **INTERESTS**

- **Taking Science Courses And Reading**
- **Volunteering On Campaigns And Helping People**