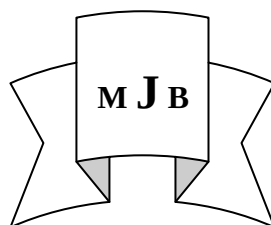


A Study of Efficacy of Disinfectants and Bacterial Contamination in AL-Hilla Teaching Hospital

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Abstract

In this study the efficiency of traditional disinfectants and antiseptics used in the hospitals against bacterial isolates was detected with help of Broth dilution method (for determination of minimum inhibitory concentration-MIC), Disc and Well diffusion method. The results of MIC method show that chlorohexidine gluconate Hibitene) was most effective disinfectants on tested bacteria and it followed by chloroxylenol (S₁) type (while chloroxylenol (S₂) and (Sp) type showed lower activity), hydrogen peroxide, sodium hypochlorite, formaldehyde, sodium dichloroisocyanurate and PVP-I, while chlorohexidine cetramid (Savlon) showed no efficiency against all tested bacteria.

Generally, *B.subtilis* and methicillin resistant *S.aureus* were found to be the most sensitive bacteria being tested in this study against disinfectants and antiseptics while *P.aeruginosa* was the most resistant bacteria to these agents.

Disc and well diffusion methods showed correlation between the concentrations of disinfectants and the inhibition zones of bacterial growth increase significantly $P < 0.05$.

الخلاصة

تضمنت هذه الدراسة تم اختبار كفاءة المعقمات و المطهرات المستعملة في المستشفيات تجاه العزلات البكتيرية بثلاث طرق هي طريقة التخفيف Broth dilution method (و ذلك لتحديد التركيز المثبط الأدنى) و طريقة أنتشار بالأقراص Disc Diffusion method . أظهرت نتائج تحديد التركيز المثبط الأدنى أن الهيتين chlorohexidine gluconate أكثر المعقمات تأثيراً على البكتيريا المفحوصة ثم جاء بعدة من حيث قوة التأثير كل من الديتول النوع (S₁) (بينما أظهر الديتول نوعي (S₁) و (Sp) فعالية أقل)، هايوكلورات الصوديوم (القاصر)، الفورمالين، sodium dichloroisocyanurate و الأيودين في حين لم يظهر معقم السافلون أي تأثير على البكتيريا المفحوصة.

أبدت بكتيريا العنقوديات الذهبية *S.aureus* حساسية تجاه معظم المعقمات و المطهرات بينما كانت بكتيريا الزوائف الزنجارية *P.aeruginosa* أكثر الأنواع البكتيرية مقاومة لتلك المعقمات. أظهرت طريقة الانتشار بالأقراص و الحفر أن هنالك علاقة ما بين تركيز المعقم و منطقة تثبيط النمو البكتيري للبكتيريا المفحوصة حيث تزداد بصورة معنوية $P < 0.05$.

Introduction

Antiseptics are agents that destroy or inhibit the growth of microorganisms in or on living tissue while disinfectant are similar but are used on inanimate objects or surface[1]. These agents such as alcohols, phenols, iodine and chlorine were used extensively in hospitals and other health care settings for infections control and prevention of nosocomial

infections[2]. An ideal disinfectant to overcome the antimicrobial resistant pathogens should have broad spectrum of antimicrobial activity [3] and the efficacy of these agents may be affected by PH, detergent base, temperature, organic matter, ionic and type of the surfactants[4]. Mechanisms of action of biocides on whatever type of microbial cell can be defined as the interaction of antiseptic or disinfectant

with the cell surface followed by penetration into the cell and action at the target site[5]. In addition, the interaction at the cell surface can produce a significant effect on viability [6] but most antimicrobial agents appear to be active intracellularly[7]. The wide spread use of these agents has promoted some speculation on the development of microbial resistance[8] and this resistance to disinfectants and antiseptics mainly intrinsic in nature whereas antimicrobial resistance is frequently conferred by plasmid or transposons, which have allowed rapid and extensive spread through the globe. Development of resistance to antimicrobial agents and biocides is particularly warning problem which is compounded by cross-resistances mechanisms (between antibiotic and between antibiotic and biocide) that may exist in certain bacteria such as pathogenic strains of *E.coli*[9]. Among bacteria, biocide sensitivity is based on the permeability of the biocide through the cell wall [10] and impermeability is

influenced by the composition of cell wall and physiologic adaptation of the microorganisms to its environment [11].

Gram-negative bacteria are generally less susceptible to biocides because of their complex cell wall[12] in which the outer membrane of Gram-bacteria act as permeability barrier in limiting or prevention the entry of many chemically unrelated types of antibacterial compounds. Germicides have multiple target site for their cidal effects on microorganisms while antibiotic have single target site[13]. It will be continued requirement for new and potent antimicrobial agents together with techniques suitable for control and destruction of microbial pathogens[14].

Materials and Methods

C-Disinfectants and antiseptics

Different types of disinfectants and antiseptics as shown in table (1) are used to test susceptibility of bacteria.

Table 1 disinfectants and antiseptics used in this study.

Source	Name
1- Hydrogen peroxide (H ₂ O ₂) 6%	Al-Teeba Company_ Baghdad, Iraq.
2- formaldehyde (formalin)10%	United Kingdom
3-Sodium hypochlorite (bleach chlorox)6%	product of Al- Baher of detergent .Baghdad,Iraq.
4-Chlorohexidine gluconate (Hibitene)	Al-Rahma pharmaceutical co. Aman –Jordan
5-Chlorohexidine cetramide (Savlon)	Ministry of health –Iraq
6-Povidone –iodin (Betadine) 4% 10%,18%	Mourad Est(Syria) Syria
7-Chloroxylenol (Dettol) 5%(3 type) 1,2 type(S ₁ ,S ₂) 3 type(Sp)	The Iraqi company for drugs industries and medical requirements-Samara– Iraq Spartin company
8-Chlorine compound (Large disc)3%	Syria

Identification of bacteria

The study focused on the most common pathogens dominate the hospitals environment and frequently cause serious NIs and those were Gram-positive bacteria represented by *Staphylococcus aureus* including Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Bacillus subtilis*. Gram-negative organisms represented by *P.aeruginosa* and *E.coli* were also investigated. Isolates were identified to the genus level based on the standard biochemical and microbiological methods such as: Cultural characteristics (colony property, hemolysis, pigment and other characteristics), Cellular characteristics (Microscopic examination): smear of bacterial isolates stain with gram stain in order for identification of staining type, shape, arrangement and presence of spore and Biochemical test: Oxidase, Catalase, Coagulase, Indol, Methyl red, Vogas –Proskauer, Simon Citrate, Urease, Gelatin liquification, Motility and Sugar fermentation (Glucose, Lactose, Arabinose, Sucrose, Mannitol)[15,16].

Antimicrobial susceptibility testing:

Susceptibility to Oxacillin 1µg/ml (Al-Razii) for *Staphylococcus aureus* isolates was determined by the standard disk diffusion according to Bauer *et.al.*, (1996) method[17] on Muller-Hinton agar incubated for 18-24 hour at 37 °C.

Sterilization test of disinfectants and antiseptics

The chemical disinfectants and antiseptics being used in this study were tested for their sterility from microorganisms for accurate susceptibility test as follow. One tenth ml of the disinfectants and antiseptics was added onto blood agar medium and was spread by spreading method. The plate incubated under aerobic condition at 37C° for 7 days[18].

Disinfectants and antiseptics susceptibility test

The antimicrobial activity of disinfectant and antiseptic was tested against 4 types of bacteria: *P.aeruginosa*, *E.coli*, MRSA and *B.subtilis*, in which bacteria tested for disinfectants and antiseptics were isolated from burns and wound infections except *B.subtilis* isolated from hospital environment and this test done by three different method :

1-Minimum Inhibitory Concentration (MIC)

2-Disc Diffusion method

1-Minimum Inhibitory Concentration (MIC) method

The MIC test was determined according to the method suggested by Baron *et.al.* (1994)[15]. depending on the turbidity of the bacterial growth. The MIC was recorded as the lowest concentration prevents the bacteria under test to grow.

2-Disc Diffusion method

The disc prepared through this study for the same disinfectants and antiseptics used in MIC test were done according to the method recommended by Wage and Hedin, (1985)[19]. The concentration of used disinfectants and antiseptics and these concentrations were 50,100,200,400,500, 800, 1000, 3000,5000 and 8000µ/disc for each hydrogen peroxide, sodium hypochlorite, formaldehyde, chloroxylenol(S₁) and chlorohexidine cetramid. The susceptibility test of these disinfectants and antiseptics discs was determinate according to Bauer *et.al.*, (1996) method[17].

Results and Discussion

In order to assess the efficiency of antiseptics and disinfectants used to sterily the skin and disinfect hospital environment, Some traditional disinfectants and antiseptics were tested toward four types of bacteria using three techniques (MIC, Disc diffusion and Well diffusion method) for this experiment as following :

1- Minimum Inhibitory Concentration (MIC)

The bacteria used for this experiment were *P.aeruginosa*, *E.coli*, MRSA and *B.subtilis*. The tested microorganisms were selected depending on their significant recurrence in nosocomial infection and their ability to resist the action of disinfectants [20]. The MIC values of Chlorohexidine gluconate (CHX) toward these bacteria were 128,128 32 and 32µg/ml respectively as show in figure(1) while the MIC values of

H₂O₂ for *P.aeruginosa*, *E.coli*, MRSA and *B.subtilis* were 1024, 512, 512 and 128µg/ml respectively figure(2) whereas the MIC values of sodium hypochlorite for these bacteria were 2048,2048,512 and 128 µg/ml respectively Figure(3). Sodium dichloroisocyanurate revealed the following MIC values 32768, 16384,128 and128µg/ml for *P.aeruginosa*, *E.coli*, MRSA and *B.subtilis* respectively as shown in figure (4).

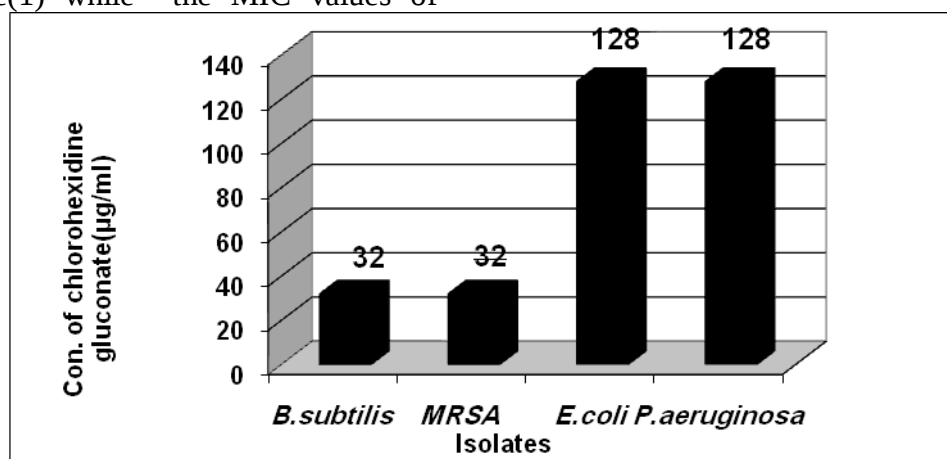


Figure 1 The MIC values of chlorohexidine gluconate on different bacterial isolates

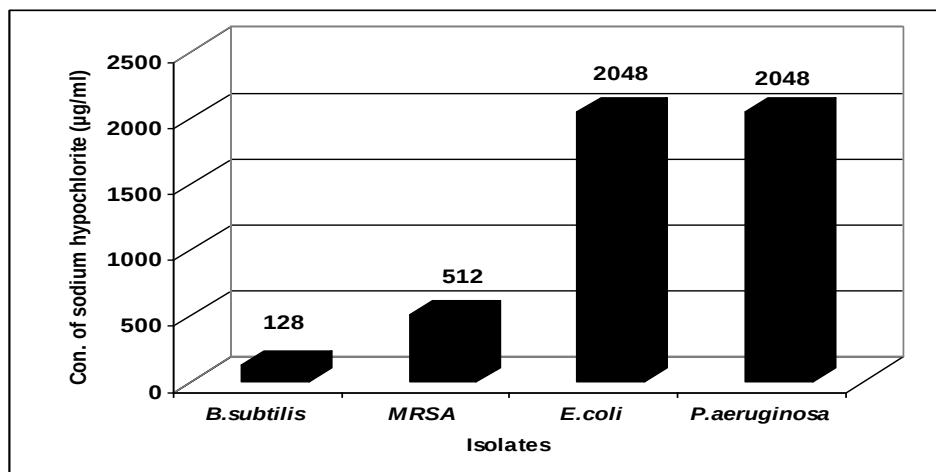


Figure 2 The MIC values of H₂O₂ on different bacterial isolates

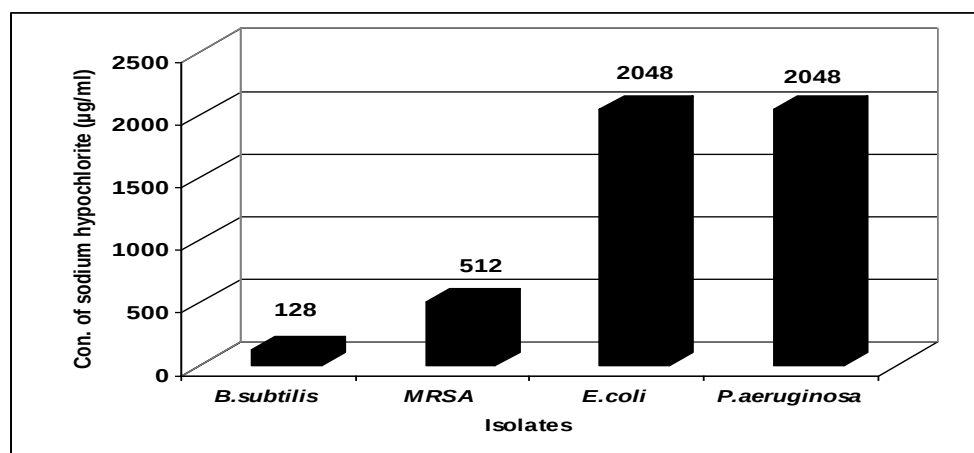


Figure 3 The MIC values of sodium hypochlorite on different bacterial isolates

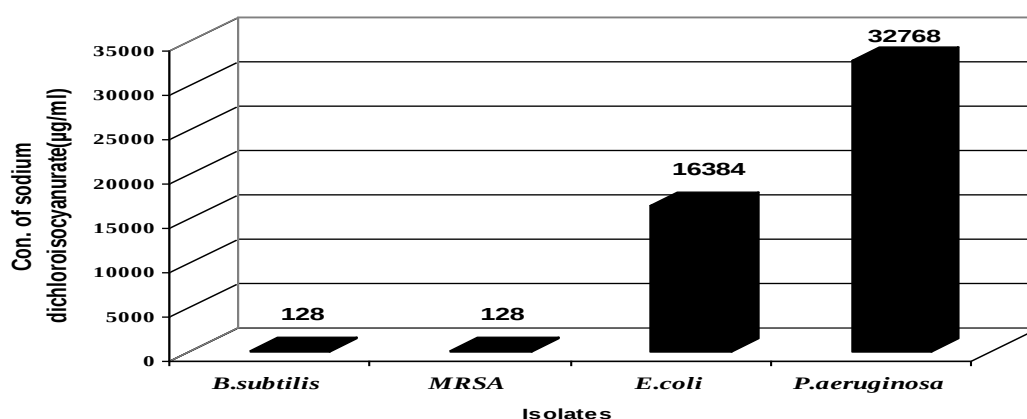


Figure 4 The MIC values of sodium dichloroisocyanurate on different bacterial isolate
The MIC values for Formaldehyde were 4096,2048,1024 and 256µg/ml for *P.aeruginosa*, *E.coli*, MRSA and *B.subtilis* respectively figure(5).

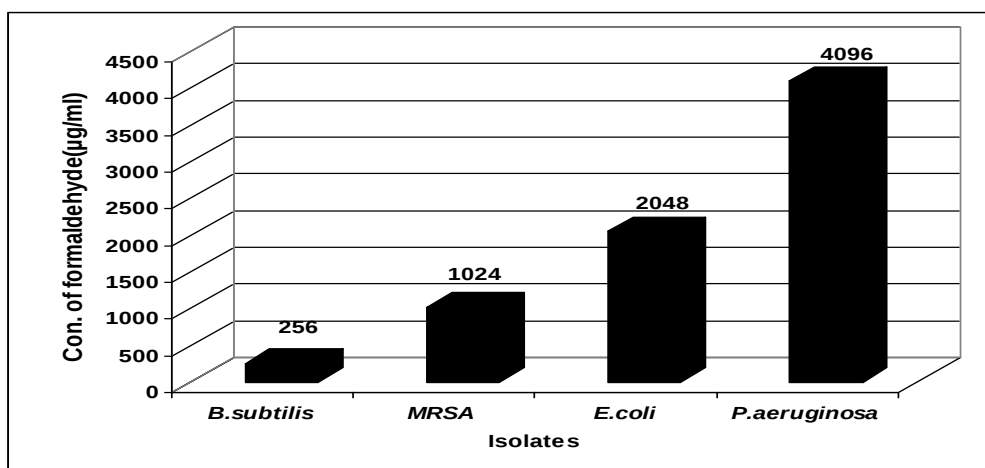


Figure 5 The MIC values of formaldehyde on different bacterial isolates

In the this study 3 types of chloroxylonol from different origins were tested. The first two types of chloroxylonol termed (S_1 and S_2) from

the Iraqi company for drugs industries and medical requirements (Samara) have MIC values towards 4 types of bacteria as shown in figure (6). In

addition, the third types of chloroxylenol (Sp) from Spartin company origins showed no considerable effect against *P.aeruginosa* and *E.coli*, while the MIC values for MRSA and *B.subtilis*

were 2048 and 1024 μ g/ml respectively. Chlorohexidine cetramid tested in this study exhibited no any efficiency against all bacterial tested even in high concentration reach to 1gm/ml.

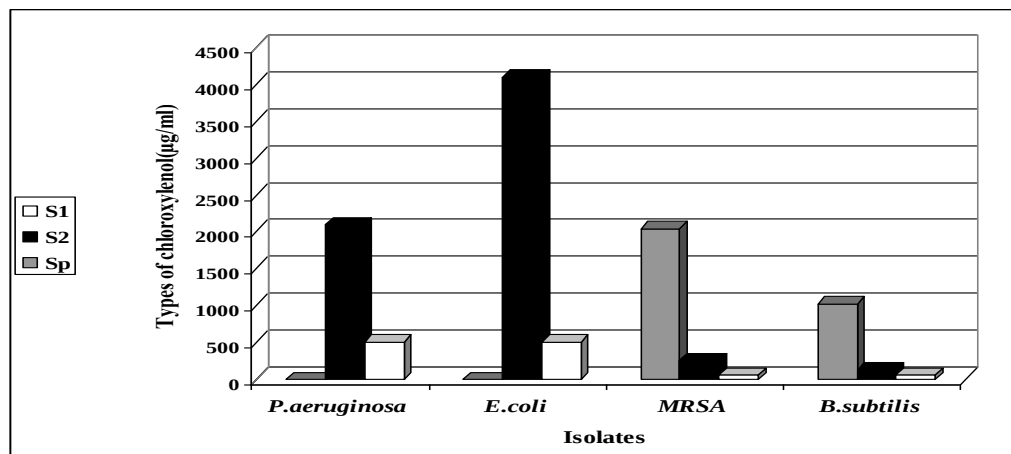


Figure 6 The MIC values of three types of chloroxylenol (S₁,S₂,Sp) on different bacterial isolates.

Chlorohexidine gluconate (CHX) was the most potent and effective disinfectant according to this study followed by chloroxylenol (S₁) type while chloroxylenol (S₂) and (Sp) types exhibited lower activity. H₂O₂, sodium hypochlorite, formaldehyde, sodium dichloroisocyanurate and PVP-I. According to the results obtained by Fakhriddeen, (2001). Chlorohexidine cetramid was the potent disinfectants against bacteria [21]. He arranged the disinfectants according to their potency as chlorohexidine cetramid, CHX, PVP-I, chloroxylenol, formaldehyde and H₂O₂. Chlorohexidine cetramid used in this study showed no considerable effect against all test bacteria and this could be attributed to the ingredients and method of formulation could affect the physical and biological properties of biocides and the presence of inactivating material in these disinfectants, storage conditions and long time period of storage may also affect the potency of disinfectants. Linton and

George, (1966) reported that many disinfectants used in the hospital lost its activity while it's still in primary jar in which all the factor mentioned above affected the efficiency of those agents [22]. Nicoletti *et.al.*, (1993) pointed out that MIC values of CHX for *P.aeruginosa*, *E.coli* and *S.aureus* were 64-128, 4-8 and 4-8 μ g/ml respectively [20]. Brumfit *et.al.*, (1985) found that MRSA isolates were more resistant than MSSA isolates to CHX, cetramid and quaternary ammonium compound [23].

Recurrent exposure of bacteria to CHX may led for adaptation and enhance their resistance to CHX whereas acquired resistance to CHX has been reported to occur in *S.aureus* and among many gram-negative bacteria including *P.aeruginosa* and *E.coli* [24] in which plasmids have been detected in MRSA strains which have altered susceptibility to a variety of biocides including CHX, cetamide, benzalkonium chloride, hypochlorite and betadine. In addition, some plasmids (i.e ,RP1) in *E.coli* has been

found to alter composition of the outer membrane and this may associated with decreased susceptibility to cetramide, CHX, and phenol[25]. *P.aeruginosa* has been described to be highly resistant to CHX.

Fakhriddien,(2001) pointed out that MIC values of H₂O₂ for *P.aeruginosa*, *E.coli*, *S.aureus* were 1024 ,512 and 512µg/ml respectively while Penna *et.al.*, (2001) mentioned that MIC values of (H₂O₂) for *E.coli* ranged between 1250-3700µg/ml and for *S.aureus* it ranged 625-938µg/ml[21][26]. Many aerobic microorganisms have developed intrinsic defense system that confer tolerance to peroxide stress particularly H₂O₂ and this includes the production of neutralizing enzymes including peroxidase, catalase and glutathione reductase to prevent cellular damage[27][28].

Penna *et.al.*, (2001) found that there were similar MIC values of chlorine compound (both sodium hypochlorite and sodium dichloroisocyanurate) for *E.coli* and *S.aureus* which ranged between (1109-1497)µg/ml[26], while the Penna *et.al.*,(2002) found that MIC values of sodium hypochlorite for *P.aeruginosa* was 2500µg/ml[29].

Dawaf,(1993) demonstrated that MIC values of chloroxylenol for *P.aeruginosa*, *E.coli* and *S.aureus* were 1024 ,1024 and 128µg/ml respectively[18]. Davies *et.al.*, (1980) referred that chloroxylenol has good activity against Gram-positive than Gram-negative bacteria and was less active against *P.aeruginosa*[30].

The MIC values of formaldehyde for *P.aeruginosa*, *E.coli* and *S.aureus* were 1024,512 and 512µg/ml respectively as it has been reported by Fakhriddien in (2001)[21][while lower relatively MIC for formaldehyde(156 µg/ ml) against both *E.coli* and *S.aureus* have been found by Penna and coworkers [26]. *Pseudomonas* spp.

and *E.coli* being resistant for formaldehyde have been isolated and identified. The resistance of these organisms against formaldehyde is conferred by a plasmid-mediated formaldehyde dehydrogenase enzyme [31].

In addition, in gram-negative bacteria, the outer membrane acts as a selective permeability barrier in limiting or preventing the entry of many unnecessary or harmful chemical compounds into the bacterial cell [32]. The changes in permeability system may lead to acquire resistance to biocidal compounds [12].

The outer membrane of *P.aeruginosa* is responsible for the high resistance in comparison with other organisms. This phenomenon is ascribed to some differences in Lipopolysaccharides (Lps) composition and in the cation content of the outer membrane, which aids in producing strong Lps-Lps links that selecting permit general diffusion through them [34]. Furthermore, *P.aeruginosa* possesses active efflux pump system acts as wide transporters for a whole range of biocides and antibiotics, that coupled with the narrow porin channels in the outer membrane of this organism, restrict diffusion of many antimicrobial agents into the cell[35].

Generally, Gram-positive bacteria observed in this study to be more susceptible to antimicrobial agents. This can be attributed to the contents of the cell wall, since it is composed of peptidoglycan and teichonic acid and neither of these appears to act as effective barrier to the entry of antiseptics and disinfectants, therefore high molecular weight substances can readily pass in to the *S.aureus* and vegetative cell of *Bacillus* spp.[36].

2-Disc Diffusion Method

To perform this experiment, discs for chloroxylenol (S1), hydrogen peroxide (H₂O₂), sodium hypochlorite,

sodium dichloroisocyanurate and formaldehyde of different concentrations were tested towards *P.aeruginosa*, *E.coli* and MRSA.

MRSA isolates exhibited considerable resistance to H_2O_2 at concentration below 1000 $\mu\text{g}/\text{disc}$ but it was sensitive at higher concentrations as shown in figure (7). *E.coli* was resistant to the concentration of H_2O_2 1000($\mu\text{g}/\text{disc}$) but it was sensitive at concentrations 3000, 5000 and 8000 $\mu\text{g}/\text{disc}$ with

inhibition zone 6, 7 and 11 mm, while *P.aeruginosa* exhibited resistance to most concentrations but it was sensitive to H_2O_2 only at concentrations 3000, 5000 and 8000 $\mu\text{g}/\text{disc}$ with inhibition zone 7, 11 and 15 mm (figure 7). Fakhriddeen, (2001) found in his study that *S.aureus*, *E.coli* and *P.aeruginosa* were resistant to lower concentrations 600 $\mu\text{g}/\text{disc}$ of H_2O_2 than concentration being used in this study [21].

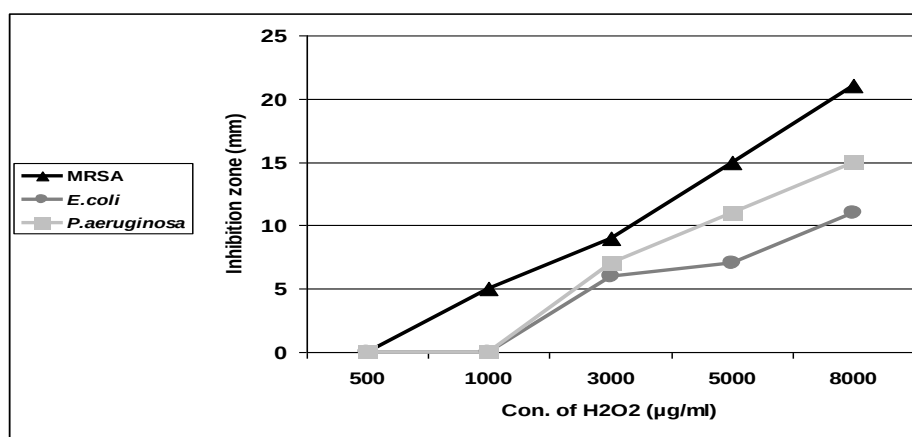


Figure 7 Inhibition zones of discs containing different concentrations of H_2O_2 on bacteria

Figure(8) shows that MRSA exhibited relative sensitivity to chloroxylenol (S_1) at concentrations of 100, 200, 300, 400 and 500 $\mu\text{g}/\text{ml}$ with inhibition zone of 8, 14, 18, 21 and 25 mm but it was not affected by concentrations below 100 $\mu\text{g}/\text{disc}$ while *E.coli* and *P.aeruginosa* revealed full resistance to all concentration of

chloroxylenol (50-500 $\mu\text{g}/\text{disc}$). Fakhriddeen, (2001) showed that *S.aureus* and *E.coli* were resistant to chloroxylenol in 10-100 $\mu\text{g}/\text{disc}$, but they were sensitive to this disinfectant at increased concentrations whereas *P.aeruginosa* was full resistant to all concentrations of chloroxylenol up to 500 $\mu\text{g}/\text{disc}$ [21].

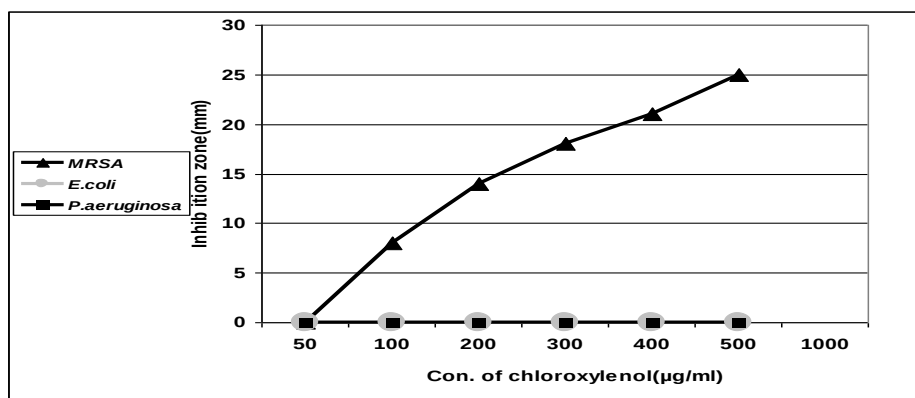


Figure 8 Inhibition zones of discs containing different concentrations of Chloroxylenol on bacteria

The results of this study showed that MRSA was resistant to all concentration of sodium hypochlorite below 3000 μ g/disc while it was sensitive to the concentrations of 3000,5000 and 8000 μ g/disc with inhibition zone 4,7 and 11 mm respectively as shown in figure(9), while *E.coli* and *P.aeruginosa* were resistant to all sodium hypochlorite

concentrations. MRSA was inhibited only at concentration ≥ 1000 μ g/disc (figure 10) of formaldehyde, while *E.coli* and *P.aeruginosa* were resistant to all concentrations of formaldehyde (50- 8000 μ g/disc). All bacterial isolates showed full resistance to sodium dichloroisocyanurate at concentrations (50-8000 μ g/disc) .

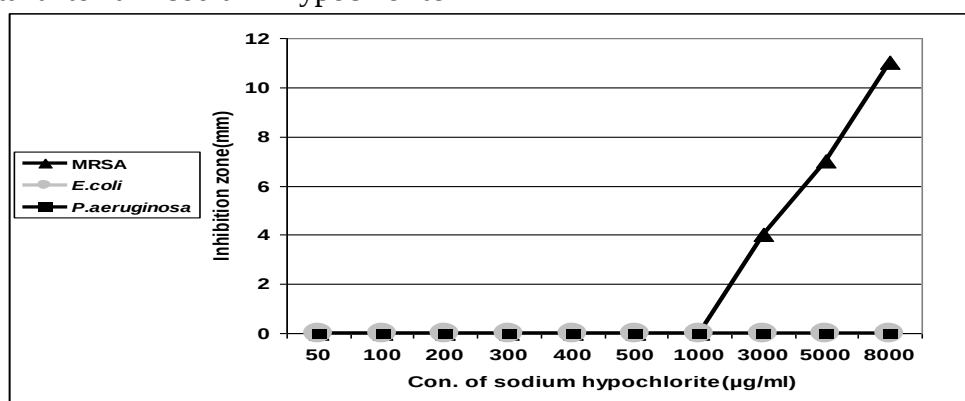


Figure 9 Inhibition zones of discs containing different concentrations of sodium hypochlorite on bacteria

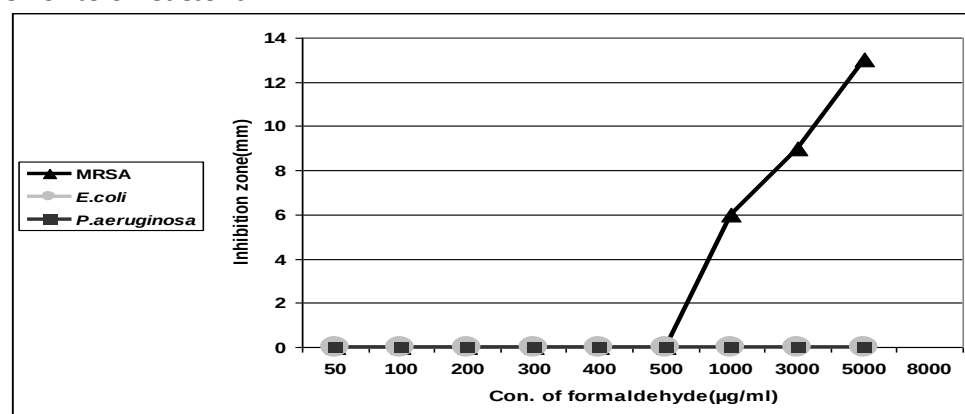


Figure 10 Inhibition zones of discs containing different concentrations of Formaldehyde on bacteria

Thus from the results expressed above, Chlorohexidine gluconate was the most effective biocidal agent against tested bacteria. Bacterial isolates especially *P.aeruginosa* and *E.coli* showed high resistance to disinfectants and antiseptics used in the hospital and this led to wound and hospital contamination.

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